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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

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Abstract

The magnitude of changes in carboxylation capacity in dominant plant species under long-term elevated CO_2 exposure (elevated pC_a) directly impacts ecosystem CO_2 assimilation from the atmosphere. We analyzed field CO_2 response curves of 16 C_3 species of different plant growth forms in favorable growth conditions in four free-air CO_2 enrichment (FACE) experiments in a pine and deciduous forest, a grassland and a desert. Among species and across herb, tree and shrub growth forms there were significant enhancements in CO_2 assimilation (A) by $+40 \pm 5\%$ in elevated pC_a (49.5–57.1 Pa), although there were also significant reductions in photosynthetic capacity in elevated pC_a in some species. Photosynthesis at a common pC_a (A_a) was significantly reduced in five species growing under elevated pC_a, while leaf carboxylation capacity (V_{cmax}) was significantly reduced by elevated pC_a in seven species (change of $-19 \pm 3\%$ among these species) across different growth forms and FACE sites. Adjustments in V_{cmax} with elevated pC_a were associated with changes in leaf N among species, and occurred in species with the highest leaf N. Elevated pC_a treatment did not affect the mass-based relationships between A or V_{cmax} and N, which differed among herbs, trees and shrubs. Thus, effects of elevated pC_a on leaf C assimilation and carboxylation capacity occurred largely through changes in leaf N, rather than through elevated pC_a effects on the relationships themselves. Maintenance of leaf carboxylation capacity among species in elevated pC_a at these sites depends on maintenance of canopy N stocks, with leaf N depletion associated with photosynthetic capacity adjustments. Since CO_2 responses can only be measured experimentally on a small number of species, understanding elevated CO_2 effects on canopy N_m and N_a will greatly contribute to an ability to model responses of leaf photosynthesis to atmospheric CO_2 in different species and plant growth forms.

Nomenclature

A = light-saturated net CO_2 assimilation at chamber pC_a
A_a = light-saturated net CO_2 assimilation per unit leaf area at current pC_a of 36 Pa
A_m = light-saturated net CO_2 assimilation per unit leaf mass at current pC_a (36 Pa)
$A_{a\text{-}56}$ = light-saturated net CO_2 assimilation per unit area at elevated pC_a of 56 Pa
$A_{m\text{-}56}$ = light-saturated net CO_2 assimilation per leaf mass at elevated pC_a of 56 Pa
FACE = free-air CO_2 enrichment
$f_{N\text{-}Rub}$ = the apparent fraction of N allocated to active Rubisco enzyme *in situ*

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J_{max} = *in situ* maximum electron transport capacity per unit leaf area
M_a = leaf mass per unit area
N_a = leaf N content per unit area
N_m = leaf N content per unit mass
pC_a = partial pressure of CO_2 in air
pC_i = partial pressure of CO_2 in intercellular air spaces
V_{cmax} = *in situ* leaf maximum CO_2 carboxylation capacity per unit leaf area
$V_{cm\text{-}m}$ = leaf maximum CO_2 carboxylation capacity per unit leaf mass

Keywords: downregulation, elevated CO_2, free-air CO_2 enrichment, leaf carboxylation capacity, leaf nitrogen, nitrogen allocation to RuBP carboxylase enzyme, photosynthesis–nitrogen relationships, photosynthetic nitrogen-use efficiency, plant functional groups

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Introduction

The carboxylation capacity of leaves drives the assimilation of CO_2 from the atmosphere (Baldocchi & Meyers, 1998; Canadell *et al*., 2000). Questions regarding the capacity for CO_2 fixation by plants and ecosystems under future, higher partial pressures of atmospheric CO_2 (pC_a) arise from uncertainties regarding whether leaf carboxylation capacity of dominant species will remain as large as in the present at pC_a of ≈37 Pa (Sage *et al*., 1989; Moore *et al*., 1999). The stimulation of photosynthesis in elevated pC_a over exposure periods of minutes to months is extremely well documented, but varies considerably among species and growth conditions (Sage, 1994; Drake *et al*., 1997). The magnitude of this stimulation strongly depends on the long-term maintenance of both the carboxylation capacity of leaves as well as the diffusional CO_2 supply to leaf internal surfaces in elevated pC_a. Early reports of losses in the initial stimulation of photosynthesis from greenhouse and controlled chamber studies over months to years (Sage *et al*., 1989) have in part been attributed to negative feedbacks on photosynthesis associated with artificial restrictions to the plant rooting zone (Curtis & Wang, 1998). However, reductions in carboxylation capacity have also been reported in field experiments in native ecosystems (Huxman *et al*., 1998; Lee *et al*., 2001; Rogers & Ellsworth, 2002) with natural rooting conditions. Mechanistically, these reductions have primarily been attributed to either carbohydrate accumulation and subsequent biochemical signal mechanisms in leaves (Moore *et al*., 1999), or from dilution of leaf N by carbohydrates and leaf structural material and/or increases in plant internal demands for N (Luo *et al*., 1994; Yin, 2002). However, there is still debate regarding how frequently reductions in carboxylation capacity are realized in field experiments (Poorter, 1998; Ainsworth *et al*., 2003).

Scores of elevated CO_2 studies have focused on the effects of elevated pC_a on a single species or small set of species on a given site (e.g., see reviews by Drake *et al*., 1997; Curtis & Wang, 1998; Saxe *et al*., 1998; Medlyn *et al*., 1999). Apart from individual case studies documenting the magnitude of elevated pC_a responses, there is a strong need to understand the prevalence and magnitude of reductions in carboxylation capacity across a range of species and functional groups or growth forms. In order to compare fundamental relationships across multiple plant species, statistical techniques for compiling individual plot-level results are required for analyzing data sets collected with different measurement conditions, instruments and protocols. For instance, metaanalyses of such data are increasingly common (Curtis, 1996; Curtis & Wang, 1998; Medlyn *et al*., 1999). However, this *post hoc* comparison approach only analyzes relative effect sizes and hence provides limited understanding of fundamental functional relationships across both ambient and elevated CO_2-grown plants.

We provide an alternative to indirect comparative statistical approaches for analyzing effects of elevated pC_a on CO_2 assimilation in multiple species at contrasting field sites, involving direct measurements following a common design protocol in free-air CO_2 enrichment (FACE) experiments. Such an approach presumably minimizes possible variation in photosynthetic parameters compared with independently conducted studies done with different procedures. This may permit a broader comparative understanding of elevated pC_a responses of plant functional groups or growth forms (Poorter, 1993) and identify trends in possible changes in photosynthetic capacity among species as well as functional relationships that could be useful for incorporation into plant and ecosystem models.

A principal functional relationship frequently utilized for predicting photosynthesis at the leaf and canopy scale in many current models of C_3 plant and

ecosystem functioning (Aber *et al*., 1996; Sellers *et al*., 1996) is the strong relationship between light-saturated net CO_2 assimilation at current pC_a per unit area (A_a) or mass (A_m) and leaf N. While the A_m–N_m relationships analyzed in these previous studies showed striking convergence toward a general, overall relationship (Reich *et al*., 1999), they provide little predictive power for estimating photosynthesis at pC_a levels other than at current ambient, or at other partial pressures of CO_2 within the leaf (pC_i) (Peterson *et al*., 1999). Moreover, fundamental A_a–N relationships may not extend to different environmental conditions forced by global change variables such as elevated atmospheric pC_a (Field *et al*., 1992). Possible reasons for changes in the A_a–N relationship in elevated pC_a include stomatal closure-induced changes in CO_2 supply to leaves, and alterations in the partitioning of N among photosynthetic enzymes as a result of downregulation of specific proteins (Stitt, 1991; Hikosaka & Hirose, 1998).

The goal of this study was to compare elevated pC_a responses measured on mature leaves under conditions favorable to photosynthesis among sites and plant growth forms. In these conditions, photosynthetic adjustments in elevated pC_a would serve as a benchmark for understanding the incidence of downregulation in the field. We measured CO_2 and water vapor exchange using common techniques and instrumentation at four FACE sites in order to minimize potential confounding effects because of methodological and instrument biases. More detailed analyses of the photosynthetic dynamics with time, environmental conditions, and seasonality are available for subsets of specific dominant species at each of these sites (Huxman *et al*., 1998; Lee *et al*., 2001; Noormets *et al*., 2001b; Rogers & Ellsworth, 2002). Thus, the intent of this study was to compare elevated CO_2 responses of recently matured leaves of diverse species measured on under proximal environmental conditions favorable to photosynthesis, as an indication of responses expected to contribute significantly to plant carbon balance. The measurements here were designed for evaluating photosynthetic properties and effects of elevated pC_a on these properties among multiple species in field FACE experiments. We hypothesized that (1) changes in CO_2 assimilation capacity after long-term growth in elevated pC_a are broadly related to CO_2-induced changes in leaf N, when they occur, and (2) plant growth forms vary in their partitioning of N to the photosynthetic apparatus, which affects their fundamental photosynthesis–N relationships (Reich *et al*., 1995), but also affects their pC_a responses. We also examined whether elevated pC_a exposure itself alters N partitioning to photosynthesis as previously hypothesized (Drake *et al*., 1997).

Material and methods

Study sites and elevated CO_2 treatments

The research sites utilize FACE for long-term CO_2 exposure of intact plant stands growing in native soils. The FACE systems at the sites were set up and operated according to the design of Lewin *et al*. (1994) and Hendrey *et al*. (1999), with modifications for the experimental design at each site (Jordan *et al*., 1999; Dickson *et al*., 2000). We denote the sites here by state location in the US (Table 1). The sites are located in the Anoka sand plain in central Minnesota (MN), the piedmont region in central North Carolina (NC), a bajada of Frenchman Flat in southern Nevada (NV) and in a glacial outwash plain in northern Wisconsin (WI). The four experimental sites described here represent FACE studies conducted in existing, unmanaged ecosystems (NC and NV) or planted ecosystems (MN and WI). A brief description of each site and the operational characteristics of the FACE systems are provided in Table 1.

The vegetation at the sites represents dominant species in typical vegetation types occurring in each region. In NC, the site consists of plots within an even-aged loblolly pine (*Pinus taeda*) forest, at age 18 years in 2001. At the NV site the vegetation is undisturbed desert shrubland dominated by evergreen *Larrea tridentata* and deciduous shrubs (*Lycium*, *Krameria* and *Ambrosia* spp.). The experimental plantings at the MN site consisted of native and naturalized tallgrass prairie species in different species mixtures including monocultures (Reich *et al*., 2001), while at the WI site the plantings were mixtures of *Populus tremuloides* with *Betula papyrifera* or *Acer saccharum*. Soils at the sites varied in texture (Table 1), pH (range 4–8) and fertility, although N is considered the principal limiting nutrient at all sites. Sites have moderate-to-low net N mineralization rates (Zak *et al*., 2003) characteristic of moderate-to-poor native soils in their respective regions. Measurements were made on plants growing in unamended soil in cases where soil manipulations were part of the FACE site design.

The treatment CO_2 regime varied somewhat among sites although target pC_a levels in FACE were approximately 56 Pa (Table 2). Specific details on the operation of the FACE systems at the sites are presented in Hendrey *et al*. (1999), Jordan *et al*. (1999) and Dickson *et al*. (2000). The NC and NV sites have evergreen vegetation and hence the FACE system operates year-round except when below freezing, whereas seasonal elevated pC_a exposures are conducted at the MN and WI sites during April–October. All sites have instrumented, ambient-only control plots with vertical vent

Table 1 A description of the location, climate and soil characteristics of each of four experimental study sites participating in the study

Site (location)	Elevation (m)	Mean annual precipitation ($mm\,yr^{-1}$)	Daily mean T (°C) in January and July	Soil order and texture
MN (45°27′N, 93°11′W)	280	660	−13.3, 20.2	Entisol; loamy sand
NC (35°58′N, 79°05′W)	170	1150	3.9, 25.7	Alfisol; clay loam
NV (36°46′N, 115°58′W)	970	140	2.2, 26.9	Aridisol; gravely sand loam
WI (45°30′N, 89°38′W)	490	730	−11.3, 19.7	Entisol; sandy loam

Sites are referred to here and in the text according to the state in which they are located.

Table 2 Background ambient pC_a during the growing season, and operational characteristics of the free-air CO_2 enrichment (FACE) system at each study site

Site	Effective plot size (m^2)	Start of experiment	Period described	Ambient pC_a (Pa)	Treatment pC_a (Pa)
NC	707	August 1996	Growing season, 1997–2001	36.7	57.1 ± 0.7
NV	491	April 1997	Frost-free season, 1997–2000	32.5	49.5 ± 3.0
MN	530*	April 1998	Growing season, 1998–2000	36.2	55.5 ± 1.2
WI	707	April 1998	Growing season, 1998–2000	34.7	56.0 ± 3.5

Ambient pC_a and treatment pC_a are for daylight hours only. Treatment pC_a was measured at the center of each of the three treatment plots at each site, at the top of the plant canopy. Additional measures of the control of CO_2 concentration in FACE experiments are given in Hendrey *et al*. (1999) and Jordan *et al*. (1999).
*Two meter squared subplots are nested within whole plots.

pipes and air-circulation systems in parallel to those used for elevated pC_a exposure. Measurements were conducted in the first and third growing seasons of operation of the FACE experiments at MN and WI, and in the second, third and fourth seasons of operation at NC and NV. Data for the longest cumulative pC_a treatment for each species were used, and the majority of CO_2 responses measured are for at least three growing seasons of CO_2 exposure (Table 2). Exceptions were made for *Lupinus* and *Populus*, both measured in the second growing season of CO_2 exposure, because of pathogen outbreaks. Measurements on the spring desert annual *Oenothera* were only made in the first year of study at the NV site during the El Niño rains of 1998 (Smith *et al*., 2000), as the species was not present during subsequent years.

Field and laboratory measurements

Responses of light-saturated leaf net CO_2 assimilation (A) to pC_i, the so-called 'A–pC_i curves', were constructed for intact leaves of each species using the Li-Cor model 6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). In all cases, leaves at the top of the plant canopy, developed in full sun were measured, typically in the morning before the onset of stomatal closure. Measurements were typically conducted on sunny days during nondrought periods, and were made during the growing season at times designed to coincide with peak photosynthetic activity, approximately mid-summer for all sites except NV. Times of high photosynthetic activity were judged from diurnal photosynthesis data for major species from each site from independent studies (Ellsworth, 2000; Noormets *et al*., 2001a; Naumburg *et al*., 2003) and from related studies at each site. The measurement protocol was chosen to indicate photosynthetic responses that would be expected to have significant impacts on plant carbon balance, rather than to select leaves most likely to show photosynthetic downregulation. The NV site is arid and receives one of the lowest mean annual precipitation levels recorded in North America (Table 1). Measurements at that site were made during periods following spring rains when soil moisture and physiological activity were relatively high (Naumburg *et al*., 2003 and unpublished site data).

Recently fully expanded leaves were measured for all the deciduous plants, and overwintering leaves were measured in spring at the time of bud-break in the evergreen species *Pinus* and *Larrea* as these leaves contribute significantly to the annual carbon balance of evergreens (Ellsworth, 2000). At all sites, sunlit, upper canopy dominant leaves were sealed inside the chamber while ensuring that chamber conditions

maintained growth pC_a, light saturation and seasonable temperatures. All measurements were made at a saturating photosynthetic photon flux density of 1500 $\mu mol\,m^{-2}\,s^{-1}$ or greater. Relative humidity inside the chambers was maintained at 60–70% at humid sites and 15–20% at the arid site. This resulted in leaf–air vapor pressure differences $<1.5\,Pa\,kPa^{-1}$ at NC, MN and WI and $<2.5\,Pa\,kPa^{-1}$ at NV, commensurate with growing season conditions. Leaf temperatures were controlled to mean daily maximum temperatures appropriate to the site and season (Table 3).

After equilibration under constant chamber conditions and growth pC_a levels, the measurements of A, C_i, stomatal conductance to water vapor, and associated parameters were recorded. Chamber pC_a was then changed and stepped through eight to 10 different levels starting close to the CO_2 compensation point and ending at pC_a above saturation. Measurements at each successive pC_a were made only after the CO_2 signal was stable, with coefficient of variation <1%. We made frequent leak tests to minimize bias in the low pC_a measurements and used teflon tape or inert clay to seal the chamber for measurements on thick leaves. To ensure direct comparability across sites, the infrared gas analyzer systems were factory calibrated, and checked against local site CO_2 standards. The procedure for fitting the Farquhar*et al*. (1980) photosynthesis model to the data for determination of the biochemical parameters V_{cmax} and J_{max} followed the approach of Wullschleger (1993) with modifications described in Appendix A.

After the field A measurements were completed, leaf tissue was harvested, sealed in plastic bags, and kept on ice or in a refrigerator until analyzed. Projected area inside the leaf chamber was measured by a scanning optical planimeter of digital images made directly from the leaf tissue (WinRhizo, Regent Instruments, Laval, Canada) or from diazo imprints of leaves. For *Pinus* species, one-sided projected area is used here for consistency with other species. After area determinations, samples were oven-dried at 70 °C for >48 h, weighed, and finely ground for chemical analysis. Total leaf N content was analyzed on all samples at the Duke University Phytotron or the University of Michigan

Table 3 Description of the 16 study species from four different free-air CO_2 enrichment experiments ('site') and four additional cooccurring species, with their mean photosynthetic characteristics (light-saturated A_a and V_{cmax}) expressed on a projected area basis for fully expanded leaves

Site	Species	Description	Sampling date	N plots	T_{leaf}	A_a ($\mu mol\,m^{-2}\,s^{-1}$)	V_{cmax} ($\mu mol\,m^{-2}\,s^{-1}$)
MN	*Achillea millefolium*	Herbaceous perennial	August 2000	3	28.2	12.2 ± 1.2	61.9 ± 6.9
MN	*Agropyron repens*	Graminaceous perennial	July 2000	3	28.0	12.3 ± 1.4	61.9 ± 7.1
MN	*Anemone cylindrica*	Herbaceous perennial	May 2000	2	25.8	9.5 ± 0.8	51.8 ± 3.8
MN	*Bromus inermis*	Graminaceous perennial	May 2000	3	25.2	15.2 ± 0.9	77.6 ± 5.0
MN	*Lupinus perennis*	Leguminous perennial	May 1999	3	25.2	16.5 ± 1.5	74.1 ± 6.5
MN	*Poa pratensis*	Graminaceous perennial	July 2000	2	25.0	12.2 ± 1.4	65.2 ± 5.3
MN	*Quercus macrocarpa*	Deciduous tree	July 2000*	3	28.0	19.0 ± 1.4	97.0 ± 8.0
MN	*Solidago rigida*	Herbaceous perennial	July 2000	3	28.0	17.7 ± 1.2	82.3 ± 4.9
NC	*Liquidambar styraciflua*	Deciduous tree	September 1999	2	28.0	12.6 ± 1.1	65.8 ± 3.1
NC	*Pinus taeda*	Evergreen conifer tree	May 2001	3	28.0	11.2 ± 0.4	63.1 ± 2.7
NC	*P. virginiana*	Evergreen conifer tree	May 2001*	3	28.0	8.9 ± 0.8	48.1 ± 2.5
NC	*Taxodium distichum*	Deciduous conifer tree	June 2001*	3	29.0	5.9 ± 0.3	40.5 ± 2.0
NC	*Festuca arundinacea*	Graminaceous perennial	June 2001*	4	30.4	17.5 ± 0.8	74.3 ± 3.9
NV	*Ambrosia dumosa*	Deciduous shrub	April 2000	3	24.2	18.6 ± 1.1	127.2 ± 9.5
NV	*Krameria erecta*	Deciduous shrub	April 2001	3	29.2	9.8 ± 1.2	109.7 ± 13.3
NV	*Larrea tridentata*	Evergreen shrub	April 2000	3	23.9	13.9 ± 1.3	95.2 ± 7.5
NV	*Oenothera primiveris*	Herbaceous annual	April 1998	3	23.9	22.8 ± 1.5	117.5 ± 10.2
WI	*Acer saccharum*	Deciduous tree	August 2000	2	26.8	6.4 ± 0.8	40.6 ± 3.5
WI	*Betula papyrifera*	Deciduous tree	August 2000	3	26.7	19.8 ± 1.2	91.2 ± 4.7
WI	*Populus tremuloides*	Deciduous tree	August 1999	3	26.2	20.9 ± 1.4	103.4 ± 5.7

Sampling date, sample size of individual study plots for a given treatment (N, with one or two plant individuals per plot), and leaf temperature (T_{leaf}) from the temperature-controlled chamber measurements are indicated. All means were calculated across pC_a treatments.

*Indicated species were not located within the actual elevated CO_2 experiment, or are species for which measurements in elevated CO_2 were not taken.

Plant-Soil Analysis Laboratory after combustion in a CHN analyzer (Model NA1500, Carlo-Erba Instrumentiazone, Milan, Italy).

Statistical analyses

Data were analyzed in analysis of variance (ANOVA) to test for main effects of site, pC_a treatment and species as well as $pC_a \times$ site interaction. The data were collected within a split-plot design with species nested within site. The significance of the pC_a treatment was tested using a nested ANOVA model with block (treatment) as error term, while the species within-site effect was tested using the residual error term. All measured variables were tested for normality and transformed appropriately (e.g., square root in most cases), when necessary. Since species were unique to their respective sites, CO_2 effects on leaf characteristics of individual species were tested when site effects were not significant ($P > 0.10$) in ANOVA, using paired *t*-tests (generally one-tailed if testing for downregulation). These tests employed the paired plot system appropriate for each FACE site according to the *a priori* experiment design. We expressed the relative effect of elevated pC_a (E) on photosynthetic variables as an enhancement ratio as a percent; $E = (P'/P) \times 100$ where P' denotes a parameter value at elevated pC_a and P denotes the control parameter value at current ambient pC_a, or as a simple response ratio (e.g., $R = (P'/P) \times 100$). Given the limited statistical power of a small number of plot replicates at each site, effects were considered marginally statistically significant for $P \leq 0.10$ and statistically significant for $P \leq 0.05$. Statistical analyses were conducted with JMP and SAS software (JMP v. 5.01 and SAS v. 9.1; SAS Institute, Cary, NC, USA).

To estimate functional relationships between pairs of variables, simple linear regression analysis was used to relate photosynthetic variables to leaf N. We used type I linear regression techniques rather than standardized major axis slope-fitting techniques (see (Peterson *et al.*, 1999) because measurement error for leaf N is relatively small. Moreover, this type of regression is appropriate since the goal was to describe a specific functional relationship between variables (Sokal & Rohlf, 1995), and it allows for comparison with previous analyses of photosynthesis–N relationships.

Results

There was nearly a fourfold range in maximum A at current ambient pC_a (A_a) among the 16 species at different FACE sites in North America (Table 3). Sites differed in the dominant growth form of species within each ecosystem (e.g., herbs vs. trees), and also varied in mean maximum A_a and other leaf-level parameters of the major dominant species (Table 3). Because of differences in leaf mass to area ratio (M_a) among species, A_m at current ambient pC_a showed a larger range than A_a (sixfold), from nearly 60 nmol C g leaf^{-1} s^{-1} in the desert species *Larrea* and *Krameria* to 350 nmol g^{-1} s^{-1} in the herb *Lupinus*.

With a ≈55% increase in pC_a in FACE (pC_a of + 20 Pa) and assuming approximately linear A over the relevant pC_a range, one may expect a similar relative enhancement of A. The observed mean instantaneous enhancement in A for the 16 species in this study deviated slightly from linearity, as A was enhanced by 51 ± 5.5% with a short-term switch in pC_a supply to the leaf chamber to 56 Pa. For plants exposed to elevated pC_a in FACE for multiple seasons compared with control plants in a paired design, there was a significant overall enhancement of A of 40 ± 7% across species with elevated pC_a of 56 Pa ($P < 0.009$, Table 4; Fig. 1).

Species varied in the magnitude of the enhancement of A after multiple years of elevated pC_a ($P < 0.0001$, Table 4), which ranged from 20% to 80% (Fig. 1). However, the multiyear A enhancement was not significant in four of the 16 species studied, across the MN, NV and WI sites (Fig. 1, one-tailed paired *t*-test, $P > 0.10$). For most species, the multiyear enhancement of A was not statistically different from the instantaneous enhancement of A with a step change in pC_a from 36 to 56 Pa. For two species (the tree *Liquidambar* and the herbaceous nitrogen fixer *Lupinus*), the multiyear A enhancement by elevated pC_a tended to exceed the instantaneous A enhancement ($0.10 > P > 0.05$). The multiyear A enhancement was significantly less than the expected instantaneous A enhancement for five of the 16 species (one-tailed *t*-test, $P < 0.05$). The lack of an increase in A at growth pC_a after multiple years at elevated CO_2 was indicative of downward adjustment in photosynthetic characteristics in major species at multiple FACE sites.

We tested whether this lack of enhancement could be attributed to treatment differences associated with a stomatal closure response under elevated pC_a by determining if growth pC_a treatment affected the relative operating pC_i of leaves. There was no significant treatment effect on the ratio of CO_2 in the leaf internal air spaces to CO_2 outside of the leaf (e.g., pC_i/pC_a), with $pC_i/pC_a = 0.70 \pm 0.02$ (± SE) for ambient-grown plants compared with $pC_i/pC_a = 0.73 \pm 0.02$ for elevated pC_a plants at $pC_a = 56$ Pa (two-tailed *t*-test, $P > 0.10$). At a common pC_a of 36 Pa, pC_i was remarkably similar among pC_a treatments across species, with $pC_i = 25.9 \pm 0.5$ Pa for ambient plants compared with $pC_i = 26.2 \pm 0.7$ Pa for elevated pC_a-grown plants. Desert shrub species had a considerably lower mean

Table 4 ANOVA results for randomized split-plot experiments on effects of elevated pC_a on leaf physiological characteristics of 16 C_3 plant species (Table 3) from four FACE sites

		Effects			
Parameter	MS error	Site	Species	pC_a treatment	Mean pC_a effect (%)
Degrees of freedom	74	3	12	1	-
A_a (growth pC_a)	0.046	ns	13.61****	56.21****	+39.9
A_a	8.15	ns	15.02****	3.56*****	−7.5
A_m (growth pC_a)	1380	ns	20.52****	5.97*	+33.6
V_{cmax}	0.685	6.68**	7.00****	4.85*	−8.3
J_{max}	2.22	ns	13.44****	ns	ns
$V_{cm\text{-}m}$	8.59	2.75*****	17.09****	10.17**	−13.6
N_m	2.7×10^{-4}	4.17*	22.13****	20.84***	−12.0
N_a	3.0×10^{-3}	7.33**	15.62****	3.32*****	−7.2
M_a	6.0×10^{-5}	6.70*	23.52****	4.26*	+7.2

All ANOVA models were highly significant ($P < 0.0001$). Values are degrees of freedom (first row), model mean-square (MS) error using type III sums of squares, F-statistics for the appropriate ANOVA term where significant ($P < 0.10$), and the relative effect size of elevated pC_a on a given parameter, respectively. Effects that are not significant (ns; $P > 0.10$) are indicated as such. The site × treatment pC_a term was not significant ($P > 0.10$) for any of the parameters analyzed and was omitted from the model. When effects of the elevated pC_a treatment are at least marginally significant ($P < 0.10$), the relative pC_a effect as a percentage is shown, calculated with the least-squares means. Parameters are: V_{cmax}, maximum CO_2 carboxylation capacity as prescribed by Farquhar *et al*. (1980); $V_{cm\text{-}m}$, maximum carboxylation capacity per unit leaf mass; J_{max}, maximum electron transport capacity; and other parameters are defined in the text. The transformations used were: ln(A_a at growth pC_a), $\sqrt{V_{cmax}}$, $\sqrt{J_{max}}$ $\sqrt{V_{cm\text{-}m}}$, $\sqrt{(N_m^{-1})}$, $\sqrt{(M_a^{-1})}$, $\sqrt{\log_{10}(N_a \times 2)}$, with other variables normally distributed without transformation.
Significance values at + $P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0005$.

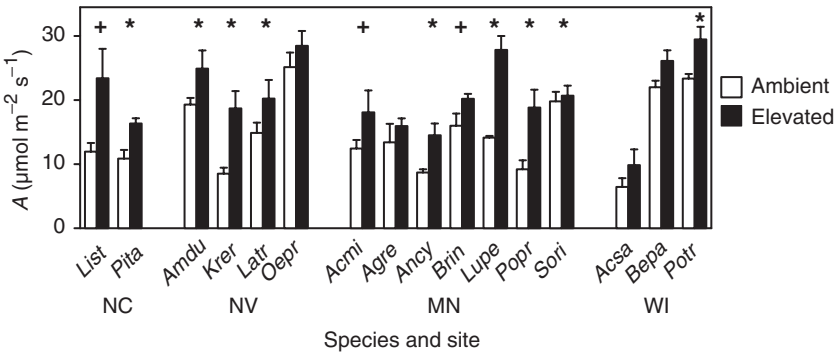

Fig 1 Maximum photosynthesis, A at growth pC_a, for 16 species in free-air CO_2 enrichment experiments at four different sites in the USA. The sites are denoted by state abbreviation, and species names from Table 3 are abbreviated according to the first two letters of each part of the scientific binomial. Mean values for plants grown and measured at ambient pC_a in control rings at each site are denoted by open bars, and means for plants grown and measured at elevated pC_a are denoted by dark bars. Elevated pC_a exposure was two or more growing seasons for all species except *Oenothera* (see text). Values shown are the mean parameter values across individual rings of a given treatment (typically $N = 3$), with standard errors for among-plot variability shown. Within a species, differences in a parameter between pC_a treatments at $P < 0.05$ (one-tailed paired t-test) are denoted by * and those for $P < 0.10$ are denoted by $^+$.

pC_i/pC_a ($pC_i/pC_a = 0.55 \pm 0.03$ across pC_a treatments) than other species, reflecting their greater water-use efficiency, and for this reason were excluded from the overall comparison of pC_i. In the absence of pC_a treatment differences in CO_2 supply to the intercellular air spaces, a lack of A enhancement in elevated pC_a must instead be related to the biochemistry of photosynthesis. In the following section, we analyzed leaf carboxylation dynamics for evidence of down-regulation.

Changes in photosynthesis and biochemical capacity in species in elevated pC_a

Assimilation at a common pC_a can be compared between pC_a treatments to test for changes in photosynthetic

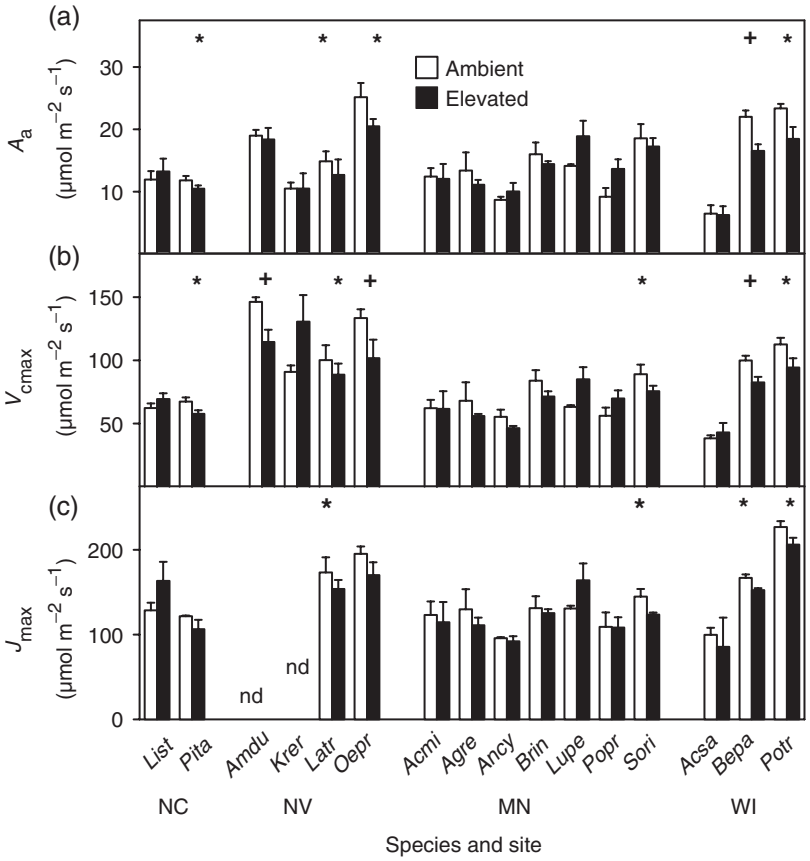

Fig 2 (a) Maximum net photosynthesis at a common pC_a of 36 Pa (A_a), (b) maximum *in situ carboxylation* velocity (V_{cmax}), and (c) maximum *in situ* electron transport capacity (J_{max}) for 16 species in free-air CO_2 enrichment experiments at four different sites. Labels are as in Fig. 1. Bars are means with standard errors for among-plot variability. Within a species, differences in a parameter between pC_a treatments at $P<0.05$ (one-tailed paired *t*-test) are denoted by * and those for $P<0.10$ are denoted by $^+$.

capacity in response to growth in different pC_a treatments (Fig. 2a). As expected, species differed significantly in mean A_a ($P<0.0001$, Tables 2 and 4). Although sites were characterized by different dominant plant growth forms (herbs including grasses, trees or shrubs), sites did not differ significantly in A_a (Tables 2 and 4). There was a marginally significant main effect of pC_a treatment on A_a ($P=0.065$, Table 4) and no significant site × pC_a treatment interaction ($P>0.10$; Table 4). In fact, three sites (NC, NV and WI) had at least one species for which there was a significant reduction in A_a with pC_a treatment (one-tailed paired *t*-test, $P<0.05$; Fig. 2a). For the most part, parallel results were obtained for V_{cmax} in response to elevated pC_a treatment (Fig. 2b).

The biochemical parameter V_{cmax} showed significant site differences ($P=0.0065$), with highest V_{cmax} observed for the five desert species at the NV site following winter rains. There was a significant main effect of elevated pC_a on V_{cmax} ($P=0.031$) and highly significant species effects ($P<0.0001$) but no significant site × pC_a interaction ($P>0.10$; Table 4). Five species showing reductions in A in elevated pC_a also showed marginally significant ($P<0.10$) or significant reductions in V_{cmax} (one-tailed paired *t*-test, $P<0.05$; Fig. 2a, b), and two additional species (*Ambrosia* and *Solidago*) also showed significant V_{cmax} reductions relative to ambient pC_a-grown plants. Thus seven species across all four sites showed statistical evidence of downregulation of V_{cmax} at elevated pC_a, with a mean effect of –19 ± 3% across these species. Six of the seven species showing downregulation had the highest V_{cmax} and leaf N per unit area in the entire set of 16 species studied. Overall there was no significant treatment pC_a effect on J_{max}, although four species that had significant reductions in V_{cmax} in elevated pC_a also showed reductions in J_{max} (one-tailed *t*-test, $P<0.10$, Fig. 2c), suggesting concurrent treatment effects in these cases.

Changes in green leaf N are implicated in the pC_a response of A in many species. Leaf N_m showed significant reductions in elevated pC_a (mass basis;

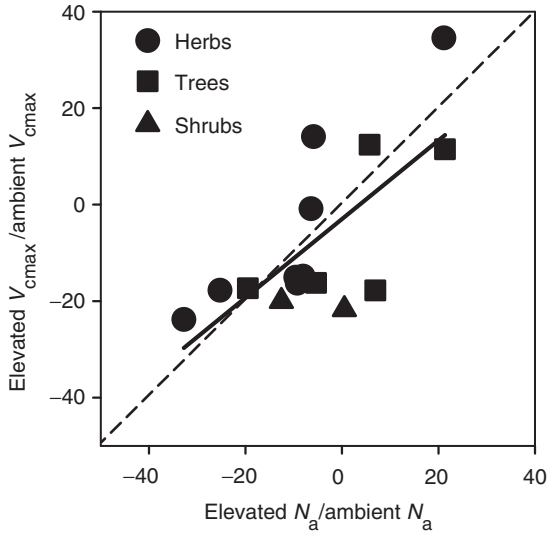

Fig 3 Relationship between the percent pC_a response of leaf N per unit area (N_a), expressed as th response ratio of N_a in elevated pC_a-grown plants to N_a in ambient pC_a (R_{Na}), and the relative response of carboxylation capacity ($R_V$cmax). Each data point represents a mean ratio for a species with some data obscured by other points. The correlation between these two ratios is shown (slope = 0.815, $r = 0.71$), as well as the 1 : 1 line.

$P = 0.031$), and a marginally significant pC_a effect on leaf N per unit area (N_a; $P = 0.072$, Table 4). There was no significant effect of pC_a on leaf carbon concentration among species (not shown). We tested for similarities in the pC_a response of V_{cmax} and leaf N per unit area (N_a) using R calculated for both of these variables, given that treatment responses were detected in both variables in ANOVA. There was a significant correlation ($P = 0.0024$; $r^2 = 0.71$) between the magnitude of relative N_a response to pC_a and the relative V_{cmax} response (Fig. 3), implicating changes in leaf N_a that were broadly associated with changes in V_{cmax} across species. The upper end of this relationship was influenced by apparent 'upregulation' of carboxylation capacity (V_{cmax}) for the N-fixing species *Lupinus*, with about a 20% increase in N_a and a 35% increase in V_{cmax}. Among all species, the correspondence between these variables was not 1 : 1 (slope = 0.82). However, among species exhibiting significant or marginally significant V_{cmax} differences with pC_a treatment (mean V_{cmax} response of –19%, see Fig. 2), the leaf N_a response ranged from a nonsignificant value of + 7% to a large and significant decrease (−33%), suggesting possible variation among species in elevated pC_a response mechanisms.

Photosynthetic–nitrogen relations among species

We explored the relationship between N_a and both A_n and V_{cmax} to understand whether long-term elevated pC_a altered fundamental plant photosynthetic–nitrogen relations. Relationships between A_a and N (on an area and mass basis) at ambient and elevated pC_a have been used as evidence of downregulation of A_a via concurrent reductions in both variables, or evidence of fundamental changes in photosynthetic nitrogen-use efficiency through changes in the slope of the A_a–N relationship (Peterson *et al*., 1999). There was no significant area-based relationship between A_a and N_a among species ($P > 0.10$; not shown). There was a significant relationship between A_m and N_m among species ($r^2 = 0.31$, $P < 0.0005$; Table 5 and Fig. 4a). The relationship between A_m at a common pC_a and N_m among ambient and elevated pC_a-grown plants was not significantly different ($P > 0.10$) in slope or intercept from relationships in two broad surveys of more than 100 C_3 plant species in the literature (Field & Mooney, 1986; Reich *et al*., 1999) (Fig. 4b).

We found statistically distinct relationships between A_m and N_m among different growth forms: the relationship for herbaceous species (including grasses) differed from that for trees, and for desert shrubs (Fig. 4a). These three groups did not differ significantly in the slope of the A_m–N_m relationship ($P > 0.10$) but showed significantly different, and progressively lower Y-intercepts ($P < 0.0001$, Table 5) from herbs to trees to desert shrubs. The reductions in Y-intercept represent plant growth form differences rather than site differences because the overall relationship comprised herbaceous species or tree species from three of the four sites, with no apparent deviation for species from different sites with their respective groups.

Elevated pC_a treatment had no significant effect ($P > 0.10$) on the overall A_m–N_m relationship among all species, nor on the A_m–N_m relationship within plant growth forms (herbaceous species, trees, and desert shrubs; $P > 0.10$ for each group, Table 5). Still, there was a highly significant effect of elevated pC_a on leaf N_m ($P < 0.005$) and on A_m ($P < 0.005$) with a weaker effect of elevated pC_a on N_a ($P = 0.065$; Table 4). The differences in elevated pC_a effects on mass vs. area-based measures of A and N in Tables 4 and 5 suggest reductions in mass-based quantities (A_m and N_m by species) as would occur with dilution by leaf carbohydrates with growth in elevated pC_a, but no alterations in the fundamental relationship between A_a and N_a. While there was no effect of elevated pC_a treatment on the A_m–N_m relationship when compared at a common measurement pC_a, measurement pC_a had a significant effect on this relationship ($P < 0.005$, Fig. 4c) as expected given the overall enhancement of A by pC_a (Fig. 2). Slopes of the $A_{m\text{-}56}$–N_m relationship were higher than those for A_m–N_m by 115% and 20% for herbs and trees, respectively (Table 5).

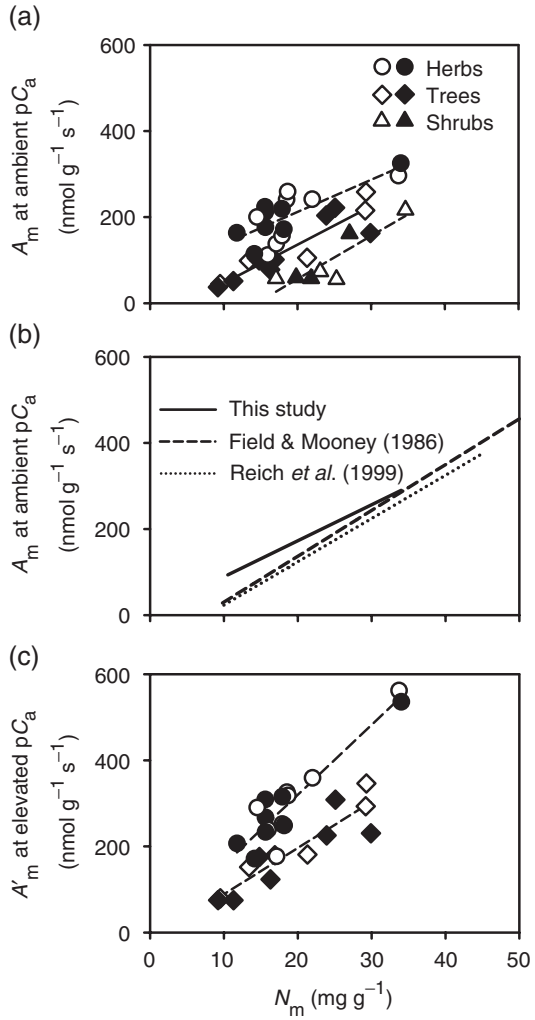

Fig 4 Relationships between A_m measured at different common pC_a levels and N_m for different growth forms for plants growing at ambient pC_a (open symbols, a, c) and two to three growing seasons of elevated pC_a (dark symbols, a, c). Data for plants in ambient pC_a include four species in Table 3 not located in free-air CO_2 enrichment (FACE) plots. (a) A_m data are compared at $pC_a = 36$ Pa; (b) Comparison of the overall relationships for herbaceous species and tree species combined from (a) with similar published relationships in the literature (Field & Mooney, 1986; Reich *et al*., 1999). (c) A_m measurements are compared at $pC_a = 56$ Pa (e.g., $A_{m\text{-}56}$). Slopes of the relationships in (b) are: $A_m = 10.2 + 8.45N_m$ for trees and herbs combined from (a); $A_m = -5.4 + 10.64N_m$, Field & Mooney (1986); and $A_m = -5.5 + 10.05N_m$, Reich *et al*. (1999). There were no statistical differences in these relationships. (a, c) Herbaceous plants (circles) and trees (diamonds) are distributed among at least two of the four sites, while shrubs (triangles) were only located at the NV site (Table 3). There was no significant difference in the relationship in (a) between herbaceous and tree species.

A statistically lower *Y*-intercept of the A_m–N_m relationship at current ambient pC_a was evident for the desert shrubs (-186 nmol g^{-1} s^{-1}) compared with the other species. This difference was likely attributable to a lower operating pC_i for A_a in the shrubs vs. herbs and trees. Mean pC_i/pC_a for desert shrubs was 0.55 ± 0.03 ($\pm$ SE) compared with a pC_i/pC_a ratio of 0.72 ± 0.02 for all other species, indicating greater relative stomatal limitations to gas exchange in desert shrub species. Herbaceous species and trees were similar to one another in pC_i/pC_a but also differed significantly in the *Y*-intercept of the A_m–N_m relationship ($P < 0.0001$).

Carboxylation capacity of different plant growth forms

The biochemical parameters V_{cmax} and J_{max} underlie photosynthetic capacity of leaves with respect to leaf N. There was a significant correlation between V_{cmax} and N_a across all species excluding shrubs ($P < 0.0025$; Table 5), although with low goodness of fit ($r^2 = 0.29$). Herb species and tree species formed distinct groups with respect to the V_{cmax}–N_a relationship (Fig. 5a) with a distinct, larger *Y*-intercept for herbs compared with trees (Table 5). This was also the case with respect to the relationship between J_{max} and N_a (Fig. 5c, Table 5). In both cases, there was no relationship between biochemical parameters and N_a for the shrub species, nor were there effects of treatment pC_a on the overall relationships or within growth form. Mass-based relationships (e.g., $V_{cm\text{-}m}$–N_m, and $J_{m\text{-}m}$–N_m) were stronger than the corresponding area-based relationships. There was a highly significant growth form effect ($P < 0.0001$) on the form of the $V_{cm\text{-}m}$–N_m relationship (Fig. 5b), with goodness of fit (r^2) within each growth form group ranging from 0.50–0.90 (Table 5). In the case of herb species and trees, the $V_{cm\text{-}m}$–N_m and $J_{m\text{-}m}$–N_m relationships were specific to growth form rather than sites, since at least one species of each growth form was located on a different site from the remaining species (Table 2), and conformed to the overall relationship.

Differences in the area-based vs. mass-based forms of the relationships between V_{cmax} and N and J_{max} and N relate to differences in N_m and N allocation to the Rubisco enzyme among different growth forms. Such differences were particularly apparent when comparing among these groups at a common M_a (Fig. 6). On average, predictions from the regressions in Fig. 6a show that there was approximately a onefold greater N_m for trees (20.9 ± 4.4 mg g^{-1}; mean $\pm$ 95% confidence interval, CI) compared with herbs (13.9 ± 3.3 mg g^{-1}), and for shrubs (31.3 ± 5.1 mg g^{-1}; mean and CI) compared with trees, all compared at a common M_a of 100 g m^{-2}. However, at this common M_a herb species

Table 5 Summary of relationships between photosynthetic parameters (dependent variables) and leaf characteristics such as for leaf N (leaf N per area, N_a; and per mass, N_m; independent variables) across species, and their regression statistics

Group	Dependent variable	Independent variable	Intercept	Slope	r^2	*P*-value	Intercept by form *P*-value	Slope by form *P*-value	Group	Group intercept	Group slope	Group r^2	Group *P*-value
All	A_a	N_a	–	–	0.043	ns	–	–	–	–	–	–	–
All	A_m	N_m	33.42	6.26	0.308	0.0006	<0.0001	ns	Herbs	61.9	7.51	0.596	0.0005
									Trees	−51.6	9.49	0.877	<0.0001
									Shrubs	−186.4	11.44	0.789	0.0180
All	$A_{m\text{-}56}$	N_m	48.8	9.10	0.309	0.0006	<0.0001	ns	Herbs	−5.21	16.25	0.857	<0.0001
									Trees	−29.24	11.42	0.875	<0.0001
									Shrubs	−51.78	7.186	0.629	0.0599
Herbs + trees	V_{cmax}	N_a	39.14	19.46	0.292	0.0025	0.0009	ns	Herbs	27.59	41.04	0.463	0.0037
									Trees	7.82	29.62	0.642	0.0010
Herbs + trees	J_{max}	N_a	68.05	38.19	0.428	<0.0001	0.0017	ns	Herbs	15.74	42.58	0.455	0.0041
									Trees	7.82	29.62	0.642	0.0010
All	$V_{cm\text{-}m}$	N_m	168.8	32.74	0.408	<0.0001	<0.0001	0.0040	Herbs	430.8	29.53	0.506	0.0020
									Trees	−131.5	41.36	0.901	<0.0001
									Shrubs	−1251.6	79.0	0.766	0.0223
All	$J_{m\text{-}m}$	N_m	350.4	64.82	0.359	0.0002	<0.0001	0.071	Herbs	271.7	91.30	0.800	<0.0001
									Trees	−47.7	78.70	0.881	<0.0001
									Shrubs	−273.6	49.17	0.910	0.0119
All	N_m	M_a	24.28	−0.0387	0.112	0.0498	<0.0001	ns	Herbs*	22.29	−0.071	0.350	0.0258
									Trees	27.75	−0.0681	0.445	0.0127
									Shrubs	44.06	−0.0974	0.879	0.0057

Relationships are shown for subsets of the plant growth forms only in cases where there were significant differences in regression parameters among growth forms, as indicated by significant effects of plant growth form on the intercept (intercept by form) or the slope term (slope by form) in regression with dummy variables. Separate relationships for the herbaceous and tree growth forms are not site specific since they are based on data from species across three different sites (see Table 1).
*N-fixing species *Lupinus* was removed from this analysis, since its N relations are obviously altered relative to other nonfixing species.figure legends.

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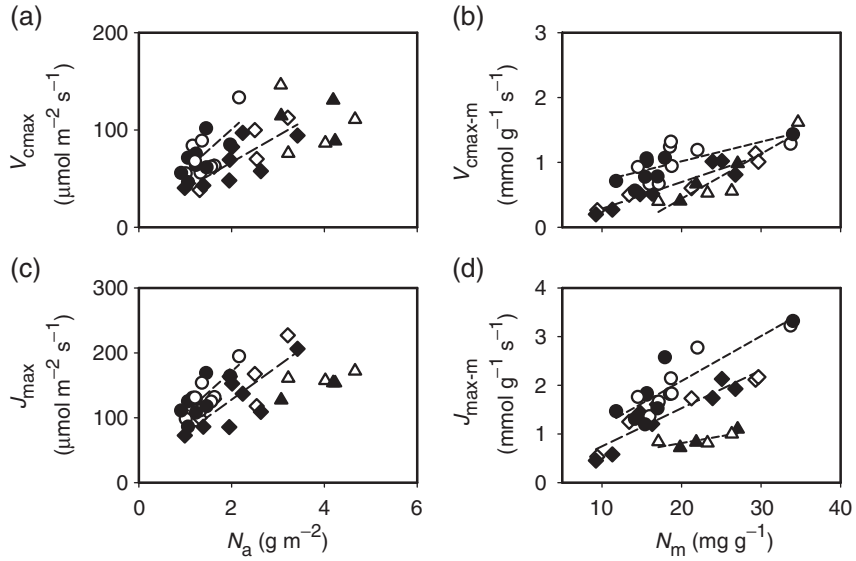

Fig 5 Relationships between $V_{cm\text{-}m}$ and N_m for different growth forms of plants growing at ambient pC_a and two to three growing seasons of elevated pC_a. Symbols as in Fig. 4 for species listed in Table 3. Regression statistics for the relationships both among and within each plant growth form shown are given in Table 5.

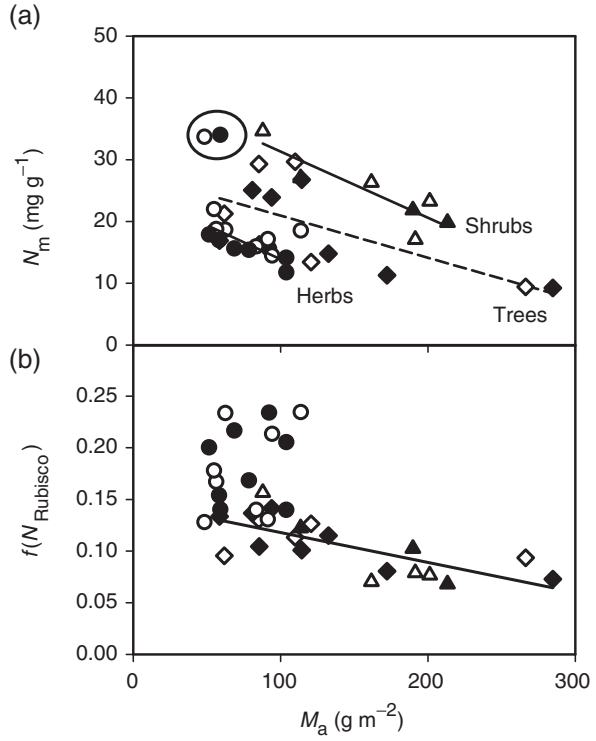

Fig 6 (a) Relationship between leaf N and leaf mass per unit area (M_a) for different plant growth forms across four free-air CO_2 enrichment sites: herbaceous species, trees and shrubs (see Table 3). Data for the N-fixing species *Lupinus* (circled) are not included in the overall relationship for herb species. (b) Differences in the apparent fraction of N invested in Rubisco $f_{N\text{-}Rub}$ for the three plant growth forms are shown in (a). Regression statistics for the relationships in (a, b) are given in Table 5. There was no statistical relationship between $f_{N\text{-}Rub}$ and M_a for herbaceous species.

showed greater photosynthetic efficiency than woody species, with one-third higher apparent fraction of N allocated to Rubisco ($f_{N\text{-}Rub} = 18 \pm 4\%$; mean $\pm$ 95% CI) than either trees or shrubs ($12 \pm 2\%$ across both groups; Fig. 6b). These apparent differences in $f_{N\text{-}Rub}$ (Appendix A) arise from differences in the slopes of the V_{cmax}–N relationships (Fig. 5). There was neither a significant pC_a treatment effect on M_a nor on the apparent fraction of N allocated to Rubisco (data not shown).

Discussion

The issue of sustainability of leaf photosynthesis enhancement responses and the importance of adjustments in the photosynthetic apparatus to elevated pC_a in the field have been debated for two decades (Pearcy & Björkman, 1983; Gunderson & Wullschleger, 1994; Ainsworth *et al.*, 2003). There appears to be a strong consensus that photosynthetic enhancements of plants in elevated pC_a can be sustained over a number of years in the field (Medlyn *et al.*, 1999; Ainsworth *et al.*, 2003), which is supported by this cross-site study of native plants in elevated pC_a in FACE (Fig. 1). While the photosynthetic enhancements we observed were strong ($+40 \pm 5\%$), there was considerable variability in this response among species. The lack of strong statistical photosynthetic enhancement in four of 16 species in our study (Fig. 1) and the significant reductions in A_a and V_{cmax} (Fig. 2) together indicate downregulation of photosynthetic enzymes for certain species in elevated pC_a in FACE that appear to be broadly related to leaf N.

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We observed significant evidence of reductions in photosynthetic capacity with multiple years of elevated pC_a consistent with photosynthetic downregulation of dominant species in major regional ecosystem types, including the dominant trees *P. taeda* and *P. tremuloides*, and the desert shrub *L. tridentata* at the NC, WI and NV sites, respectively (Fig. 2). Downregulation has been reported previously for *Larrea*, *Pinus* and grassland species (Huxman *et al*., 1998; Lee *et al*., 2001; Rogers & Ellsworth, 2002), but here we augment these earlier studies with a larger set of dominant and codominant species in FACE experiments. While the photosynthetic downregulation response was elicited in 2–3 years of elevated pC_a exposure in these species, it is an open question as to whether this downregulation will be maintained over longer time periods. Interannual variation in growth conditions and N availability may generate variation in the magnitude of downregulation (Ainsworth *et al*., 2003; Naumburg *et al*., 2003).

Our results demonstrating field downregulation of photosynthesis contrast with those of Curtis & Wang (1998) and earlier studies where no overall evidence of photosynthetic downregulation was found in chamber-based elevated pC_a studies with minimal or no root constraints. However, a similar summary of European studies found statistical evidence of photosynthetic downregulation after multiple years of elevated pC_a (Medlyn *et al*., 1999). It is currently popular to invoke a sink limitation hypothesis related to feedback effects of carbohydrate accumulation (Stitt, 1991) to describe the occurrence of downregulation, but our data represent measurements during favorable growing periods for these species corresponding to times of year when growth and carbohydrate sinks are expected to be large. Therefore, our data represent a conservative estimate of the magnitude of photosynthetic downregulation during the physiologically active period of the year for these species. Overwintering leaves were measured during growth periods for the two evergreen species *Larrea* and *Pinus*, and photosynthetic downregulation is relatively common in aging evergreen leaves (Griffin *et al*., 2000; Crous & Ellsworth, 2004), although physiological explanation for the age-related downregulation phenomenon is still lacking. Also, the results here for upper-canopy sunlit leaves of all species at times of year of high photosynthetic activity do not necessarily imply that downregulation of photosynthetic capacity does or does not occur in other leaves in the plant canopies or at other times of the year, although our sampling was designed to focus on leaves and times of year expected to be significant for canopy CO_2 assimilation.

Causes for the observed photosynthetic responses to elevated pC_a may include selective downregulation of particular photosynthetic enzymes (Moore *et al*., 1999; Rogers & Ellsworth, 2002), general reductions in leaf soluble protein and leaf N content, or both (Luo *et al*., 1994). Dilution of leaf N is common in field experiments (Körner, 2000; Yin, 2002) and is consistent with our evidence (Fig. 3; Table 4). We observed significant pC_a treatment effects on $V_{cm\text{-}m}$, N_m and M_a among species, with similar amounts of reduction in $V_{cm\text{-}m}$ and N_m (e.g., about 12%), but somewhat less enhancement in M_a (Table 4). We interpret this evidence as supporting the leaf N dilution hypothesis, whereby leaf chemical composition is affected through the accumulation of soluble carbohydrates as a product of photosynthetic enhancement and/or relative tissue source–sink carbon demands (Körner *et al*., 1995). However, in addition to these mass-based quantities, the area-based measures V_{cmax} and N_a also showed significant pC_a treatment effects of similar magnitude to each other (Fig. 2, Table 4) and such effects are not strictly consistent with N dilution as a sole mechanism of photosynthetic downregulation, although clearly this is an important contributing factor (Luo *et al*., 1994).

Only a subset of seven of the 16 species, those with highest photosynthetic capacity, showed significant evidence of photosynthetic downregulation in elevated pC_a and it is unlikely that the same mechanism is responsible for this in all of these species. For instance, downregulation in *P. taeda* has been observed in overwintering leaves with 3–5 years of elevated pC_a despite a lack of changes in total leaf N_m (Rogers & Ellsworth, 2002; Crous & Ellsworth, 2004). Still, the close association between changes in V_{cmax} and change in leaf N across many disparate species (Fig. 3) supports the interpretation that changes in leaf N with elevated pC_a exposure, regardless of the mechanism of this change, likely represent a dominant mechanism of photosynthetic adjustments. Increased V_{cmax} in the N-fixing species in elevated pC_a likely results from stimulation of N fixation in elevated CO_2, a commonly observed response for this group (Lee *et al*., 2003). Differential species sensitivity to N limitations and the resulting expression in leaf N, together with differences in carbohydrate processing capabilities among species, may result in variation in elevated pC_a-induced photosynthetic downregulation among species at a site. However, a small set of leaf traits appear to describe these photosynthetic responses to elevated pC_a well across species.

The overall relationship between the photosynthetic parameter A_a and leaf N, as well those between biochemically based parameters $V_{cm\text{-}m}$ and $J_{m\text{-}m}$ (mass-based) and leaf N, was largely unaffected by elevated pC_a (Fig. 5, Table 5). The nature of the observed downregulation thus appears to have been primarily

tied to changes in leaf N in most species rather than changes in the fundamental relationships ($V_{cm\text{-}m}$ and $J_{m\text{-}m}$ as a function of leaf N_m) as would be predicted by the protein-specific downregulation hypothesis (Rogers & Ellsworth, 2002). Given the lack of pC_a effects on $f_{N\text{-}Rub}$ (Fig. 6), a protein-specific downregulation of the Rubisco enzyme is unlikely to be common, at least as a broad descriptor of the apparent downregulation we observed across herbs, trees and shrubs in elevated pC_a.

Although there was no evidence of elevated pC_a effects on $f_{N\text{-}Rub}$, we cannot rule out the hypothesis that elevated pC_a-induced downregulation may promote N redistribution (see (Drake *et al*., 1997) such that downregulation of specific photosynthetic proteins in elevated pC_a provides N that can be reallocated toward other protein-requiring systems. Sage (1994) and Medlyn *et al*. (1999)showed that this reallocation must be very efficient since the two major photosynthetic complexes, carboxylation and ribulose 1,5-bisphosphate (RuBP) regeneration in electron transport, are typically matched to one another. This appears to be the case for the species in elevated pC_a in FACE experiments as well, since there was a strong overall relationship between V_{cmax} and J_{max} that was unaffected by elevated pC_a (mean J_{max}/V_{cmax} ratio = 1.81 ± 0.06, $r^2 = 0.79$, $P = 0.0001$; data not shown), despite the lack of an overall significant elevated pC_a effect on J_{max} in ANOVA (Table 4). While elevated pC_a increases photosynthetic N-use efficiency in many species (Fig. 4), broad evidence of N redistribution from photosynthetic components to other leaf or plant functions and identification of the N-containing components that increase in elevated pC_a is still lacking (Stitt & Krapp, 1999). Even so, if internal N redistribution to other functions occurs it still does not compensate overall plant growth N demands in elevated pC_a-grown plants, since invariably leaf N_a is often not maintained (BassiriRad *et al*., 2001).

Decreases in leaf N_a and associated changes in V_{cmax} in elevated pC_a among the set of species in this study (Fig. 3, Table 4) suggest that elevated pC_a-driven photosynthetic adjustments in these species are not simply the result of a dilution phenomenon because of mass accumulation in the leaf. Changes in leaf N_a in elevated pC_a can occur in plants because of reductions in soil N availability or N uptake relative to canopy N demand (Zak *et al*., 2000; BassiriRad *et al*., 2003). While elevated pC_a effects on soil N availability have been widely hypothesized and sometimes observed in smaller-scale elevated pC_a experiments (Diaz *et al*., 1993; Hu *et al*., 2001), Zak *et al*. (2003) found no evidence of changes in soil N mineralization and immobilization at the NC and WI FACE sites used in our study after 3 years of elevated pC_a using a soil ^{15}N dilution technique. However, a general trend toward depletion of leaf $\delta^{15}N$ in elevated pC_a in these FACE experiments (BassiriRad *et al*., 2003) may suggest increased plant internal N demand and/or a corresponding reduction in plant-assimilated inorganic N, although the immediate cause of these changes is unclear. Models predict that such responses should intensify with long-term elevated pC_a because of different response time constants associated with different components of ecosystem N cycles (McMurtrie *et al*., 2001).

Relationships between the biochemical parameters of leaf photosynthesis (V_{cmax} and J_{max}) and leaf N may provide important basic insights into the functional significance of variation in leaf form and leaf N within and among different growth forms when grown in long-term elevated pC_a (Roderick *et al*., 1999). The systematic differences we observed among growth forms in photosynthetic N use (e.g., $V_{cm\text{-}m}$ and $J_{m\text{-}m}$ vs. N_m; Fig. 5) can be partly explained by differences in the apparent N investment to the Rubisco enzyme between herbaceous species with high $f_{N\text{-}Rub}$ and trees and shrubs (generally lower $f_{N\text{-}Rub}$; Fig. 6). Photosynthesis and carboxylation capacity per unit N tend to decrease as M_a increases because Rubisco represents a smaller fraction of leaf N in thicker, denser leaves (Poorter & Evans, 1998). For trees and shrubs, the strong correlation between the apparent fraction of leaf N allocated to Rubisco with M_a (Fig. 6) may represent increasing mesophyll limitations in leaves with higher M_a (Reich *et al*., 1998; Evans & Poorter, 2001), which may lead to growth form differences in the intercepts of the $V_{cm\text{-}m}$ vs. N_m relationships we observed. We suggest that these differences are related to differences in the amount of structural, nonphotosynthetic N among these different growth forms, particularly at high M_a where relatively high overall N_m (for a given M_a) combined with a low fraction of N in Rubisco leads to low photosynthetic N-use efficiency (Fig. 6).

High fractional N investments in Rubisco are favored in particularly fast-growing plant types such as herbaceous species that lack perennial aboveground structures, since such plants maximize relative growth rate through high net assimilation rate as achieved through high leaf-level A (Poorter, 1998). Lower fractional N investments in Rubisco in trees and shrubs, particularly for thick leaves with high M_a imply possibly diffusional limitations in such leaves, or inactivation of Rubisco or both (Roderick *et al*., 1999; Evans & Poorter, 2001). These differences among different growth forms have important implications for nitrogen-use efficiency and species responses to elevated pC_a on nutrient-poor sites.

Invariant functional relationships between photosynthetic parameters and leaf N have great utility in

modeling photosynthesis in different vegetation types (Wohlfahrt *et al*., 1999), but differences among different plant growth forms suggest that general functional relationships between photosynthesis and leaf N (Reich *et al*., 1999; Fig. 2) may have different biochemical determinants in different vegetation types. The results strongly suggest that fundamental relationships between N and physiology under ambient pC_a may broadly apply in models to photosynthetic responses of plants under elevated pC_a when constrained by tissue N concentration and M_a.

Conclusions

Standardized measurements of 16 forest, grassland and desert species at four FACE sites showed that there was strong evidence of photosynthetic enhancement ($+40 \pm 5\%$ for A_a) in elevated pC_a in FACE after about 3 years. However, concurrent with photosynthetic enhancement in most species there was statistically significant photosynthetic downregulation in five of the 16 species growing in elevated pC_a for 2–3 years across FACE sites. Downregulation of photosynthesis was apparent in terms of a marginally significant effect of treatment pC_a on A at a common pC_a (-8% among all species, $P = 0.06$) as well as a significant effect of treatment pC_a on carboxylation capacity (V_{cmax}, $P = 0.03$, -8% effect among all species). Similar magnitudes of elevated pC_a responses of A_a, V_{cmax}, N_a and M_a implicate leaf N dilution driven by carbohydrate accumulation as responsible for this downregulation, particularly in species with high carboxylation capacity and leaf N_a. Decreases in V_{cmax} and A_a were significantly related to the effects of elevated pC_a on N_a, suggesting an inability of plants to maintain internal leaf N in elevated pC_a in the long term. Mass-based parameters $V_{cm\text{-}m}$ and N_m also decreased significantly in elevated pC_a, consistent with a dilution mechanism driven by carbohydrate accumulation. Thus the broad mechanisms of photosynthetic downregulation among species at difference FACE sites are closely tied to an inability to maintain leaf N on both a mass and area basis in canopy leaves in long-term elevated pC_a.

Fundamental differences in photosynthetic and carboxylation capacity–N relationships were apparent for different plant growth forms, but not for plants growing under different pC_a conditions. The differences among growth forms resulted from apparent differences in N allocated to carboxylation, which varied from low total leaf N but high $f_{N\text{-}Rub}$ in herbs to progressively more total leaf N and also a greater proportion of nonphotosynthetic N in trees and desert shrubs. These differences in photosynthetic N use have important implications for modeling photosynthesis in different vegetation types, and also affects the relative responsiveness of photosynthetic downregulation under elevated pC_a to changes in soil or plant internal N. These and other plant-elevated pC_a responses are central to debates about future C sequestration by the terrestrial biosphere. Understanding ecological controls on leaf and canopy N in response to long-term elevated pC_a, both within and among different plant growth forms, will greatly contribute to an ability to predict the magnitude of long-term leaf and canopy photosynthetic responses to rising atmospheric pCO_2.

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Appendix A: modeling analysis of field A–pC_i curves

We fitted the Farquhar *et al*. (1980)photosynthesis model to the data separately for each measured leaf, and compared V_{cmax} among sites and species. Data from field A–pC_i curves were used to parameterize the biochemical model of C_3 photosynthesis described by Farquhar *et al*. (1980) with recent modifications (Medlyn *et al*., 1999; Bernacchi *et al*., 2001). According to the model, light-saturated leaf CO_2 assimilation rate (A) is limited either by regeneration of RuBP in the photosynthetic carbon reduction cycle or by the catalytic activity of RuBP carboxylase (Rubisco) when the chloroplast RuBP concentration is saturating. Thus the initial slope of the relationship between A and pC_i (here for $pC_i < 23$ Pa) is considered to be the region of limitation by Rubisco activity, from which the maximum carboxylation activity of Rubisco with saturating RuBP (V_{cmax}) is calculated by least-squares regression. Given that the Rubisco enzyme is characterized by relatively conservative kinetic properties among different lineages of C_3 plant species, the temperature dependencies of the kinetic parameters k_c, k_o and the compensation point between photosynthesis and respiration in the absence of photorespiration (Γ^*) were calculated with modifications proposed by Bernacchi *et al*. (2001). From V_{cmax} and the corresponding leaf N, the apparent fraction of N allocated to active Rubisco enzyme ($f_{N\text{-}Rub}$) is calculated, assuming a composition of 16.67% N, eight active sites and a k_{cat} value for the enzyme of 3.3 (Evans, 1989).

After solving for V_{cmax}, the maximum rate of electron transport at near saturating pC_i (J_{max}) was calculated as the best fit for the entire A–pC_i curve solved by iteration (Wullschleger, 1993). The current analysis assumes that

the partial pressure of CO_2 at the chloroplast surface (pC_{chl}) is near that in the intercellular air space (pC_i), recognizing that there is a finite leaf internal liquid- and gas-phase conductance not considered here (Evans & Von Caemmerer, 1996). Thus, V_{cmax} represents apparent carboxylation rate for Rubisco in leaves at its native activation state. There was no evidence of strong statistical biases among curves in the saturation pC_i point for A (not shown), as might be suggested if there were large differences in internal conductances among the species here. Apparent $f_{N\text{-}Rub}$ was compared among different plant growth forms at a common M_a to minimize bias because of internal conductance limitations in thick leaves.