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The Penalty of a Long, Hot Summer. Photosynthetic Acclimation to High CO_2 and Continuous Light in "Living Fossil" Conifers[1]

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Deciduous forests covered the ice-free polar regions 280 to 40 million years ago under warm "greenhouse" climates and high atmospheric pCO_2. Their deciduous habit is frequently interpreted as an adaptation for minimizing carbon losses during winter, but experiments with "living fossils" in a simulated warm polar environment refute this explanation. Measured carbon losses through leaf abscission of deciduous trees are significantly greater than losses through winter respiration in evergreens, yet annual rates of primary productivity are similar in all species. Here, we investigate mechanisms underlying this apparent paradox by measuring the seasonal patterns of leaf photosynthesis (A) under pCO_2 enrichment in the same trees. During spring, A increased significantly in coastal redwood (*Sequoia sempervirens*), dawn redwood (*Metasequoia glyptostroboides*), and swamp cypress (*Taxodium distichum*) at an elevated pCO_2 of 80 Pa compared with controls at 40 Pa. However, strong acclimation in Rubisco carboxylation capacity ($V_{c,max}$) completely offset the CO_2 response of A in all species by the end of 6 weeks of continuous illumination in the simulated polar summer. Further measurements demonstrated the temporary nature of acclimation, with increases in $V_{c,max}$ during autumn restoring the CO_2 sensitivity of A. Contrary to expectations, the acclimation of $V_{c,max}$ was not always accompanied by accumulation of leaf carbohydrates, but was associated with a decline in leaf nitrogen in summer, suggesting an alteration of the balance in plant sources and sinks for carbon and nitrogen. Preliminary calculations using A indicated that winter carbon losses through deciduous leaf abscission and respiration were recovered by 10 to 25 d of canopy carbon fixation during summer, thereby explaining the productivity paradox.

Geological evidence shows that today's permanent polar ice sheets are a recent phenomenon, appearing some 34 million years ago (Ma) in Antarctica and 3 Ma in the Arctic (Zachos et al., 2001). Earlier periods of global warmth extending back to 280 Ma enabled forests to cover the polar regions and reach latitudes as high as 85° in both hemispheres (Spicer and Chapman, 1990). These ancient high latitude forests quite likely grew in an environment unlike any on Earth today, with mean winter temperatures above freezing (Pole and Macphail, 1996; Markwick, 1998; Tripati et al., 2001; Dutton et al., 2002), and an atmospheric pCO_2 enriched over current ambient levels (Royer et al., 2001). However, in common with modern polar vegetation, they would have experienced strong seasonality in daylength (Creber and Chaloner, 1985). For example, trees at a latitude of 69° are exposed to continuous sunlight for 6 weeks in the summer and an equal period of darkness in winter (Beerling and Osborne, 2002).

Fossils suggest that polar forests were largely deciduous (Spicer and Chapman, 1990), and this is frequently interpreted as an adaptation for minimizing canopy respiration during the warm, dark polar winter (Chaney, 1947; Hickey, 1984; Wolfe and Upchurch, 1987; Spicer and Chapman, 1990). However, comparative analyses of evergreen and deciduous trees have recently overturned this paleobotanical dogma (Royer et al., 2003). In a controlled, high-latitude environment, simulating a typical warm climate of 100 to 40 Ma, the quantity of carbon lost through complete abscission of deciduous leaf canopies greatly exceeds that lost through wintertime respiration and abscission from evergreen canopies (Royer et al., 2003). Despite the higher carbon loss, deciduous trees achieve similar rates of net primary production (P_n) to their evergreen counterparts (Royer et al., 2003). This observation raises an apparent paradox that could be important for understanding the deciduous habit of polar forests.

We examined the seasonal pattern of photosynthesis to investigate the characteristics of this paradox. Photosynthesis may be lower in evergreen than deciduous leaves for a number of reasons: internal shading and diffusional limitation by strengthening tissues, investment in defense against herbivores rather than photosynthetic proteins, or low stomatal conductance to conserve water during drought (Reich et al., 1999). However, our study emphasized the physiological mechanisms underlying seasonal changes in photosynthesis and the action of elevated pCO_2. We examined three closely related "living fossil" species of conifer: the evergreen coastal redwood

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(*Sequoia sempervirens*), the deciduous dawn redwood (*Metasequoia glyptostroboides*), and the deciduous swamp cypress (*Taxodium distichum*). All are members of the *Taxodiaceae*, and genera known as co-occurring fossils from Late Cretaceous and early Paleogene Arctic forests (e.g. Schweitzer, 1980). The trees were grown for 3 years in a simulated warm polar climate and photoperiod regime equivalent to a latitude of 69°N, under elevated (80 Pa) or control (40 Pa) levels of atmospheric pCO_2 (Beerling and Osborne, 2002). The elevated pCO_2 treatment corresponded to a conservative estimate for the Late Cretaceous and high value for the early Paleogene (Royer et al., 2001).

Earlier measurements in the same experiment indicated no consistent stimulation of net leaf photosynthesis (*A*) under pCO_2 enrichment during the period of continuous summer illumination, suggesting strong acclimation of the photosynthetic system. A contrasting, positive effect of pCO_2 on *A* during the shorter days of fall demonstrated important seasonal variation in acclimation processes (Beerling and Osborne, 2002). Acclimation to continuous light and elevated pCO_2 is typically characterized by a decline in the leaf carboxylation capacity and expression of Rubisco (Pilgrim and McClung, 1993; Drake et al., 1997), mediated by a negative feedback from the accumulation of sugars or starch (Dorais et al., 1996; Paul and Foyer, 2001). However, additional mechanisms do also seem to be important, particularly an alteration in the partitioning of nitrogen to leaves (Makino et al., 1997; Osborne et al., 1998). Based on these findings, we expected that photosynthesis in our experiment would be regulated by three key mechanisms, and we hypothesized that: 1) a decrease in the leaf carboxylation capacity underpins the acclimation of *A* to continuous illumination and elevated pCO_2; 2) that seasonal changes in the carboxylation capacity are linked to the accumulation of soluble sugars and/or starch in leaves during summer, particularly under pCO_2 enrichment; and 3) the seasonal suppression of photosynthetic capacity is additionally associated with a decrease in the partitioning of nitrogen to leaves.

We focused on elucidating the seasonal patterns and relative importance of these mechanisms throughout the 2002 growing season. Finally, in an effort to address the P_n paradox, we developed a simple scaling approach to place the carbon costs of litter production and canopy respiration in the context of whole-canopy photosynthesis.

RESULTS

Seasonal Changes in *A*

Light-saturated values of *A* at 25°C (A_{sat}) were significantly greater than controls in all three species when grown and measured in an elevated pCO_2 (Fig. 1; Table I). The largest response to pCO_2 was 138%
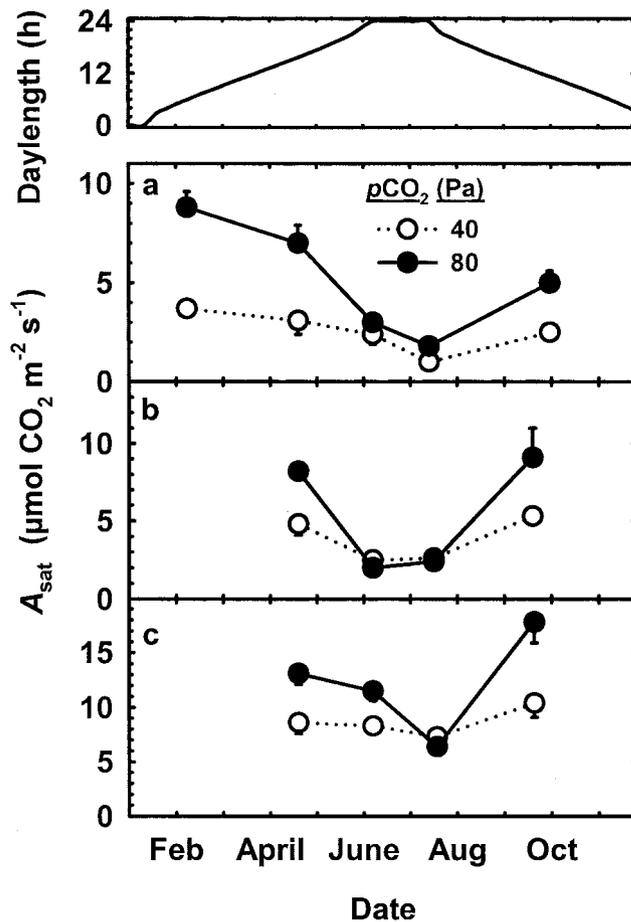

Figure 1. Seasonal changes in daylength (h) and A_{sat} (micromoles CO_2 per meter squared per second) for coastal redwood (a), dawn redwood (b), and swamp cypress (c), all grown and measured at a pCO_2 of 40 or 80 Pa. Measurements were made on recently expanded leaves under standardized conditions, with a leaf temperature of 25°C, and values are the mean ± SEM for four replicate growth rooms.

during February in coastal redwood, when this evergreen species was first exposed to short periods of illumination after the continuous darkness of a simulated polar winter (Fig. 1A). High sensitivity of *A* to pCO_2 at this time resulted from a significant increase in leaf stomatal conductance under pCO_2 enrichment (*t* test; $t_6 = 2.5$; $P = 0.044$), which allowed intercellular pCO_2 (C_i) to rise by 122% compared with controls. By April, when leaves of the deciduous species first emerged, the stimulation of *A* due to elevated pCO_2 had decreased to 122% in coastal redwood, was 72% in dawn redwood, and was 53% in swamp cypress. However, this response changed significantly during the subsequent season of canopy growth between April and October (Table I). The sensitivity of A_{sat} to pCO_2 during growth declined with increasing daylength in spring, and was completely lost in coastal redwood and dawn redwood at the onset of continuous illumination in June (Fig. 1, A and B). A similar seasonal decline occurred more slowly in swamp cypress with a smaller, 40% stimulation of

Table I. *Results of two-way, repeated-measures analysis of variance for leaf photosynthesis and biochemistry*

The two-way component tested effects of growth pCO_2 treatment and species, whereas the repeated measures component examined the seasonal changes between measurements in April, June, July, and September (Season). We also considered the interactions between these effects, although the three-way interaction between pCO_2, species, and Season was never statistically significant, and is not shown. For all tests, we assumed that individual growth rooms were independent, and we used the mean measure for two plants in each. The assumptions of normality and homogeneity of variances were met through log_{10} transformations of variables where necessary.

	pCO_2		Species		Season		$pCO_2 \times$ Species		$pCO_2 \times$ Season		Species $\times$ Season	
	$F_{1,18}$	P	$F_{2,18}$	P	$F_{3,18}$	P	$F_{2,18}$	P	$F_{3,18}$	P	$F_{6,18}$	P
Photosynthesis												
A_{sat}	48.9	<0.001	170.7	<0.001	41.2	<0.001	3.2	0.067	10.6	<0.001	4.8	0.001
$V_{c,max}$	27.9	<0.001	138.6	<0.001	42.7	<0.001	5.7	0.012	0.4	>0.5	3.9	0.003
J_{max}	13.2	0.002	103.8	<0.001	82.2	<0.001	1.3	0.308	1.5	0.224	5.3	<0.001
$Aday$	11.3	0.004	50.0	<0.001	4.3	0.009	1.7	0.208	1.7	0.185	2.3	0.049
$Acan$	7.2	0.015	18.3	<0.001	33.3	<0.001	1.1	0.350	2.2	0.098	6.3	<0.001
Biochemistry												
Soluble sugars	1.4	0.248	60.6	<0.001	15.8	<0.001	2.5	0.107	4.9	0.005	1.4	0.239
Starch	3.2	0.092	5.5	0.014	2.7	0.054	0.1	>0.5	0.4	>0.5	1.1	0.401
Total nonstructural carbohydrates (NSC)	3.1	0.094	7.3	0.005	2.5	0.068	0.2	>0.5	0.4	>0.5	1.1	0.394
Total N	1.6	0.222	48.0	<0.001	77.1	<0.001	0.2	>0.5	3.4	0.008	3.4	0.008
Leaf mass per unit area (LMA-NSC)	2.4	0.137	8.4	0.003	22.1	<0.001	1.1	0.359	1.7	0.178	3.9	0.003
Total C-NSC	5.3	0.037	18.2	<0.001	12.9	<0.001	0.7	>0.5	1.4	0.260	2.2	0.066

A_{sat} in elevated pCO_2 during June, and a complete loss of CO_2 sensitivity only in July (Fig. 1C). Measurements of A_{sat} in September demonstrated a reversal of the insensitivity to pCO_2 in all three species, with values again significantly greater than controls in leaves grown and measured under CO_2 enrichment (Fig. 1).

Photosynthetic Acclimation

Analysis of the response of A_{sat} to C_i revealed a significant decrease in the photosynthetic capacity of leaves between April and July in all species. This was manifested as a decrease in the apparent activity of Rubisco in vivo ($V_{c,max}$; Fig. 2, A–C; Table I) and the

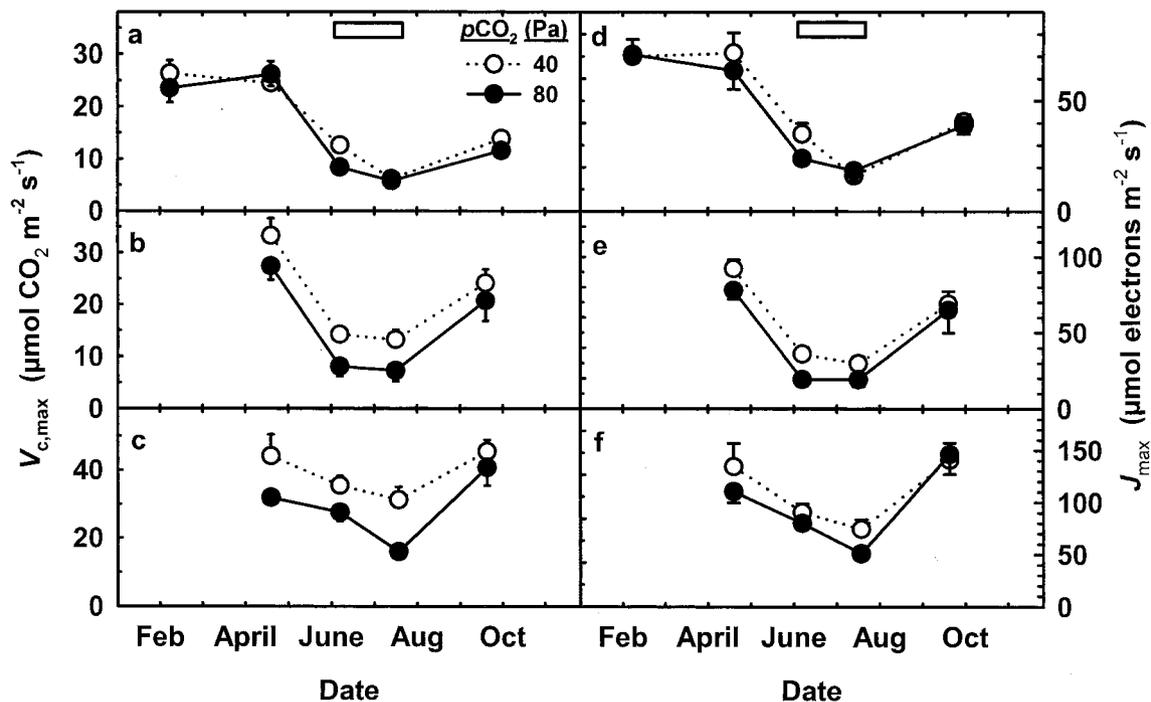**Figure 2.** Seasonal changes in $V_{c,max}$ (micromoles CO_2 per meter squared per second; a–c) and J_{max} (micromoles electrons CO_2 per meter squared per second; d–f) estimated by analyzing the response of A_{sat} to C_i in recently expanded leaves. Values are the mean ± SEM for coastal redwood (a and d), dawn redwood (b and e), and swamp cypress (c and f) grown at a pCO_2 of 40 or 80 Pa. Bars on the top of graphs indicate the period of continuous light.

apparent in vivo capacity for RubP regeneration via electron transport (J_{max}; Fig. 2, D–F; Table I; Farquhar et al., 1980). The downward seasonal trends in $V_{c,max}$ and J_{max} were reversed after the period of continuous summer illumination, with capacities recovering to, or exceeding, their springtime level (Fig. 2; Table I). $V_{c,max}$ and J_{max} were lower in dawn redwood and swamp cypress under pCO_2 enrichment than in controls, a response that did not vary significantly through the year (Fig. 2, B and C; Table I). In contrast, $V_{c,max}$ was largely unresponsive to pCO_2 treatment in coastal redwood (Fig. 2A), whereas J_{max} was lower throughout the year under elevated pCO_2 than in controls (Fig. 2D; Table I).

Further examination of the leaf A/C_i responses suggested that A_{sat} at the growth pCO_2 was limited by the carboxylation efficiency in all species (data not shown). This limitation meant that A was affected chiefly by changes in $V_{c,max}$ throughout the experiment, and not by variation in J_{max}, ruling out photoinhibition and photodamage as primary causes of the decreases in A. The summer decline in $V_{c,max}$ (Fig. 2, A–C) diminished the sensitivity of A to C_i in all species and both pCO_2 treatments. Responses of A to C_i became so flattened in the summer that differences in A between CO_2 treatments could not even be detected in coastal redwood (Fig. 1A), where $V_{c,max}$ was largely unaffected by pCO_2 (Fig. 2A). This effect

was more pronounced in dawn redwood and swamp cypress grown under pCO_2 enrichment, and the additional decrease in $V_{c,max}$ was of a similar magnitude to the seasonal response (Fig. 2, A–C). Together, these reductions in $V_{c,max}$ completely removed the positive effect of elevated pCO_2 on A.

Leaf Biochemistry

Relationships between the acclimation of A in summer and the accumulation of carbohydrates in leaves differed markedly between species. In contrast to our expectation of sugar accumulation during summer, the soluble sugar content declined significantly from April to a minimum in June or July in all species, and tended to rise again in September (Fig. 3, A–C; Table I). Thus, acclimation was sufficient to offset the effects of continuous light on source activity, ensuring that sugar production did not outstrip sink demands for carbon in summer. The anticipated increase in soluble sugars under elevated pCO_2 occurred only in swamp cypress during summer, with no large effects in coastal redwood or dawn redwood (Fig. 3, A–C). This pattern resulted in a significant interaction between pCO_2 and season, but no overall effect of pCO_2 (Table I). The starch content of leaves during summer tended to be greater under pCO_2 enrichment than in controls, especially in the deciduous species dawn
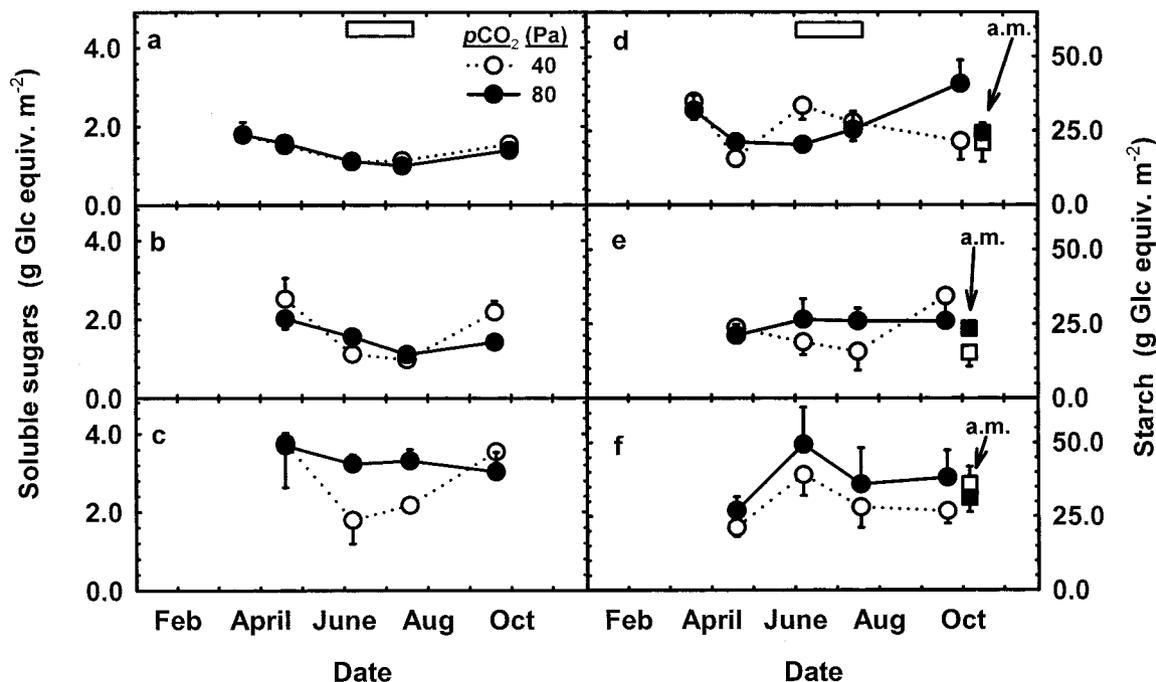

Figure 3. Seasonal changes in the soluble sugar (a–c) and starch (d–f) content of recently expanded leaves (both grams of Glc equivalents per meter squared). Values are the mean ± SEM for coastal redwood (a and d), dawn redwood (b and e), and swamp cypress (c and f) grown at a pCO_2 of 40 Pa (○ and □) or 80 Pa (● and ■). Measurements were made for leaves sampled immediately after the end of the light period, except during the continuous illumination of summer, when samples were taken between 12:00 PM and 3:00 PM (○ and ●). Additional samples were taken immediately before the start of the light period in September and are denoted by the square symbols (□ and ■) labeled "a.m." Soluble sugars are the combined total of Glc, Fru, and Suc contents. Bars on the top of graphs indicate the period of continuous light.
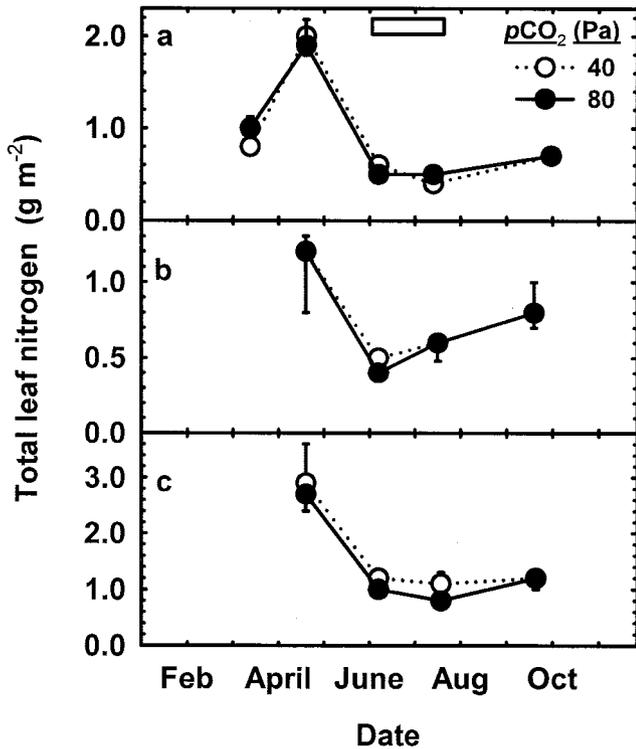
Figure 4. Seasonal changes in the nitrogen content of recently expanded leaves (grams per meter squared) for coastal redwood (a), dawn redwood (b), and swamp cypress (c) grown at a pCO_2 of 40 or 80 Pa. Values are the mean ± SEM for four replicate growth rooms. Bars on the top of graphs indicate the period of continuous light.

redwood and swamp cypress (Fig. 3, E and F), but this result was not statistically significant (Table I; $P < 0.10$). Analyses provided some evidence in coastal redwood and swamp cypress for an increase in starch at the beginning of the dark period in September, especially under pCO_2 enrichment (Fig. 3, D and F). However, repetition of these measurements shortly before dawn of the next day showed that the accumulation was only temporary (Fig. 3, D and F), with the additional starch under pCO_2 enrichment completely removed by export or metabolism during the night (Zeeman and ap Rees, 1999).

The total nitrogen content of new leaves declined significantly in all species between the initial flush of growth in April and the onset of continuous illumination in June, but recovered partially by September (Fig. 4; Table I). Measurements of total nitrogen in the leaves of coastal redwood during February suggested a continuation of the reversal through the winter in this evergreen species (Fig. 4A). There was no significant overall effect of growth pCO_2 treatment on total leaf nitrogen (Table I). However, seasonal effects of pCO_2 on leaf nitrogen were highly significant (Table I), with leaves of all species in June containing lower levels of nitrogen under high pCO_2 than in the controls (Fig. 4).

To test the hypothesis that acclimation is driven by a general accumulation of total NSC in leaves, we plot seasonal changes in $V_{c,max}$ against NSC in Figure 5. $V_{c,max}$ does not show a significant regression with

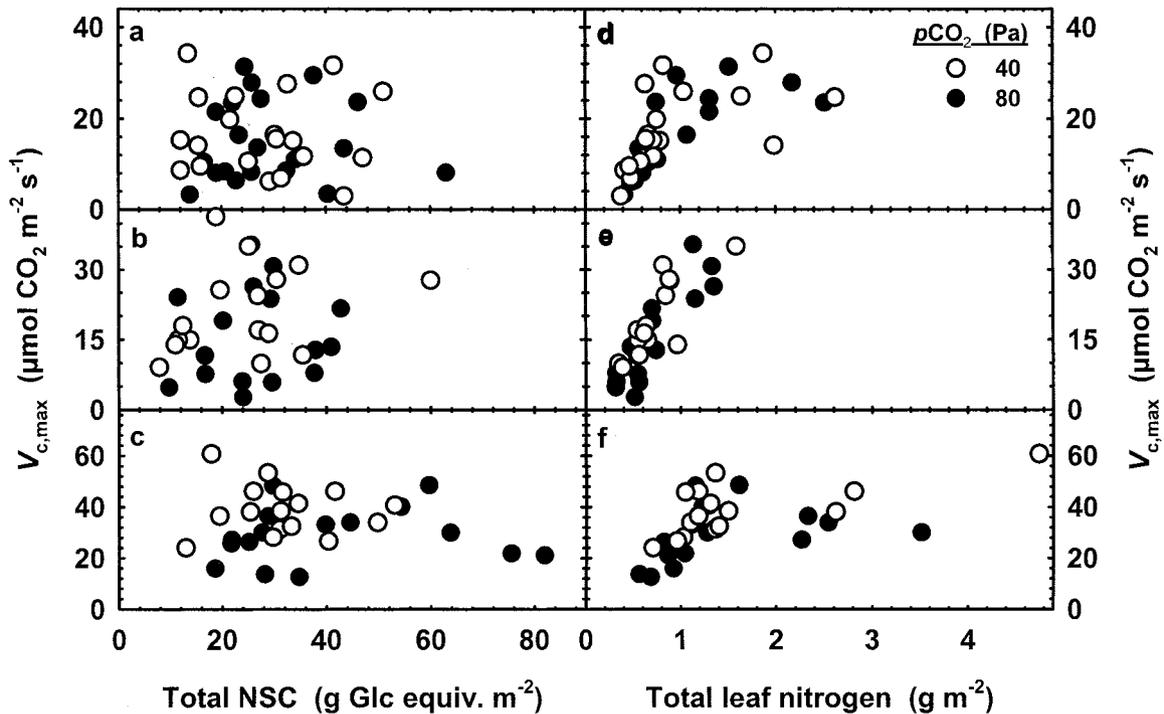**Figure 5.** The relationship between $V_{c,max}$ and leaf NSC and nitrogen contents. Values are individual replicate values from all times of the year in Figures 2A–C, 3, and 4. Relationships between $V_{c,max}$ and NSC (a–c), and $V_{c,max}$ and nitrogen contents (d–f) are shown for coastal redwood (a and d), dawn redwood (b and e), and swamp cypress (c and f) grown at a pCO_2 of 40 or 80 Pa.
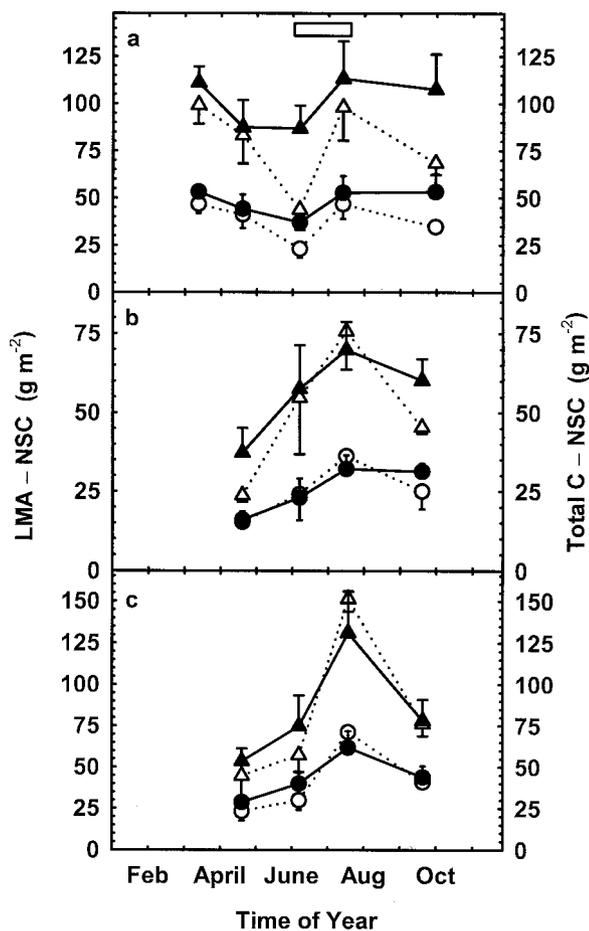
Figure 6. Seasonal changes in LMA ($\triangle$ and $\blacktriangle$) and leaf carbon content ($\bigcirc$ and $\bullet$) in recently expanded leaves, corrected to remove the influence of NSC accumulation. Values are the mean $\pm$ SEM for coastal redwood (a), dawn redwood (b), and swamp cypress (c) grown at a pCO_2 of 40 Pa ($\triangle$ and $\bigcirc$) or 80 Pa ($\blacktriangle$ and $\bullet$). Bars on the top of graphs indicate the period of continuous light.

NSC in any species (Fig. 5, A–C), in conflict with the hypothesis that predicted a significant negative slope. In contrast, $V_{c,max}$ has a close relationship with leaf nitrogen (Fig. 5, D–F), with a significant positive linear regression at nitrogen contents lower than 1.5 g m^{-2} in all species and treatments, suggesting strong limitation of carboxylation capacity. Two-way analysis of variance shows that the slope of these regression relationships does not differ significantly among species ($F_{2,66} = 0.8$; $P > 0.05$) or pCO_2 treatments ($F_{1,66} = 0.3$; $P > 0.05$), whereas independence of $V_{c,max}$ at leaf nitrogen values greater than 1.5 g m^{-2} suggests a saturation of the response (Fig. 5, D–F).

Changes in LMA may indicate structural changes in the construction of leaves or simply the accumulation of NSC under pCO_2 enrichment (Roumet et al., 1999; Yin, 2002). To separate these confounding effects and to isolate changes in structure alone, we subtracted the total mass of NSC from measured values of LMA and leaf carbon content (Fig. 6). Our

corrected results revealed marked changes in the construction of leaves through the growing season. The initial flush of growth in April produced relatively large, soft leaves in the deciduous species dawn redwood and swamp cypress (C.P. Osborne, personal observation), characterized by low values of LMA (Fig. 6, B and C). A significant increase in LMA through the period of continuous illumination suggests a switch to the production of tougher, denser, or thicker leaves in summer (Fig. 6; Table I). The evergreen coastal redwood showed a more complex seasonal pattern of LMA, with a decline between April and June, especially in control plants, followed by an increase in July (Fig. 6A). LMA declined again in the autumn, reaching values lower than the summer for all combinations of species and pCO_2 treatments except coastal redwood under pCO_2 enrichment (Fig. 6). These plants continued to produce leaves through September with LMA more than 50% greater than controls (Fig. 6A). Measurements in February showed similarly high values in elevated pCO_2 and control plants of coastal redwood (Fig. 6A), indicating a toughening of foliage during winter in this evergreen species. The overall effect of pCO_2 treatment on LMA was not statistically significant (Table I).

Seasonal patterns of LMA were accompanied by similar variation in total leaf carbon (Fig. 6, A–C; Table I). In contrast with LMA, leaf carbon content was significantly greater under pCO_2 enrichment than in controls (Fig. 6, A–C; Table I). Because these values excluded NSC, they suggest strongly that seasonal and CO_2-induced changes in leaf construction were driven by significant modifications in the investment of structural carbon to new foliage.

DISCUSSION

Our results demonstrate that living fossil conifer species are unable to capitalize on the full potential of pCO_2 enrichment for carbon fixation in the summer. Instead, the major effects of elevated pCO_2 on photosynthesis occur during the shorter days of spring and autumn when A increases by 53% to 122% in response to a doubling of pCO_2. This CO_2 sensitivity of A falls within the upper half of the range observed in previous experiments on trees growing at midlatitudes (Gunderson and Wullschleger, 1994; Curtis, 1996). Our analysis of the A/C_i response shows that the mechanism for high CO_2 sensitivity in our experiment is a strong constraint of A by carboxylation efficiency, resulting in substrate limitation of CO_2 fixation, even in plants under pCO_2 enrichment. The regulation of A by decreases in $V_{c,max}$ supports our first hypothesis of a common causal factor for acclimation to continuous light and elevated pCO_2.

Leaf acclimation to elevated pCO_2 was most pronounced during the continuous light of the polar summer, as anticipated by our second hypothesis. However, the mechanism varied between species,
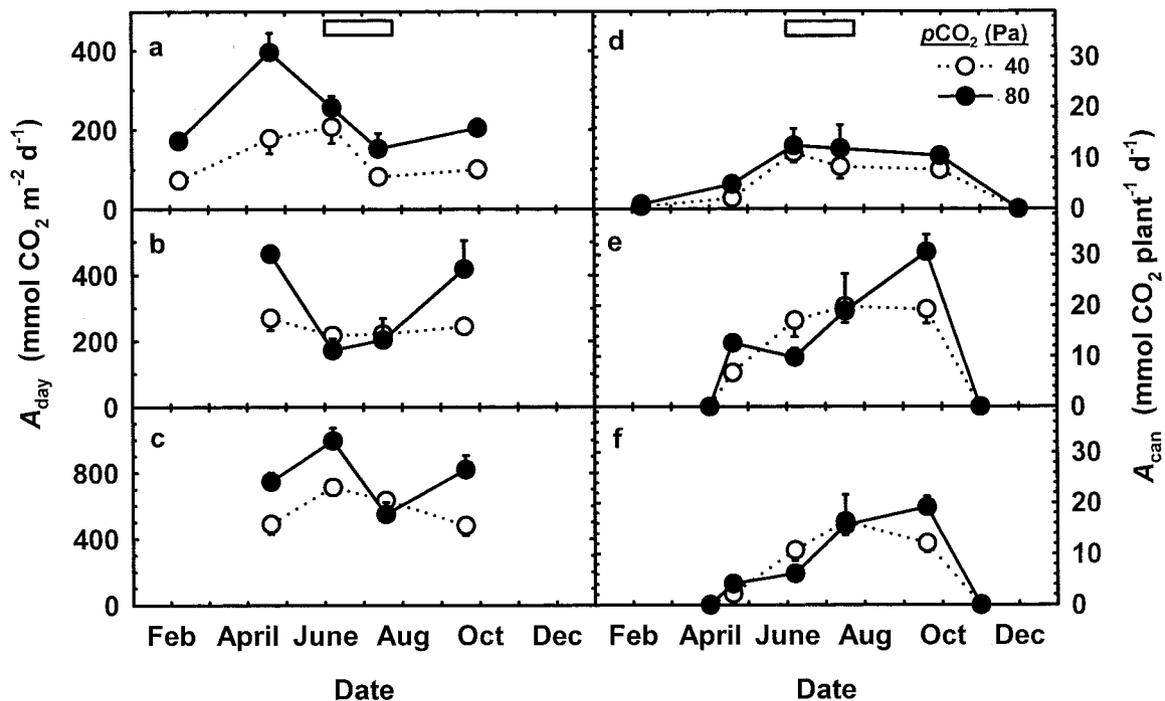
Figure 7. Scaling of leaf photosynthesis in time and space. Estimates of total daily CO_2 fixation by individual leaves (A_{day}, millimoles CO_2 per meter squared per day) are shown in a through c, and gross daily CO_2 fixation by each plant (A_{can}; millimoles CO_2 per plant per day) are shown in d through f. Zero values were set for A_{can} during the leafless period in deciduous species (e and f) and the dark period in the evergreen (d). Remaining values are the mean ± SEM for coastal redwood (a and d), dawn redwood (b and e), and swamp cypress (c and f) grown at a pCO_2 of 40 or 80 Pa. Bars on the top of graphs indicate the period of continuous light.

and carbohydrates did not increase universally under pCO_2 enrichment and continuous light as expected. Acclimation of photosynthesis during the summer was accompanied by an accumulation of soluble sugars only in swamp cypress under elevated pCO_2, and patterns of starch and total NSC accumulation in leaves were not directly related to the acclimation response in any species (Figs. 3, D–F and 5, A–C). Therefore, as with previous studies (e.g. Nie et al., 1995), a coarse linkage between levels of soluble sugars, starch, and the down-regulation of photosynthesis is ruled out by our experiment. However, we recognize the potential for a more subtle regulation involving the metabolism of hexoses or Suc (Moore et al., 1999; Stitt and Krapp, 1999; Paul and Foyer, 2001).

Biochemical differences between the new leaves emerging in summer and spring suggest an imbalance between plant sources and sinks for carbon and nitrogen during the period of continuous light. The general decline observed in $V_{c,max}$, J_{max}, and leaf nitrogen during summer (Figs. 2 and 4) supports our third hypothesis. It implies a down-regulation of carbon source strength and more sparing consumption of nitrogen by growth in response to a surplus of carbon over nitrogen availability. Decreases in the $V_{c,max}$ of several conifer species under pCO_2 enrichment are caused by a decline in the amount of active Rubisco, without change in the activation state of the

enzyme (Van Oosten et al., 1992; Tissue et al., 1999; Griffin et al., 2000; Laitinen et al., 2000; Rogers and Ellsworth, 2002). These observations imply that the decrease in $V_{c,max}$ in our plants was caused by a change in the amount, rather than activity, of Rubisco, although this suggestion remains to be tested directly. The coupling of $V_{c,max}$ and leaf nitrogen in our experiment (Fig. 5, D–F) indirectly supports this suggestion because Rubisco typically accounts for 10% to 30% of total leaf nitrogen (Evans, 1989; Warren et al., 2000).

As a preliminary test of the P_n paradox, we calculated seasonal changes in canopy photosynthesis at two levels of pCO_2 to compare with the carbon costs of litter production and winter respiration. To achieve this aim, we scaled from leaf to canopy by incorporating changes in photoperiod, canopy leaf dynamics and growth room temperature (Fig. 7; Table I). The effects of photoperiod on A_{day} showed marked seasonal variation. Short days in February significantly reduced A_{day} compared with A_{sat} in coastal redwood (Fig. 7A), but the strong CO_2 sensitivity of A_{sat} in the spring and fall was reflected in A_{day}, and was not constrained by photoperiod in any species (Fig. 7,A–C; Table I). The effects of photoperiod in summer were similarly weak, with long summer days only partially compensating for the acclimation of A_{sat}, and A_{day} declining during summer

(Fig. 7, A–C). In contrast, the estimates of A_{can} indicate a significant constraint of photosynthetic rates by cool springtime temperatures in all species (Fig. 7, D–F). Strong photosynthetic responses to pCO_2 remained in dawn redwood and swamp cypress only during fall (Fig. 7D–E), when growth room temperatures were around 20°C (Royer et al., 2003). This pattern differed in coastal redwood, where A_{can} under pCO_2 enrichment was typically higher than controls throughout the year, with the exception of June, when photosynthetic sensitivity to CO_2 was lost (Fig. 7D). The seasonal changes calculated for A_{can} provide a good match with measurements of canopy gas exchange (D.L. Royer, C.P. Osborne, and D.J. Beerling, unpublished data), suggesting that our simple procedure incorporates the important scaling factors.

Comparison of the carbon costs associated with wintertime respiration and leaf abscission with the estimates of canopy photosynthesis suggests an answer to the P_n paradox. The complete winter abscission of leaf canopies in dawn redwood and swamp cypress, respectively, accounted for 380 and 470 mmol C plant^{-1} in our experiment, significantly greater than the total carbon loss through wintertime respiration and abscission of 25 mmol C plant^{-1} in coastal redwood (Royer et al., 2003). Perhaps surprisingly, this marked differential in winter carbon costs carried little penalty for P_n because it was small relative to canopy carbon fixation (Fig. 7, D–F). Direct measurements of whole-plant CO_2 exchange confirmed this finding, suggesting annual gross photosynthesis of 2.8 to 3.8 mol C plant^{-1} year^{-1} in 40 Pa pCO_2 and 3.8 to 4.3 in 80 Pa pCO_2, and annual whole-plant respiration of 1.1 to 1.4 mol C plant^{-1} year^{-1} in both CO_2 treatments (D.L. Royer, C.P. Osborne, and D.J. Beerling, unpublished data). Our calculations show that the carbon required for growth of the deciduous leaf canopies could be fixed in around 10 to 25 d, a period representing only 5% to 15% of the 6-month growing season. Therefore, preliminary scaling of A indicates a possible solution to the P_n paradox, and suggests that the deciduous leaf habit may not be as critical to the carbon balance of polar forests as previously postulated (Chaney, 1947; Hickey, 1984; Wolfe and Upchurch, 1987; Spicer and Chapman, 1990).

Our experiment is designed to provide insight into the possible photosynthetic responses of high-latitude forests to pCO_2 in ancient greenhouse climates. We show significant acclimation of $V_{c,max}$ under pCO_2 enrichment and the continuous light of a simulated polar summer, which restricts stimulation of A by elevated pCO_2 to the spring and fall. The seasonal relationship between soluble sugars, starch, and photosynthetic acclimation is complex, indicating subtle regulation mechanisms rather than a coarse linkage between carbohydrate accumulation and photosynthetic capacity. However, decreases in $V_{c,max}$ during the summer are coupled with a decline in leaf nitrogen, suggesting a mechanistic link between acclimation and regulation of the plant carbon: nitrogen balance. Simple scaling from A to canopy photosynthesis provides preliminary support for the contention that seasonal carbon fixation in deciduous trees is large relative to the carbon required annually for leaf growth.

MATERIALS AND METHODS

Plant Material

Coastal redwood (*Sequoia sempervirens* [D. Don] Endl), dawn redwood (*Metasequoia glyptostroboides* Hu & Cheng), and swamp cypress (*Taxodium distichum* Rich) were grown from 1-year-old saplings for 3 years in 2-liter pots in controlled-environment growth rooms. Four replicate growth rooms each simulated an ancient polar environment and were split into two parts, one providing pCO_2 enrichment to 80 Pa, and the other a control pCO_2 of 40 Pa. Temperature within the growth rooms was warmed by 5°C compared with the outside air, giving a mean annual temperature of 15.1°C, and mean temperatures for the coldest and warmest months of 8.5°C and 21.8°C, respectively, while maintaining natural variability on diurnal, seasonal, and interannual timescales. Mean temperatures matched estimates for high latitudes of the Cretaceous and early Paleogene derived from a range of fossil proxies (Pole and Macphail, 1996; Markwick, 1998; Tripati et al., 2001; Dutton et al., 2002). A daytime photon flux of 300 to 400 μmol m^{-2} s^{-1} was provided as a square wave using sodium lamps, and radiant heat flux was minimized by pumping cool water through a glass jacket surrounding the bulb (Sunbeam Hydrostar; Avon Gro-Lite Systems, Bristol, UK). The photoperiod was controlled using segmental timing switches and was changed weekly to simulate a latitude 69°N (Beerling and Osborne, 2002). Fertilizer was applied twice daily as Rorison's Nutrient Solution via a drip irrigation system, with a gradual increase in strength from 10% to 50% during the experiment as plants grew larger. Further details of the environmental control mechanisms in growth rooms and the cultivation of plants are given by Beerling and Osborne (2002).

Observations throughout the year showed that leaf growth was initiated by flush growth of the shoot in April and continued through September in all species (D.L. Royer, personal communication). All of the measurements reported here were made on recently expanded foliage at the top of the canopy, and therefore reflect seasonal changes in the physiological development of successive leaf cohorts, rather than changes during the ontogeny and senescence of a single cohort. Values for coastal redwood in February are the exception, representing leaves persisting after growth during the previous fall.

Gas Exchange

Leaf CO_2 and water vapor fluxes were measured during the 3rd year of plant exposure to experimental treatments. The response of A_{sat} to variation in C_i was determined under standardized conditions, using a fully controlled microenvironment cuvette incorporated into an open gas-exchange system (CIRAS-1; PP Systems, Hitchin, Herts, UK). Calibration of the infrared gas analyzer in this system was checked regularly for CO_2 using a volumetrically mixed reference gas (Certified Standard±5%; BOC Gases, Guildford, Surrey, UK), and for water vapor by recycling air through ferrous sulfate (FeSO$_4$.7H$_2$O) at a known temperature.

The leaf was illuminated using a quartz halide source providing a photon flux of 600 μmol m^{-2} s^{-1}, previously shown to saturate photosynthesis. Leaf temperature was calculated using an energy balance and was regulated to 25°C with a feedback control system, and leaf-air vapor pressure deficit was maintained at 1.0 to 1.5kPa by controlling the vapor pressure of chamber air. A and C_i were calculated using the equations of von Caemmerer and Farquhar (1981), and were used to estimate $V_{c,max}$ and J_{max} following Wullschleger (1993), with the kinetic properties of Rubisco from Bernacchi et al. (2001) and equations from von Caemmerer (2000). Measurements were made using two plants from each of the four replicate growth rooms.

Biochemical Analyses

Two leaves were sampled from different plants in each growth room, frozen in the dark within 1 h of the end of the light period, and stored at −80°C until NSC analysis. During the summer when there was no dark period, these leaf samples were taken between 12:00 PM and 3:00 PM. The area of each was measured using image analysis software (TpsDig, F.J. Rohlf; State University of New York, Stony Brook) from a high-resolution digital photograph taken before freezing (Coolpix 995; Nikon, Kingston upon Thames, Surrey, UK).

Soluble sugars were extracted from leaves by two successive 20-min incubations with hot, buffered ethanol (70°C, pH 7.4, 80%, v/v). Previous tests had shown that no further sugars were released by additional ethanol incubations. The extracts were dried under vacuum and redissolved in distilled water. Glc, Fru, and Suc were assayed after the sequential addition of hexokinase, phosphogluco-isomerase, and invertase. The assay was linked to the activity of Glc-6-P dehydrogenase and changes in A_{340} caused by the production of NADH (Scholes et al., 1994). The remaining leaf material was homogenized in 500 μL of distilled water (800M mixer/mill; Glen Creston, Middlesex, UK) and was autoclaved for 30 min. Starch content was determined in this mixture by incubating 100 μL for 5 h at 37°C with a buffered solution of hydrolytic enzymes (100 μL of 500 mM MES-KOH, pH 4.5, 50 μL of 4 mg mL^{-1} amyloglucosidase, and 50 μL of 90 mg mL^{-1} α-amylase) and assaying for Glc as above (Scholes et al., 1994). Soluble sugars were obtained as the sum of Glc, Fru, and Suc contents, with total NSC being the sum of soluble sugars and starch.

The leaves used for gas exchange measurements were dried at 40°C, weighed for the calculation of LMA, and ground to a fine powder (800M mixer/mill; Glen Creston). Total carbon and nitrogen contents were measured using a stable isotope ratio mass spectrometer (PDZ Europa 20-20, Cheshire, UK).

Canopy Photosynthesis

We estimated canopy photosynthesis using a simple scaling model, accounting for photoperiod, canopy leaf area, and limitation by temperature under growth room conditions. Total daily CO_2 fixation by individual leaves (A_{day}, millimoles CO_2 per meter squared per day) was calculated as the product of A_{sat} (Fig. 1) and photoperiod, and gross daily CO_2 fixation by each plant (A_{can}; millimoles CO_2 per plant per day) was calculated as the product of A_{day} and canopy leaf area (Royer et al., 2003). Rates of photosynthesis measured at 25°C were adjusted for A_{can} using the temperature functions for $V_{c,max}$ of Bernacchi et al. (2001) and the mean monthly temperatures for growth rooms (Royer et al., 2003). We recognize that this simple approach fails to account for the interaction between temperature and pCO$_2$ (Long, 1991). Close agreement between our calculations and direct measurements of canopy CO_2 exchange suggest that additional factors, such as self-shading and leaf ontogeny, do not exert a major effect on canopy photosynthesis in these young trees.

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