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Physiological Ecology of Mesozoic Polar Forests in a High CO_2 Environment

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Fossils show that coniferous forests extended into polar regions during the Mesozoic, a time when models and independent palaeo-CO_2 indicators suggest that the atmospheric CO_2 concentration was at least double that of the present day. Consequently, such polar forests would have experienced high CO_2 interacting with an extreme variation in light. Here we describe an experiment investigating this plant–environment interaction for extant tree species that were important components of polar forests, and give results from the first year of treatment. Specifically, we tested the hypotheses that growth in elevated CO_2 (1) stimulates photosynthesis; (2) reduces photoinhibition during the polar summer; and (3) reduces respiration of above- and below-ground plant organs. Our results indicate that CO_2 fertilization generally does not affect photosynthesis under continuous daylight characteristic of the polar summer but does increase it when the period of illumination is shorter. Growth in elevated CO_2 did not alter the potential for photoinhibition. CO_2 enrichment significantly reduced leaf and root respiration rates by 50 and 25 %, respectively, in a range of evergreen taxa. Incorporating these observed CO_2 effects into numerical simulations using a process-based model of coniferous forest growth indicates that a high palaeo-CO_2 concentration would have increased the productivity of Cretaceous conifer forests in northern Alaska. This results from decreased respiratory costs that more than compensate for the absence of high CO_2–high temperature interactions during the polar summer. The longer-term effects of CO_2 enrichment on seasonal changes in the above- and below-ground carbon balance of trees are discussed.

Key words: Atmospheric CO_2, carbohydrates, fossil plants, photosynthesis, photoinhibition.

INTRODUCTION

Marine oxygen isotope data indicate that the Earth has been in a 'greenhouse' mode for approx. 80 % of the past 500 million years (Spicer and Chapman, 1990; Frakes *et al*., 1992). Between the Mesozoic and early Tertiary [250 to 50 million years ago (Ma)], the plant fossil record shows the presence of tall, dense coniferous forests on the high latitude landmasses (Spicer and Chapman, 1990). Such forests extended to 65–85°N during the Jurassic and Cretaceous periods in Alaska and northern Russia (Spicer and Herman, 2001), and to the high latitudes (75 to 85°S) of the southern hemisphere, including Antarctica (Jefferson, 1982; Francis, 1986; Falcon-Lang *et al*., 2001), South America (Archangelsky, 1963), New Zealand (Pole, 1999; Thorn, 2001) and Australia (Douglas and Williams, 1982). Amongst the best-studied high latitude fossil forests are those from Antarctica and the Canadian High Arctic. Analyses of fossil woods and *in situ* stumps of canopy-forming trees indicate that adaptation to the polar environment allowed high primary productivity, as shown by wide growth rings in fossil woods and large fossil stumps, indicative of growth to over 30 m in height (Jefferson, 1982; Francis, 1986; Falcon-Lang and Cantrill, 2000, 2001). Palaeobotanical investigations further suggest a geographical difference in the dominant leaf habit between hemispheres, with mid-Cretaceous polar forests in Alaska and northern Russia being a mixture of deciduous and evergreen vegetation, and those in Australia and Antarctica being predominantly evergreen (Falcon-Lang, 2000; Falcon-Lang and Cantrill, 2000, 2001).

The polar environment, with its highly seasonal light regime, provides unusual conditions for tree and forest growth. Ancient forests located at 80°N, for example, experienced a growing season some 3 months shorter than those at 65°N (Fig. 1A), a severe constraint on photosynthetic carbon acquisition, and thus to survival during the long polar winter (Creber and Chaloner, 1985). To survive the extended darkness of the polar winter trees must store sufficient energy reserves, in the form of carbohydrates, to fuel their metabolism (Penning de Vries, 1975; Read and Francis, 1992). Furthermore, those same high latitude forests must endure 2 months more continuous polar sunlight compared with their counterparts at 65°N (Fig. 1B), with an associated increased risk of photoinhibition, i.e. reduced CO_2 uptake caused by a decline in photosynthetic efficiency in high light conditions (Long *et al*., 1994). This risk is compounded by the negative feedback operating on CO_2-uptake if leaf carbohydrates accumulate (Sheen, 1994; Hymus *et al*., 1999).

One crucial difference between the environment of today's polar vegetation and that of Mesozoic polar forests is the concentration of atmospheric CO_2. Geochemical modelling of the long-term carbon cycle and independent CO_2 indicators suggest that for most of the past 250 Ma the atmospheric CO_2 concentration was several times higher than at present (reviewed in Royer *et al*., 2001). It is well established that many key physiological processes of trees are strongly influenced by a CO_2-enriched atmosphere

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(Curtis and Wang, 1998; DeLucia *et al*., 1999; Norby *et al*., 1999; LaDeau and Clark, 2001). Leaf photosynthesis is typically stimulated by 40–80 % during growth with double the present concentration of CO_2 (Drake *et al*., 1997; Curtis and Wang, 1998; DeLucia *et al*., 1999; Norby *et al*., 1999). Therefore, increased CO_2 concentration has the potential to counteract the limitation on the growth of polar forests imposed by the short, high-latitude growing season. Experimental evidence also suggests that the higher atmospheric CO_2 may decrease photoinhibition (Hymus *et al*., 1999; Roden *et al*., 1999; Terry *et al*., 2000), suggesting an important interaction for ancient polar forests experiencing continuous irradiance, with the potential to cause photoinhibition during the summer. In addition, the carbon balance of polar forests will be determined not only by photosynthesis but also by respiration, which itself can be influenced directly and indirectly by elevated CO_2 (Wullschleger *et al*., 1994; Bunce, 2001). For example, atmospheric CO_2 enrichment reduces the mitochondrial respiration rates of leaves, roots and stems, through direct inhibition of enzyme activity (Drake *et al*., 1999).

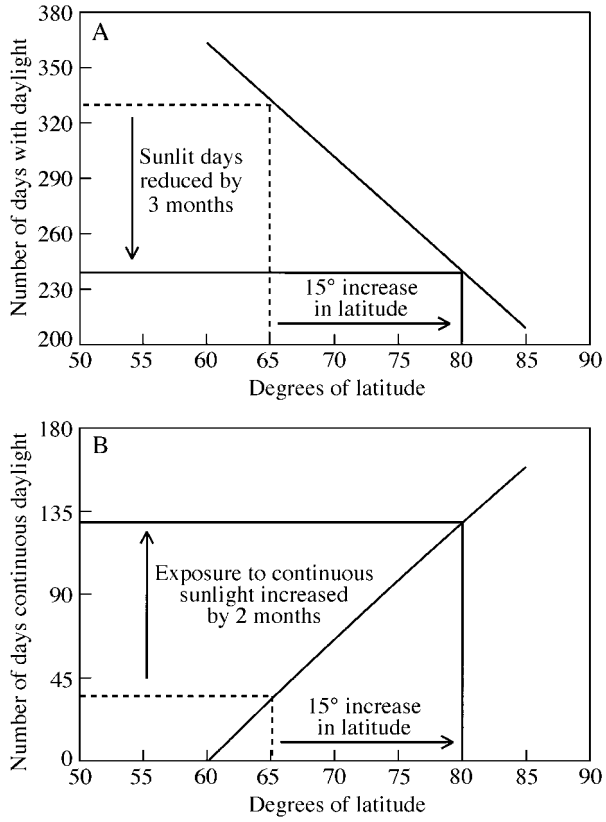

FIG. 1. Changes in length of the growing season (A) and the polar summer (B) as a function of latitude, calculated from equations describing sun–earth geometry (Monteith and Unsworth, 1990). Broken lines indicate the growing season and polar summer lengths at 65°N, with solid lines indicating the situation 15° closer to the pole at 80°N.

These potential interactions are important for understanding polar forest ecology. It has been suggested that the deciduous habit of polar forests reduced respiratory costs allowing plants to tolerate winter darkness and mild temperatures (Axelrod, 1984; Creber and Chaloner, 1985). But if respiration is decreased by elevated CO_2, the requirement for a deciduous habit is diminished. Alternatively, if elevated CO_2 affects respiration indirectly, for example by altering substrate availability and the demand for respiratory products, the respiration rates of leaves grown at elevated CO_2 may increase (Drake *et al*., 1999).

These physiological considerations show that the effects of palaeo-CO_2 concentrations may be especially important in understanding polar forest physiology and should be assessed (Beerling, 1998, 2000). However, the responses of trees to CO_2 enrichment have not yet been assessed experimentally under a light regime relevant to polar forests. Here we describe an experimental approach investigating the effects of a high atmosphere CO_2 concentration on the physiology of some extant genera of plants that were important components of northern and southern hemisphere polar forests (Table 1). We recognize that, whilst the morphology of extant and fossil taxa are similar, the species may not be the same, but we assume that their physiological characteristics are similar. The responses of young trees to

TABLE 1. *Woody plant species grown under polar light conditions in a simulated Cretaceous environment with and without CO_2 enrichment*

Species	Family	Life form	Native habitat
Ginkgo biloba (maidenhair tree)	Ginkgoaceae	Deciduous tree	Uncertain, widely planted in urban environments, but probably sub-tropical forests of southern China*
Metasequoia glyptostroboides (dawn redwood)	Taxodiaceae	Deciduous conifer	Mesothermal forested regions of S.E. China†
Sequoia sempervirens (coastal redwood)	Taxodiaceae	Evergreen conifer	Low hills, near coastal regions of California‡
Taxodium distichum (swamp cypress)	Taxodiaceae	Deciduous conifer	Tidal creeks, flood plains, southern USA
Araucaria araucana (monkey-puzzle)	Araucariaceae	Evergreen conifer	Forests in the southern Andes, Chile§
Nothofagus cunninghamii (southern beech)	Nothofagaceae	Evergreen tree	Cool/temperate forests, Australia/Tasmania¶

* Tredici *et al.* (1992).
† Chaney (1948); Chu and Cooper (1950).
‡ Waring and Franklin (1979).
§ De Laubenfels (1988).
¶ Read (1999).

atmospheric CO_2 enrichment during their first year's growth in a simulated polar light regime (equivalent to latitude = 69°) are reported. Utilizing this facility, we tested the hypotheses that elevated CO_2 (1) stimulates photosynthetic carbon gain and carbohydrate storage; (2) lowers the potential for photoinhibition during polar summers; and (3) suppresses rates of plant respiration. The experimental results have been incorporated into a process-based model of conifer forest growth (Osborne and Beerling, 2000) to provide a first assessment of the possible consequences of the interactions between CO_2 and light for the productivity of mid-Cretaceous north Alaskan coniferous forests.

MATERIALS AND METHODS

Experimental design and performance

The controlled environment facility (Tapton Experimental Gardens, University of Sheffield, UK) consists of four glasshouses, each divided into two isolated sections. This provides eight independent replicated environments: four maintained at ambient CO_2 (400 μmol mol^{-1}) and four at the target CO_2 concentration (800 μmol mol^{-1}). Control of temperature, humidity and CO_2 in each section was achieved using a programmable datalogger (CR10 Measurement and Control System; Campbell Scientific, Inc., Logan, Utah, USA). CO_2 was measured with an infrared gas analyser (IRGA) mounted in each glasshouse (CO_2 Gas Monitor, ADC 2000 Series; The Analytical Development Company Limited, Hoddesdon, Herts., UK). The calibration of each IRGA was checked monthly using a volumetrically mixed reference gas (Certified Standard ± 5 %; BOC Gases, Guildford, Surrey, UK). The CO_2 concentration was increased by frequent injection of pure CO_2 gas (BOC Gases, UK) into the circulating air of each section. During the first year of operation (treatments began in April 2000), daily mean CO_2 concentrations were maintained to within ± 5 % of the target values (Fig. 2A).

Air temperature was measured in the immediate vicinity of plants using a shaded, ventilated copper–constantan thermocouple, and controlled in each section by air-conditioner units (Bradley Refrigeration, Sheffield, UK), which also served to mix and circulate air. Growth temperature within all sections was modified to track outside ambient values, but with a +5 °C warming to mimic the seasonal cycle simulated in high latitudes by general circulation modelling (GCM) studies for the mid-Cretaceous (Valdes *et al*., 1996; Price *et al*., 1997; Beerling, 2000). A minimum winter temperature of 5 °C prevents plants being damaged by frosts. The system provides an effective means of simulating an annual climate representative of the Cretaceous (Fig. 2B).

Atmospheric humidity near the plants was measured using a ventilated wet–dry bulb psychrometer with copper–constantan thermocouples, and the relative humidity within each glasshouse section was maintained to a minimum of 75 % using a horticultural misting system (LBS Horticulture, Colne, Lancs., UK).

Seasonal changes in daylength at 69°N were achieved by complete replacement of glass in the glasshouses with opaque, insulated panels and the installation of an automated lighting system to regulate the intensity and duration of lighting throughout the year. Two water-cooled light units (Sunbeam Hydrostar; Avon Gro-Lite Systems, Bristol, UK) were deployed in each of the eight sections, each unit providing 300–400 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) over approx. 2 m^2 ground area. Thus lighting was supplied and controlled artificially all year round. Daylength was adjusted weekly to simulate the seasonal changes calculated from standard equations (Monteith and Unsworth, 1990) for 69°N (Fig. 2C).

Plant materials

The tree species (Table 1) were grown from seed at Llangwm Arboretum (Usk, UK), and 1-year-old saplings were acclimated to the Sheffield climate in a polythene tunnel for 2 months before transfer to the glasshouses in April 2000. Each tree species was represented by two individuals per glasshouse, a total of eight per CO_2 treatment. Plants were grown in 2 l pots in a medium designed to have good water holding and pH-buffering capacity, and a high mineral content, and consisting of lime-free silica sand (Pioneer Supamix Ltd, Nuneaton, UK), fine vermiculite (LBS Horticulture) and peat (Midland Irish Peat Moss Ltd, Rathlowen, Co. Westmeath, Ireland) in the ratio 13 : 5 : 2. As gymnosperms are naturally associated with vesicular–arbuscular (VA) mycorrhizas (Khan and Valder, 1972), even in Antarctic Triassic fossils (Stubblefield *et al*., 1987); and contemporary *Nothofagus* is commonly ecto-mycorrhizal (Warcup, 1980), mycorrhizal symbioses were established in the experiment by inoculating roots with spores of generalist fungal species during potting (MycorTree Root Dip; Plant Health Care, Berkhamsted, Herts., UK).

Plants were drip irrigated regularly using an automated system (Nutriculture Ltd, Mawdsley, Lancs., UK), and supplied weekly during the growing season with a nutrient solution appropriate for VA mycorrhizal systems (10 % Rorison's Nutrient Solution).

Leaf gas exchange and carbohydrate status

Leaf photosynthetic responses and carbohydrate contents of five tree species grown with either 400 or 800 μmol mol^{-1} CO_2 were determined during their first year under a polar light regime. All measurements on leaves were made using a portable open gas exchange system (CIRAS-1; PP-Systems, Hitchen, Herts., UK) on foliage that developed under the experimental conditions. Gas exchange was measured at a PAR flux of 600 μmol m^{-2} s^{-1}, previously shown to saturate photosynthesis (data not shown), a leaf temperature of 25 °C and a leaf-air vapour pressure deficit of 1.0 kPa. Measurements were made during the summer (plants under continuous light) and the autumn (plants under a daylength of 10 h), at CO_2 concentrations of 400 and 800 μmol mol^{-1}, i.e. at the growth CO_2 concentrations.

Following the method of Scholes *et al*. (1994), leaf carbohydrate content was determined three times during the year: in summer and autumn, in parallel with leaf

photosynthesis measurements and in midwinter, during continuous darkness. For photosynthesis and carbohydrate measurements, the mean of two leaves, i.e. one leaf sampled from each of two plants, was used as the replicate, giving $n = 4$ measurements at each CO_2 concentration for each species.

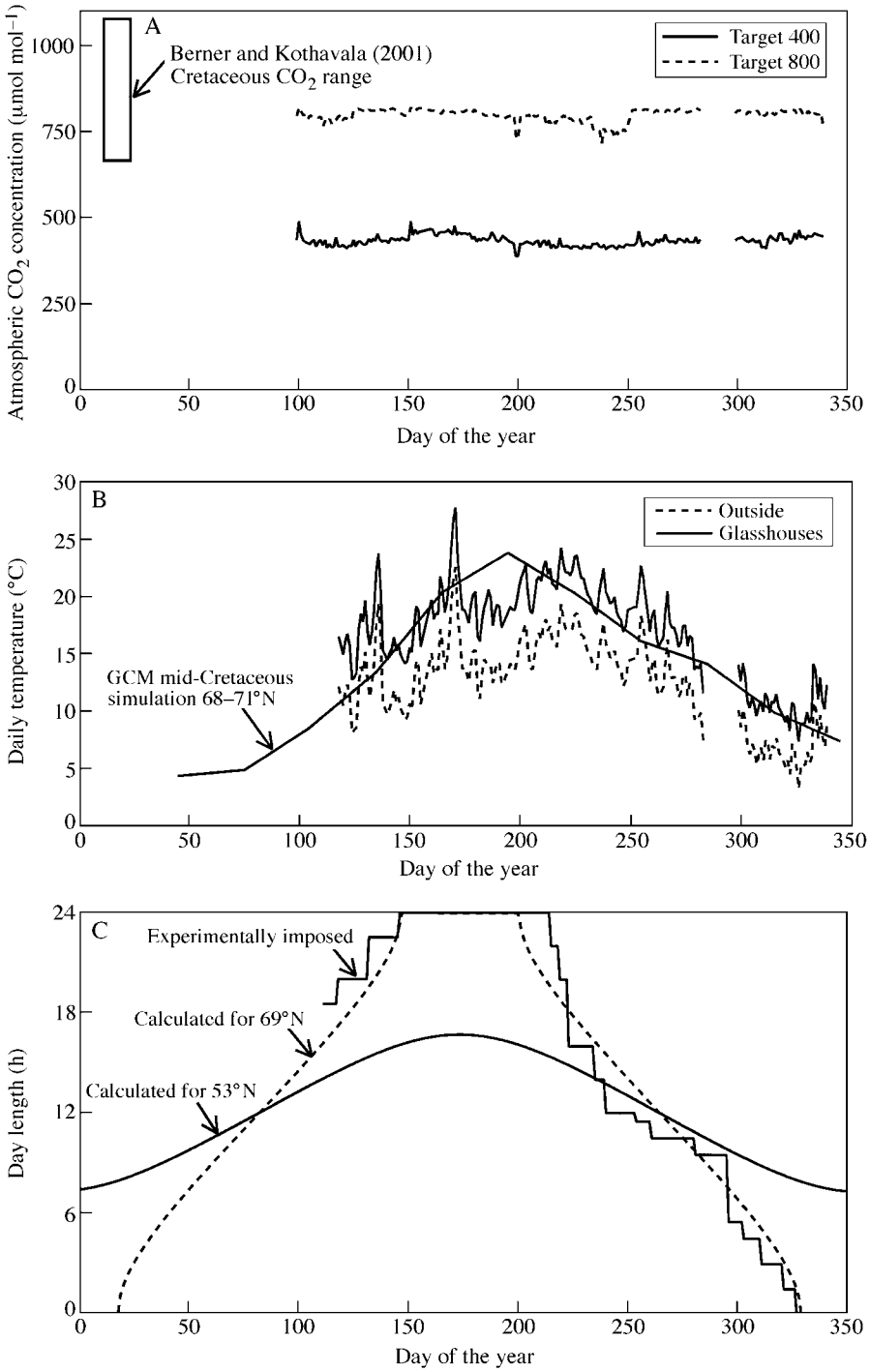

FIG. 2. A, Measurements of atmospheric CO_2 concentrations in the ambient and elevated CO_2 glasshouses during the first year (2000) of treatment. Values are means of four replicates at each concentration. The vertical box denotes the range of uncertainty in CO_2 predictions of a geochemical model for the mid-Cretaceous. B, Seasonal temperature (solid line) within both the ambient and elevated CO_2 glasshouses during 2000. For comparison, the outside air temperature in Sheffield (UK) is shown (broken line). Temperatures at 69°N from a climate modelling study for the mid-Cretaceous (Valdes *et al.*, 1996) are shown for comparison. C, Calculated and experimentally imposed changes in day length at 69°N. Also shown, for comparison, is the seasonal change in daylength experienced at Sheffield (53°N).

Photoinhibition

The maximum quantum efficiency of photosystem II photochemistry (Genty *et al*., 1989) was assessed *in situ* using periodic measurements of the dark-adapted variable to maximum fluorescence ratio (F_v/F_m) using a portable modulated system (FMS-2 Field Fluorescence Monitoring System; Hansatech Instruments Ltd, King's Lynn, UK), with replication as for photosynthesis. To determine the effects of growth CO_2 concentration on F_v/F_m, plants were exposed for 2 h to warm temperatures (32 °C) and a PAR of 1000 μmol m^{-2} s^{-1}, under the CO_2 concentration in which they grew (i.e. either 400 or 800 μmol mol^{-1}). A baseline set of measurements was established before and after this treatment from plants placed in cool (22 °C), low light (PAR = 150 μmol m^{-2} s^{-1}) conditions under growth CO_2 concentrations.

Plant respiration

Measurements were made on *Sequoia sempervirens* and *Nothofagus cunninghamii*, as evergreen taxa representing elements of the northern and southern hemisphere polar forests, respectively, using a portable open gas exchange system (CIRAS-1; PP-Systems, Hitchin, Herts., UK). A mean value for each leaf was obtained from 30 measurements made at 1-min intervals under the mean ambient temperature for January (7 °C), with replication as for photosynthesis. Direct or indirect effects of CO_2 were measured by reciprocal transfer of plants grown in ambient CO_2 to elevated CO_2, and of plants grown in elevated CO_2 to ambient CO_2. The CO_2 efflux from leaves was measured as before, immediately following the transfers.

Rates of root respiration were measured for *Araucaria araucana* and *S. sempervirens* during early and late autumn, when daylengths were 12 and 3 h, respectively. These two species were selected because of their differential accumulation of carbohydrates in leaves, which could influence root carbohydrate content (Ekblad and Högberg, 2001). Respiration rates of excised roots were measured as the quantity of O_2 consumed using a liquid phase oxygen electrode (Model LD 2/2 oxygen electrode; Hansatech Instruments, King's Lynn, UK) maintained at a constant temperature of 20 °C. Roots were then dried at 40 °C for 48 h to obtain dry mass, and respiration rates were expressed as μmol O_2 consumed g^{-1} dry matter h^{-1}.

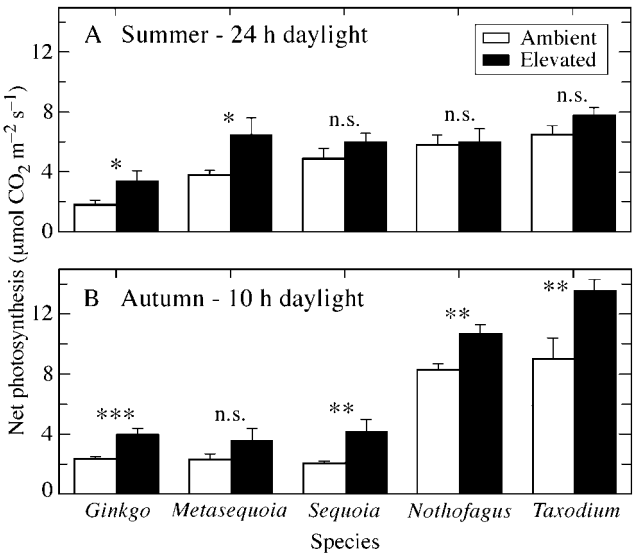

FIG. 3. Response of net photosynthesis to CO_2 enrichment in five tree species during their first exposure to a continuous PAR flux of the polar summer light regime (A) and the short days of autumn (B).

RESULTS

Leaf gas exchange and carbohydrate status

After 4 weeks of continuous light during the polar summer, CO_2 enrichment stimulated photosynthesis by 47 % in *Ginkgo biloba* and 42 % in *Metasequoia glyptostroboides* ($P = 0.05$). However, this did not occur in *S. sempervirens*, *N. cunninghamii* and *Taxodium distichum* (Fig. 3A), indicating marked photosynthetic acclimation in these species. In contrast, photosynthetic rates in the autumn were significantly higher in elevated than in ambient CO_2 for all species except *M. glyptostroboides* (which was showing visible signs of leaf senescence by this time) (Fig. 3B). The response to CO_2 in species previously showing acclimation shows that acclimation is reversible, and suggests that the earlier acclimation was not caused by limited rooting volume. In addition, photosynthetic rates in *N. cunninghamii* and *T. distichum* were higher in autumn than in summer (Fig. 3A, B), suggesting that photosynthetic activity was limited during the summer in these species irrespective of CO_2 treatment. Responses to CO_2 were not related to leaf habit, either in the summer or autumn.

The effect of CO_2 on total non-structural carbohydrate (TNC) content of leaves in the polar summer was an increase in *G. biloba* and *M. glyptostroboides* by 73 % and 59 %, respectively, compared with their counterparts in ambient CO_2 (Fig. 4A), but there were no significant effects in the other species. This pattern mirrors the photosynthetic responses (Fig. 3A). By contrast, TNC content in autumn was similar in all species (Fig. 4B) with no effects of growth in elevated CO_2, a pattern seemingly unrelated to the autumnal photosynthetic responses (Fig. 3B). In winter, the TNC contents of the evergreen species were depleted relative to the summer and autumn (Fig. 4C) due to respiration under continuous darkness without photosynthetic replenishment. Growth CO_2 concentration had no effect on the TNC content of leaves of *S. sempervirens* and *N. cunninghamii*, but increased it in the leaves of *A. araucana* (Fig. 4C).

Effects of CO_2 on photoinhibition

All species, from both ambient and elevated CO_2, showed a progressive time-dependent reduction in F_v/F_m on exposure to saturating irradiance, reflecting a decrease in the efficiency of photochemical energy dissipation. The decrease in F_v/F_m was largely reversed after a return to cool, low light conditions (Fig. 5). Four of the six tree species

grown in elevated CO_2 showed no significant ($P < 0.05$) differences in the response of F_v/F_m to high irradiance when compared with their ambient CO_2-grown equivalents (Fig. 5). The two species showing differences in F_v/F_m due to CO_2 treatment responded in different ways: in *S. sempervirens*, F_v/F_m was greater in elevated CO_2 conditions on

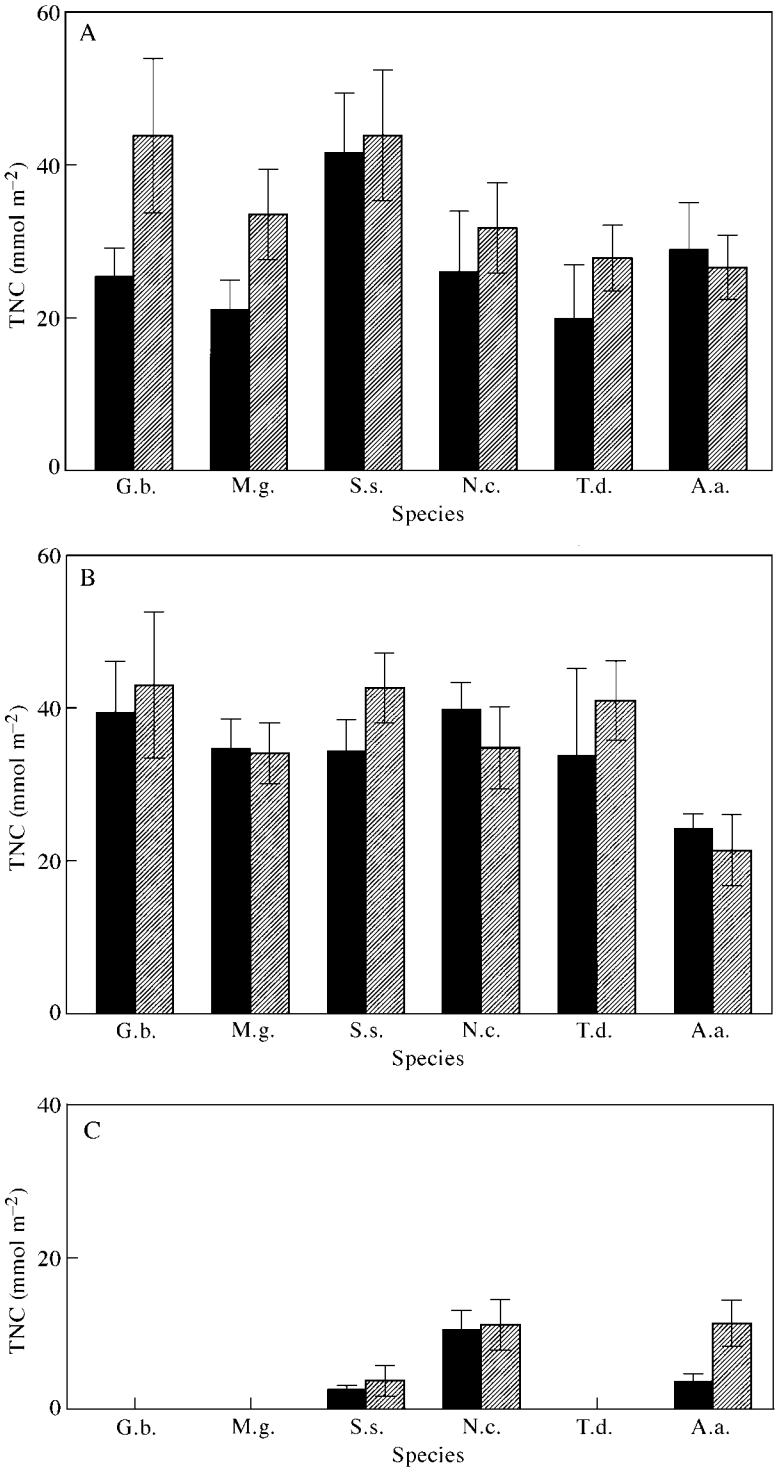

FIG. 4. Effects of CO_2 enrichment on leaf total non-structural carbohydrate (TNC) content. Results are shown for six tree species during their first year of exposure to the continuous days of a polar summer light regime (A), the shorter days during the autumn (B) and for evergreen taxa during the winter (C). A.a., *Araucaria araucana*; G.b., *Ginkgo biloba*; M.g., *Metasequoia glyptostroboides*; N.c., *Nothofagus cunninghamii*; S.s., *Sequoia sempervirens*; T.d., *Taxodium distichum*.

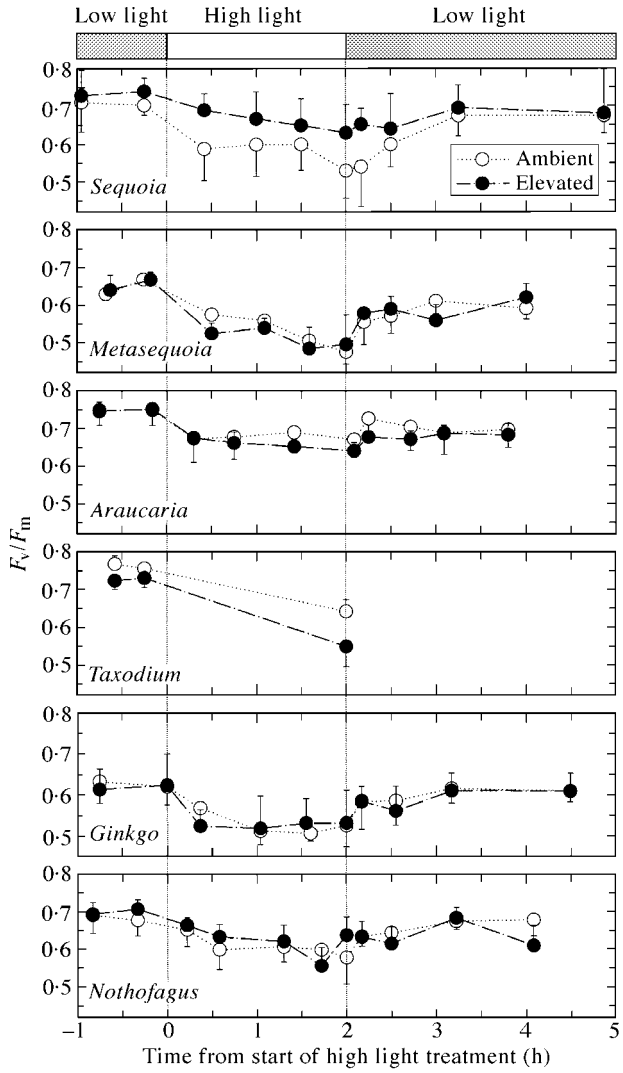

FIG. 5. Effects of CO_2 enrichment on the variable to maximum fluorescence ratio (F_v/F_m) of six tree species exposed to high irradiance. CO_2 effects were significant ($P < 0.05$) for *Sequoia* and *Taxodium* only.

exposure to high irradiance compared with ambient CO_2 controls, whereas in *T. distichum*, F_v/F_m was lower in plants grown in elevated CO_2 (Fig. 5).

Plant respiration

After 2 months of permanent darkness in elevated CO_2, dark respiration rates of *S. sempervirens* leaves were reduced by 61 % ($P < 0.05$) compared with plants grown under ambient CO_2 (Table 2). *N. cunninghamii* responded similarly, with respiration rates being reduced by 38 %, although this was not statistically significant because of large variability between individuals (Table 2). Transfer of plants grown in ambient CO_2 to elevated CO_2 markedly suppressed rates of leaf respiration in both *S. sempervirens* and *N. cunninghamii*, whilst the transfer from elevated- to ambient-CO_2 increased them (Table 2).

Respiration rates of roots of *S. sempervirens* and *A. araucana* grown in elevated CO_2 were lower in both the early and late autumn than those of plants grown in ambient CO_2 (Table 3). Overall, root respiration was suppressed by 10 and 40 % in early and late autumn, respectively, in both species, but as with leaf respiration, the large variation between individuals resulted in a significant effect of CO_2 treatment on *A. araucana* during the autumn (Table 3).

DISCUSSION

Understanding polar forest ecology of the Mesozoic requires that key aspects of tree physiology, and particularly the effects of light, atmospheric CO_2 and their interactions with physiology be considered (Beerling, 1998, 2000). To do so requires information on plant taxa of this extinct biome. As this is impossible, we have used closely related extant species. Our experiment is designed to provide data allowing us to address this fundamental requirement by assessing three determinants of the plant carbon balance (photosynthesis, photoinhibition and respiration). Contrary to expectations, we found that stimulation of leaf photosynthesis by elevated CO_2 was not generally evident during the polar summer, i.e. under conditions of continuous sunlight (24 h), but did occur under shorter days (10 h). Measured TNC contents of leaves were not directly

TABLE 2. *Responses of leaf respiration (R, μmol m^{-2} s^{-1}) of* Sequoia sempervirens *and* Nothofagus cunninghamii *to CO_2 enrichment in a polar light regime during midwinter*

	CO_2 concentration during growth			
	Ambient CO_2 (400 μmol mol^{-1})		Elevated CO_2 (800 μmol mol^{-1})	
CO_2 concentration during measurement	*S. sempervirens*	*N. cunninghamii*	*S. sempervirens*	*N. cunninghamii*
Ambient CO_2	0.13 ± 0.02	0.32 ± 0.01	0.11 ± 0.03	0.26 ± 0.10
Elevated CO_2	0.05 ± 0.01	0.19 ± 0.06	0.05 ± 0.01	0.20 ± 0.06

Values are means ± s.e. of eight plants. Two-way analysis of variance indicated a significant ($P < 0.01$) effect of CO_2 concentration during measurement on leaf respiration of *S. sempervirens*, but no effect of the CO_2 concentration during growth. CO_2 concentrations during growth and measurement had no significant effects on the respiration rates of *N. cunninghamii* leaves. There were no significant interactions between measurement and growth CO_2.

TABLE 3. *Effect of CO_2 enrichment on root respiration rates, measured as O_2 consumption (μmol g^{-1} dry matter h^{-1}) at 20 °C, for two evergreen conifer taxa during contrasting day lengths (12 and 3 h)*

	Early autumn		Late autumn	
Species	Ambient CO_2 (400 µmol mol^{-1})	Elevated CO_2 (800 µmol mol^{-1})	Ambient CO_2 (400 µmol mol^{-1})	Elevated CO_2 (800 µmol mol^{-1})
Sequoia sempervirens	85.1 ± 7.2	73.5 ± 15.0	82.0 ± 19.7	52.8 ± 10.9
Araucaria araucana	59.6 ± 6.6	55.4 ± 4.4	40.9 ± 3.7	30.5 ± 0.9

Values are means of eight replicates ± 1 s.d. Analysis of variance indicated CO_2 effects on respiration were significant ($P < 0.05$) for *Araucaria araucana* in the late autumn. All other CO_2 effects were not significant at $P = 0.05$.

correlated with absence of CO_2 stimulation of photosynthesis (Figs 3 and 4), in contrast with previous studies (Drake *et al*., 1997; Curtis and Wang, 1998). The reasons for the unresponsiveness of TNC to CO_2 are uncertain, but possibly the storage capacity of leaves was similar in both ambient and elevated CO_2 treatments, and saturated during the continuous light of the polar summer. If this is the case, the TNC content of trunk wood might have increased in elevated CO_2, since the trunk is a major site for storage in trees (Larcher, 1995).

Growth in elevated CO_2 can increase photochemical energy use when there is a large demand for assimilates but not when demand is low (Hymus *et al*., 1999). This indicates the potential for increased photoinhibition during periods of carbohydrate accumulation. Measured TNC contents of leaves were highest in the polar summer (Fig. 4) under conditions of continuous irradiance. Our measurements of F_v/F_m were therefore made a time when the risk of CO_2-induced photoinhibition was high. Despite this, there was scant evidence to suggest that growth in an elevated CO_2 atmosphere, representative of the Mesozoic, affected photoinhibition during the polar summer in either evergreen or deciduous taxa (Fig. 5).

The observed reductions in rates of leaf (Table 2) and root (Table 3) respiration for plants grown with CO_2 enrichment are consistent with results from numerous studies of plants exposed to elevated CO_2 (Lambers *et al*., 1996; Norby *et al*., 1999). Reductions in the respiration rates of roots of plants grown in elevated CO_2 are likely to be caused by acclimation of the respiratory system, by decreased supply of respiratory substrate or by decreased demand for the products of respiration (Ryan, 1991; Amthor, 2000). Interestingly, however, the effect of elevated CO_2 on leaf respiration (50 % decrease) in our polar experiment was greater than that shown by a number of tree species (mean 19 %) in experiments with regular 12–16 h daylengths (Drake *et al*., 1999). This difference is consistent with the idea (Bunce, 2001) that the CO_2-sensitivity of mitochondrial respiration is greatest after a period of prolonged darkness, when respiration becomes substrate-limited (Fig. 4). Data from the reciprocal transfer experiment (Table 2) indicate that dark respiration rates of leaves of *S. sempervirens* and *N. cunninghamii* were directly suppressed by CO_2 inhibiting the biochemistry of respiratory pathways, possibly by reducing activity of cytochrome-*c*-oxidase and succinate dehydrogenase (Drake *et al*., 1999). Analyses of carbohydrates in leaves suggest that reserves accumulated during the polar summer were largely depleted during the winter in these two species (Fig. 4C). Thus, limited stores of TNC, and of respiratory substrate in leaves during winter, may explain why no indirect effects of CO_2 on leaf respiration rates were observed then.

Suppression of respiration of above- and below-ground plant organs by elevated CO_2, if a consistent feature of the experimental treatment, implies that the high concentration of atmospheric CO_2 in the Mesozoic played an important role in decreasing respiratory demand of an evergreen canopy during the mild winters. Furthermore, it is possible that plants acclimated with decreased respiration to a warm winter climate, as seen in the field (e.g. Mooney and Brayton, 1966) and in controlled environment studies (Tranquillini *et al*., 1986). Suppression of plant respiration by the joint action of a high CO_2 atmosphere (Tables 2 and 3), and physiological acclimation to warmer climates, suggests that the deciduous habit in northern hemisphere polar forests may not have been such a crucial adaptation for reducing canopy respiratory CO_2 losses as previously suggested (Axelrod, 1984). Critical to this conclusion is further investigation of CO_2-related effects on leaf and root respiration, especially in the longer term (Bunce, 2001).

A more complete assessment of the effects of CO_2 on whole plant carbon balance, particularly the distribution of dry matter between above- and below-ground parts, is clearly required. The complexity of whole tree responses to elevated CO_2 is exemplified by controlled environment microcosm experiments with *Pseudotsuga menziesii* (Lin *et al*., 2001). They showed that increased root growth under elevated CO_2 more than compensated for reduced respiration rates per unit weight of root, so that the total respiration of the rhizosphere increased under high CO_2. In the context of polar forests, measurements throughout the year of carbon fluxes associated with shoots and roots of trees and with the soil are an important requirement of future studies, and will be made as the experiment progresses.

Simulation modelling

Collectively, the effects of CO_2 on carbon gain by photosynthesis and carbon loss by respiration in extant species show the potential of CO_2 to modify the primary production of ancient polar forests. We assessed

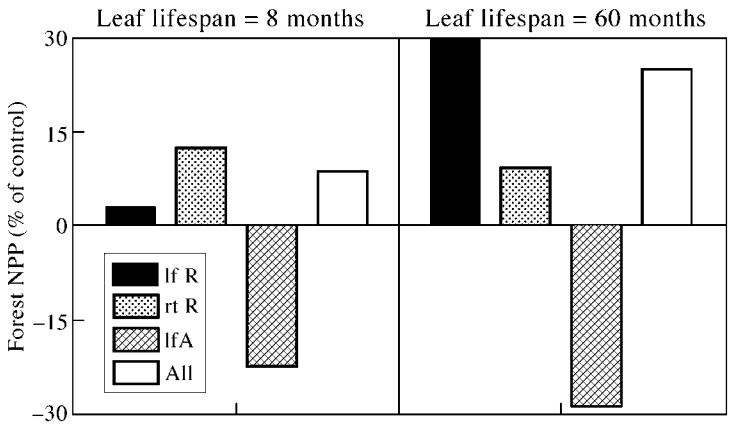

FIG. 6. Simulated changes in the net primary productivity (NPP) of evergreen and deciduous Cretaceous coniferous forests resulting from the inclusion of the observed effects of CO_2 on respiration and photosynthesis in a simulation model. Changes are expressed relative to the control simulation. Codes for each simulation are: the effects of a 50 % decrease in leaf respiration (lf R); the effects of a 25 % reduction in root respiration (rt R); absence of CO_2 stimulation of photosynthesis during the polar summer (lfA); and all three of the previous treatments combined (all).

this potential using a process-based generic model of coniferous forest productivity (Osborne and Beerling, 2000), and a simulated Mid-Cretaceous climate for northern Alaska (72°N), as in the study conditions (Beerling, 2000). Northern Alaska was selected because analyses of the fossil record indicate that Cretaceous forests in this region included a mixture of deciduous and evergreen taxa (Falcon-Lang, 2000; Falcon-Lang and Cantrill, 2000, 2001). The model of Osborne and Beerling mathematically describes forest carbon, nitrogen and water fluxes by scaling up widely applicable relationships between leaf lifespan and function (Reich *et al.*, 1997). It is therefore uniquely sensitive to prescribed leaf lifespan, and includes representation of the environmental influences on photosynthesis, respiration and stomatal activity, and is sensitive to soil nutrient and water status. From climatic and soil information it predicts the structural (leaf area index) and functional [net primary productivity (NPP), canopy transpiration, etc.] characteristics of coniferous forests.

Five simulations were performed to account for the separate and combined effects of elevated CO_2. In each case, leaf lifespans of either 8 (deciduous) or 60 months (evergreen) were used in the model.

Simulation 1. Control, no changes to the conifer growth model, CO_2 concentration of 800 μmol mol^{-1}.

Simulation 2. As for the control, but with a 50 % reduction in leaf respiration rates (Table 2).

Simulation 3. As for the control, but with a 25 % reduction in root respiration rates (Table 3).

Simulation 4. As for the control, but without photosynthetic CO_2 stimulation during the polar summer, when daylength was greater than 20 h (Fig. 3).

Simulation 5. As for the control, but with effects 2–4 applied together.

Each effect of elevated CO_2 has a different impact on the simulated productivity of north Alaskan coniferous forests (Fig. 6). Reductions in leaf and root respiration by elevated CO_2 increased forest NPP, whilst the absence of stimulation of photosynthesis by CO_2 during the summer reduced NPP (Fig. 6). These effects of CO_2 are largest for trees with long-lived foliage (evergreen) (Fig. 6). Decreased leaf respiration increases forest NPP, relative to the control simulation, because it reduces the light compensation point of photosynthesis. This allows a positive net carbon gain for a longer period of each day (Long and Drake, 1991; Osborne *et al.*, 1997) and in a greater fraction of the canopy (Osborne *et al.*, 1998). Reduced root respiration rates decrease the demand for carbohydrates, allowing root growth to increase, with a resulting improvement in nutrient and water uptake efficiency. If photosynthesis is not increased by elevated CO_2 during the warm polar summer, NPP is reduced (Fig. 6), because stimulation of photosynthesis by elevated CO_2 is strongly dependent upon temperature (Long, 1991), and is therefore greatest in summer. When all three physiological effects of CO_2—leaf and root respiration and photosynthesis—operate together, their non-linear interactions result in a net increase in forest productivity with the decreased respiratory costs more than compensating for loss of high CO_2–high temperature interactions during the polar summer (Fig. 6).

We conclude that the effects of elevated CO_2 observed in six species of deciduous and evergreen trees, representing some ancient taxa of polar forests, are likely to be important modifiers of whole tree carbon balance. According to the results of our simulations, trees with long-lived foliage will show the greatest benefit from growth in a high CO_2 environment (Fig. 6). This is because the growing season of above- and below-ground plant organs is longer in evergreen than in deciduous trees. They are therefore better

placed to exploit the interaction between high CO_2 and warm spring and autumnal temperatures to increase photosynthetic carbon gain, whilst at the same time incurring reduced respiratory costs of fine root production. These considerations provide some physiological clues that might explain the geographical separation between the northern and southern hemispheres of polar forests with a predominately evergreen or deciduous leaf habit.

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