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Carbon loss by deciduous trees in a CO$_2$-rich ancient polar environment

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Fossils demonstrate that deciduous forests covered the polar regions for much of the past 250 million years[1] when the climate was warm and atmospheric CO$_2$ high[2]. But the evolutionary significance of their deciduous character has remained a matter of conjecture for almost a century[3]. The leading hypothesis[1,4–7] argues that it was an adaptation to photoperiod, allowing the avoidance of carbon losses by respiration from a canopy of leaves unable to photosynthesize in the darkness of warm polar winters[8–11]. Here we test this proposal with experiments using 'living fossil' tree species grown in a simulated polar climate with and without CO$_2$ enrichment. We show that the quantity of carbon lost annually by shedding a deciduous canopy is significantly greater than that lost by evergreen trees through wintertime respiration and leaf litter production, irrespective of growth CO$_2$ concentration. Scaling up our experimental observations indicates that the greater expense of being deciduous persists in mature forests, even up to latitudes of 83°N, where the duration of the polar winter exceeds five months. We therefore reject the carbon-loss hypothesis as an explanation for the deciduous nature of polar forests.

Plant fossils provide tangible evidence for the widespread occurrence of deciduous forests in the polar regions throughout the Mesozoic and Palaeogene[1]. These high-latitude forests flourished in a CO$_2$-rich atmosphere[2], under winter temperatures considerably warmer than those of today[8–11] and more closely resembling the northern coast of the Mediterranean[12] (Fig. 1). The carbon loss hypothesis[1,4–7] maintains that the quantity of carbon lost when a canopy of leaves is shed annually (that is, the deciduous habit) is less than the carbon losses by canopy respiration during the warm, dark winter months and the annual abscission of only a fraction of leaves (that is, the evergreen habit). However, because no modern analogues exist for these ancient polar environments, the hypothesis remains untested. Experiments offer the most direct means of testing, but are necessarily shorter in duration than the lifetime of the trees. To address differences in carbon budgets over longer timescales applicable to mature forests, and across a wide latitudinal gradient, requires application of suitable process-based models of forest biogeochemistry. Here, therefore, we present an integrated study that tests the carbon-loss hypothesis by combining plant-growth experiments in a simulated high-latitude environment[13] with numerical modelling simulations of conifer forests[14].

We ran our experiments using three deciduous species (the taxodiaceous conifers *Metasequoia glyptostroboides* Hu & Cheng and *Taxodium distichum* (L.) Rich, and the broadleaved gymnosperm *Ginkgo biloba* L.) and two evergreen species (the taxodiaceous *Sequoia sempervirens* (D. Don) Endl., and the southern-hemisphere angiosperm *Nothofagus cunninghamii* (Hook.) Oerst.). All of these represent reasonable modern analogues for their congeneric ancestors, which were dominant elements in Cretaceous and early Palaeogene polar landscapes up to a latitude of 85°N (refs 1, 6). One-year-old saplings of each species were grown in a high-latitude (69°N) photoperiod and a simulated Cretaceous/early Palaeogene seasonal temperature regime for three years, in atmospheres of either 400 p.p.m.v. or 800 p.p.m.v.

CO$_2$ (Fig. 1, see ref. 13 for details). Because plant respiration typically increases with temperature[15], a severe thermal challenge to the carbon-loss hypothesis was realized by experimentally imposing the warmest of the cold-month mean temperatures reconstructed from fossil biota[8–11] (Fig. 1). Whole-tree net carbon exchange rates were tracked throughout the year using flow-through chambers, and the production and carbon content of leaf litter measured in the autumn (see Methods).

During the six weeks of continuous darkness in both the 2001–2002 and 2002–2003 simulated polar winters, carbon losses by leaf and stem respiration in the evergreen species were consistently small (Fig. 2a), equivalent to only 1–2% of annual net primary productivity (NPP) (Fig. 2d). Nevertheless, they were significantly larger than those of the leafless stems of the deciduous species (2002–2003 winter: $F_{1,6} = 90$, $P < 0.001$; 2001–2002 winter: $F_{1,6} = 140$, $P < 0.001$). Importantly, all of the trees survived three successive polar winters, in agreement with an earlier study[16], and maintained a normal rhythm of leaf expansion and abscission in each growing season. The quantity of carbon lost in the production of deciduous leaf litter was nearly an order of magnitude greater than the comparatively minor litter turnover in evergreens ($F_{1,6} = 45$, $P < 0.001$) (Fig. 2b). No significant effects of CO$_2$ concentration in the growth chambers on either respiration or litter production were evident.

An initial evaluation of the carbon-loss hypothesis can be achieved by summing the quantity of carbon lost in leaf litter production and wintertime respiration for trees with each leaf habit (Fig. 2a,b). These figures demonstrate that all three deciduous tree species lost almost an order of magnitude more carbon than their evergreen counterparts in the same environmental setting (Fig. 2c), despite temperatures above freezing throughout the winter (Fig. 1). For the deciduous species, the loss of carbon originally invested into leaf production (Fig. 2b), along with the wintertime respiration of leafless stems (Fig. 2a), was a substantial fraction of the NPP (Fig. 2d) (14–25%), and significantly more than in the evergreens (1–3%) ($F_{1,6} = 59$, $P < 0.001$). This contrast is well illustrated by the closely related evergreen *S. sempervirens* and deciduous *M. glyptostroboides*, which both attained similar NPPs (Fig. 2d). The *M. glyptostroboides* leaf canopy lost 16 times more carbon than that of *S. sempervirens* at a CO$_2$ concentration of 400 p.p.m.v., and 14 times more at 800 p.p.m.v. (Fig. 2c). Respiration accounted for 90% of this loss in *S. sempervirens*, whereas litter production was 98% of the loss in *M. glyptostroboides*.
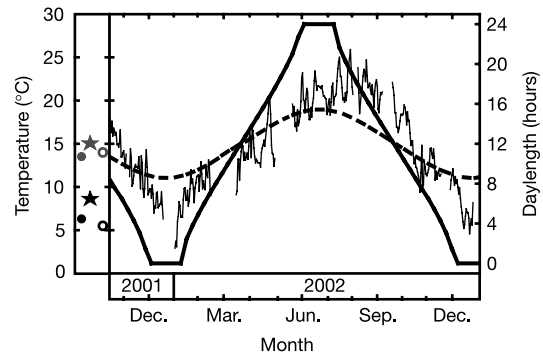
Figure 1 Environmental data for growth rooms. The thin line shows daily mean temperatures 2001–2002, and symbols in the left-hand column correspond to proxy estimates of coldest month mean temperature (black symbols) and mean annual temperature (grey symbols). Closed circles are estimates from geochemical analyses of early Palaeogene high-latitude marine molluscs[10,11], open circles are estimates from Cretaceous and early Palaeogene vertebrates[8,9], and stars are corresponding mean temperatures inside the growth rooms. The thick line denotes the simulated photoperiod at 69°N, and the dashed line shows for comparison that at 45°N.

An evaluation of the relative merits of leaf habit on the carbon budgets of mature forests can be obtained by scaling up our measurements in space and time. The wintertime respiratory burden of an evergreen canopy, avoided by deciduous trees, will scale with canopy size, as described by the leaf area index (LAI, m^2 leaf area per m^2 ground area). In contrast, evergreens have the advantage of a substantially reduced rate of leaf litter production, to an extent dependent on the number of leaves produced each year, foliage longevity and tree age. We assessed the importance of our experimental findings for mature forests by addressing these two scaling issues and calculating the carbon cost (CC, g C per m^2 ground area) incurred by evergreen forests in a polar environment as $CC = LAI \times (R + LCD \times \tau)$, where R is the canopy respiration rate integrated for the period of continuous darkness (g C per m^2 leaf area), LCD is litter carbon density (g C per m^2 leaf area) and τ is the fraction of the canopy shed annually (dimensionless) (see Methods). For deciduous trees, where $R = 0$ and $\tau = 1$, the calculation of CC simplifies to $CC = LAI \times LCD$. Our analyses assume that the energetic costs of leaf construction for evergreen and deciduous species are similar, in agreement with observations[17].

According to these scaling procedures, the difference in carbon cost between evergreen and deciduous forest stands is diminished relative to that measured directly in our experiments (Fig. 3a,b) because the scaled calculations more completely account for carbon losses by leaf fall from an evergreen canopy. Nevertheless, the cost of producing a deciduous canopy of leaves remains more than twice that incurred by evergreen trees through canopy respiration and turnover (Fig. 3a). Growth in a CO_2-enriched atmosphere enhances the difference in carbon costs between leaf strategies (Fig. 3b) through a significant increase in LCD ($F_{1,30} = 9.6$, $P = 0.004$). Since there was no significant effect of CO_2 treatment on leaf tissue carbon concentration (g C per g leaf tissue) ($F_{1,30} = 0.51$, $P = 0.48$), the rise in LCD was driven solely by a significant increase in leaf mass per unit area (g dry matter per m^2 leaf tissue) ($F_{1,30} = 9.7$, $P = 0.004$), in agreement with other CO_2 studies on forest species at lower latitudes[18]. If this effect operated in ancient polar forests at times of increased atmospheric CO_2, it may have exacerbated the carbon cost of having short-lived leaves.

Our experiments and scaling calculations are directly applicable

to a latitude of 69 °N. However, the carbon-loss hypothesis predicts a greater penalty for evergreen trees at higher latitudes as cumulative canopy respiration increases with the duration of the polar winter. To investigate this issue at the scale of mature forests, we performed a series of simulations using a process-based numerical model of forest biogeochemistry[14] forced with a Cretaceous climate and geography[19] encompassing a range of latitudes (68–83 °N; see Methods) where mean temperatures were above freezing during winter darkness (Fig. 3c). In agreement with expectation, we find that wintertime respiration increases with latitude, despite the cooler temperatures, because of the lengthening polar winter (Fig. 3c,d). Nevertheless, this adverse effect on evergreens is negligible relative to the cost of annual canopy turnover in mature trees. As a consequence, the quantity of carbon required by deciduous conifers for annual canopy replacement continues to remain 50–100% greater, regardless of latitude (Fig. 3).

Proxy CO_2 data and geochemical modelling indicate a CO_2-rich atmosphere for much of the Jurassic and Cretaceous periods[2], an interval when polar forests flourished and produced wide maximum growth ring widths[20]. In our experiment, elevated CO_2 significantly ($F_{1,20} = 6.6$, $P = 0.02$) stimulated whole-tree NPP by between 17 and 73% (Fig. 2d). High CO_2 therefore might have partially compensated for the short high-latitude growing season experienced by these forests. Comparison of the NPP values for evergreen and deciduous taxa indicates that, despite a higher carbon cost, deciduous trees have a productivity similar to that of evergreens. This suggests that deciduous trees compensate for higher canopy carbon losses through increased rates of photosynthetic carbon gain.

Our results overturn the long-standing and pervasive conjecture that leaf habit in ancient polar forests was an adaptation to photoperiod. Moreover, it has been recognized that Antarctic polar forests appear to have contained a higher proportion of evergreens than their Arctic counterparts[7,21,22], a disparity also incompatible with the carbon-loss argument. We suggest that alternative explanations for the dominance of deciduous trees in these ecosystems must now be sought[23,24]. The categorization of fossil forests as either evergreen or deciduous appears to represent an overly simplistic dichotomy in this context, since it assumes
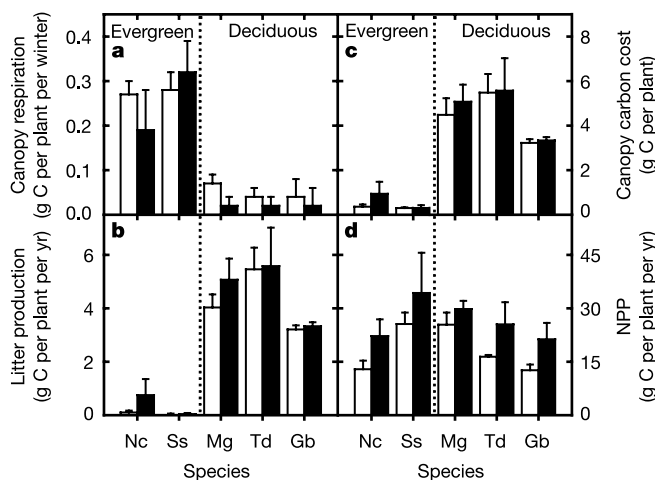
Figure 2 Experimental analyses of the carbon costs of leaf habit at 69 °N for two atmospheric CO_2 levels. Open bars, 400 p.p.m.v.; closed bars, 800 p.p.m.v. **a**, Canopy respiration during the 6-week period of continuous darkness of the 2002–2003 winter. **b**, Litter production in 2002. **c**, Total carbon costs of the polar winter, defined as the sum of **a** and **b**. **d**, Annual net primary productivity (NPP). Values are means of four replicates ±1 s.e. ($n = 3$ for NPP) for these species: Nc, *Nothofagus cunninghamii*; Ss, *Sequoia sempervirens*; Mg, *Metasequoia glyptostroboides*; Td, *Taxodium distichum* and Gb, *Ginkgo biloba*.
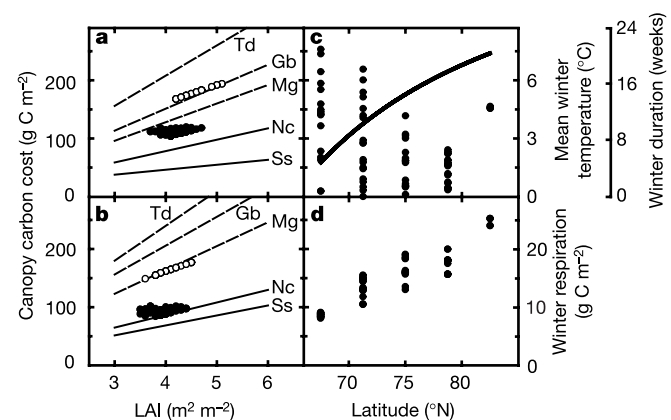
Figure 3 High-latitude forest carbon budgets and climates. Carbon costs of a polar winter for mature evergreen and deciduous leaf canopies calculated by scaling experimental observations (dashed lines, deciduous species; solid lines, evergreen species, defined as in Fig. 2.) with leaf area index (LAI) for two atmospheric CO_2 levels. **a**, 400 p.p.m.v. and **b**, 800 p.p.m.v. Also shown are stand-level forest model simulations using a mid-Cretaceous climate. Open circles, leaf lifespan = 6 months; closed circles, leaf lifespan = 60 months across a range of latitudes (68–83 °N). **c**, Mean Cretaceous climate model temperatures of the winter dark period (data points) and length of polar winter (solid line). **d**, Modelled wintertime canopy respiration of evergreen trees (leaf lifespan = 60 months).

similar function in all evergreen leaves, irrespective of their longevity. In fact, studies across biomes with different evolutionary and climatic histories demonstrate that this categorization is unlikely to be correct because leaf lifespan is strongly correlated with physiological, whole-tree and ecosystem processes[14,25,26]. A palaeontological view of leaf lifespan is now afforded through anatomical analyses of growth rings in fossil woods[27], providing a critical means of linking past and present forest physiology. □

Methods

Whole-tree CO₂ flux measurements

The net carbon exchange rates of trees were tracked throughout the year under growth conditions using eight custom-built chambers, based on a modified design of ref. 28. Three replicate plants per CO_2 treatment were used, and the remaining two chambers used for controls, one from each CO_2 treatment, consisting of a pot with the same soil mixture as those with plants. Hourly measurements were made for each species over the course of a 24-h period, and repeated ten times throughout the year. Daily carbon budgets were estimated from the resulting 400 measurements, and then integrated to produce annual budgets (NPP) after taking into account the annual carbon loss by litter production. The carbon contribution of litter was estimated in sub-samples of leaf litter, using a stable-isotope ratio mass spectrometer (PDZ Europa 20–20, Cheshire).

CO_2 effects were analysed using a two-way ANOVA with replication ($CO_2 \times$ species); leaf habit effects were analysed using a two-way ANOVA with unequal but proportional subclass sizes ($CO_2 \times$ leaf habit) (ref. 29). None had significant interaction terms.

Scaling experimental results to mature stands

The following values for τ and measured LCD (mean ± s.e.) were used: for *N. cunninghamii* (at 400 p.p.m.v. CO_2: $\tau = 0.33$, LCD = 51.2 ± 2.6; at 800 p.p.m.v. CO_2: 0.33, 56.2 ± 7.1), for *S. sempervirens* (at 400 p.p.m.v. CO_2: 0.2, 44.0 ± 3.8; at 800 p.p.m.v. CO_2: 0.2, 70.1 ± 12.5), for *M. glyptostroboides* (at 400 p.p.m.v. CO_2: 1.0, 34.1 ± 2.6; at 800 p.p.m.v. CO_2: 1.0, 42.3 ± 3.0), for *T. distichum* (at 400 p.p.m.v. CO_2: 1.0, 53.0 ± 3.3; at 800 p.p.m.v. CO_2: 1.0, 60.4 ± 10.3) and for *G. biloba* (at 400 p.p.m.v. CO_2: 1.0, 38.9 ± 2.8; 800 p.p.m.v. CO_2: 1.0, 52.0 ± 1.7). See Fig. 2a for canopy respiration data (R). Measured rates of belowground respiration did not differ significantly between the two leaf habits during the polar winter ($F_{1,6} = 0.32$, $P = 0.59$), and so were not included in the scaling procedures.

Conifer forest modelling

The University of Sheffield Conifer Model (USCM) uses generalized relationships between leaf lifespan and function, and incorporates a full set of responses to atmospheric CO_2 and climate, as well as feedbacks with soil water and nutrient content[14]. USCM predictions of key properties and processes of conifer forests (NPP, LAI, evapotranspiration, nitrogen uptake and carbon partitioning) are validated well for forest sites across a wide climatic gradient using monthly climate data (temperature, precipitation and relative humidity), soil nutrient status and leaf lifespan information as inputs[14]. We forced the USCM with monthly climate data from the mid-Cretaceous climate simulation by the UK Universities Global Atmospheric Modelling Programme general circulation model[19]. Soil nutrient data for the Cretaceous period were derived using the Century soil biogeochemistry routines[30].

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Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium

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Tetrachloroethene (PCE) and trichloroethene (TCE) are ideal solvents for numerous applications, and their widespread use makes them prominent groundwater pollutants. Even more troubling, natural biotic and abiotic processes acting on these solvents lead to the accumulation of toxic intermediates (such as dichloroethenes) and carcinogenic intermediates (such as vinyl chloride)[1–4]. Vinyl chloride was found in at least 496 of the 1,430 National Priorities List sites identified by the US Environmental Protection Agency, and its precursors PCE and TCE are present in at least 771 and 852 of these sites, respectively[5]. Here we describe an unusual, strictly anaerobic bacterium that destroys dichloroethenes and vinyl chloride as part of its energy metabolism,