Ecophysiological responses of woody plants to past CO₂ concentrations

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Summary An approach is detailed for calculating historical rates of CO₂ uptake and water loss of leaves from measurements of leaf δ¹³C composition and climatic information. This approach was applied to investigate leaf gas exchange metabolism of woody taxa during the past 200 years of atmospheric CO₂ increase and in response to the longer-term atmospheric CO₂ increases plants experienced over the Pleistocene. Reconstructed net assimilation rates and water use efficiencies increased in response to increasing atmospheric CO₂ concentrations in both sets of material, whereas stomatal conductance, showing the combined responses of changes in stomatal density and leaf assimilation rates, was generally less responsive. Woody temperate taxa maintained a nearly constant c/cᵢ ratio in response to the increase in atmospheric CO₂ concentrations over both timescales, in part, as a result of changes in stomatal density. The reconstructed leaf-scale physiological responses to past global climatic and atmospheric change corroborated those anticipated from experimental work indicating the adequate capacity of experiments, at least at the scale of individual leaves, to predict plant responses to future environmental change.

Keywords: assimilation rate, climate change, CO₂ uptake, leaf δ¹³C composition, Pinus flexilis, Salix herbacea stomatal conductance, water use efficiency.

Introduction

The possible impact of predicted future atmospheric CO₂ increases on plants and ecosystems is being investigated by the coordinated development of global vegetation modeling and ecophysiological experimental research programs (Steffen et al. 1992). Equally important, however, is an understanding of how leaf photosynthesis and transpiration have responded to past CO₂ increases (Beerling and Woodward 1993). This objective is being pursued through paleoecophysiology. The paleoecophysiological approach investigates changes in leaf morphology, especially stomatal density (Woodward 1987, Beerling et al. 1993b, Van de Water et al. 1994), and water use efficiency, based on stable carbon isotope composition (δ¹³C) (Peñuelas and Azcón-Bieto 1992, Beerling et al. 1993a, Beerling and Woodward 1993, Woodward 1993, Ehleringer and Cerling 1995) from historical collections of leaves.

Paleoecophysiology can now be extended further to investigate leaf-scale fluxes of CO₂ and water vapor in the past. There is a sound theoretical basis for relating leaf gas exchange processes to leaf δ¹³C composition (Farquhar et al. 1982, Farquhar et al. 1989). After accounting for the isotopic fractions associated with diffusion and enzymatic reactions, leaf δ¹³C composition is determined principally by the ratio of intercellular (cᵢ) to ambient CO₂ concentrations (cᵅ), representing a time-integrated measure of CO₂ uptake and water loss (Ehleringer 1993). Changes in the concentration of atmospheric CO₂ and δ¹³C/δ¹²C ratio have been reported from ice core studies (Friedli et al. 1986, Neftel et al. 1988, Leuenberger et al. 1992), and the isotope fractionation associated with diffusion and enzymatic reactions has been determined experimentally (see Farquhar and Lloyd 1993). Consequently, it is now possible to extract values of cᵢ directly from historical sequences of leaves analyzed for δ¹³C. To reconstruct CO₂ uptake rates of leaves growing in the past requires δ¹³C-derived cᵢ values to be fed into a mechanistic biochemical model of photosynthesis (Farquhar et al. 1980) that can be used, in conjunction with appropriate climatic information, to drive an empirical model of leaf stomatal conductance to water vapor (Ball et al. 1987). This new approach differs from that of Beerling (1994) in that rates of CO₂ uptake and water loss from leaves are constrained by δ¹³C composition and are not entirely dependent on climate (see Schemes A and B, Figure 1). The central aim of the present work is to understand the effects of long-term changes in global climate and atmospheric composition, including ecosystems and the atmosphere.

The new approach has been applied to two historical sequences of leaves (Beerling et al. 1993a, 1993b, Van de Water et al. 1994). The first set is a collection of herbarium leaves of the woody dwarf shrub Salix herbacea L. (dwarf willow) that spans the past two centuries during which a rapid increase in atmospheric CO₂ concentration occurred (Friedli et al. 1986). The second set covers a longer time sequence, 30,000 years, and provides a history of leaf metabolism of Pinus flexilis James. (limber pine) during the Pleistocene (Van de Water et al. 1994). During this period, ice core studies have revealed major changes in the concentration of atmospheric CO₂ (Neftel et al. 1988, Leuenberger et al. 1992) with low glacial concentrations of about 200 ppm and higher concentrations of about 280 ppm in the early Holocene.
Materials and methods

Theory

Leaf stable carbon isotope composition, $\delta^{13}C_p$, is controlled by gas exchange according to (Farquhar et al. 1982):

$$\delta^{13}C_p = \delta^{13}c_a - a - (b - a)(c_d/c_a).$$  \hspace{1cm} (1)

where $a$ and $b$ are fractionation constants due to diffusion through the stomata (4.4) and net CO$_2$ fixation by Rubisco (29), respectively, $\delta^{13}c_a$ is the $^{13}$C/$^{12}$C ratio of atmospheric CO$_2$ concentration, $c_d$ is the ambient CO$_2$ partial pressure, and $c_a$ is the intercellular CO$_2$ partial pressure. When $\delta^{13}c_d$ and $c_a$ are both known, Equation 1 can be solved for $c_d$ as:

$$c_d = \frac{-(\delta^{13}c_p - \delta^{13}c_a + a)}{blc_a - alc_a} .$$  \hspace{1cm} (2)

The isotopically derived value of $c_d$ is next fed into the Farquhar et al. (1980) biochemical model of leaf photosynthesis. According to the model, leaf photosynthesis is described by:

$$A = \left(1 - \frac{0.5O}{\tau c_1}\right) \min(W_c, W_j) - R_d,$$  \hspace{1cm} (3)

where $A$ is net leaf photosynthetic rate (µmol CO$_2$ m$^{-2}$ s$^{-1}$), $c_1$ and $O$ are the partial pressures of CO$_2$ and O$_2$ in the intercellular leaf space, respectively, $W_c$ and $W_j$ are the rates of carboxylation-limited Rubisco (ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase) activity and RuBP regeneration by electron transport, respectively, $R_d$ is the rate of CO$_2$ release in the light resulting from processes other than photorespiration, and $\tau$ is the specificity of Rubisco.

Because it is uncertain whether Rubisco activity or electron transport is limiting photosynthesis when dealing with historical values of $\delta^{13}C$-$c_1$, two leaf photosynthetic rates are calculated, one limited by Rubisco activity and the other limited by RuBP regeneration through electron transport, and according to Equation 3, the minimum of the two is chosen.

Rubisco activity limits the carboxylation rate according to:

$$W_c = \frac{V_{max} c_1}{c_1 + K_c \left[1 + \frac{O}{K_o}\right]},$$  \hspace{1cm} (4)

where $V_{max}$ is the maximum rate of carboxylation, and $K_c$ and $K_o$ are the Michaelis-Menten constants for carboxylation and oxygenation, respectively.

The rate of carboxylation when limited solely by the rate of RuBP regeneration in the Calvin cycle, $W_j$, is mediated through the rate of electron transport ($J$):

$$W_j = \frac{J c_1}{4 \left[c_1 + \frac{O}{\tau}\right]},$$  \hspace{1cm} (5)

Light dependency of the model is achieved through its effects on electron transport according to:

$$J = \frac{\alpha I}{\Gamma},$$  \hspace{1cm} (6)

where $J_{max}$ is the maximum rate of electron transport, $\alpha$ is the efficiency of light conversion on an incident light basis (mol electrons per mol photons) (0.24), and $I$ is the photosynthetically active radiation (PAR) (µmol m$^{-2}$ s$^{-1}$). The temperature responses of $K_c$, $K_o$, $\tau$, $V_{max}$ and $J_{max}$ described by Harley et al. (1992) have been used.

Equations 1–6 provide a theoretical framework for estimating leaf net assimilation rates from $\delta^{13}C$ composition. From the CO$_2$ uptake rate (A, µmol m$^{-2}$ s$^{-1}$), the response of stomatal conductance is predicted, after modification by environmental conditions (Ball et al. 1987, Leuning 1990, Aphalo and Jarvis 1993), as follows:

$$g_s = g_o + \left(\frac{g_o A H}{c_d - 1}\right),$$  \hspace{1cm} (7)

where $g_s$ is the stomatal conductance to water vapor (mmol m$^{-2}$ s$^{-1}$), $g_o$ is the stomatal conductance when $A$ is zero at the light compensation point, $g_1$ is an empirical sensitivity coefficient, $H$ is the relative humidity (%) of the air surrounding the leaf, $c_d$ is the atmospheric CO$_2$ concentration (Pa), and $H$ is the CO$_2$ compensation point (Pa). Both $g_o$ and $g_1$ have the temperature responses and constraints reported by Woodward et al. (1995), and $H$ has a temperature response general to plants with a C$_3$ photosynthetic pathway (Leuning 1990). The term $(c_d - 1)$, in the denominator of Equation 7, improves the responses of stomatal conductance to low CO$_2$ concentrations (Leuning 1990), appropriate for simulations under low glacial CO$_2$ concentrations; however, there remain problems with some of the responses of Equation 7, particularly to relative humidity (Aphalo and Jarvis 1993). Note that a known value of $c_d$ permits the calculation of the gas exchange rates of the plants directly rather than requiring the iterative procedure adopted when the value of $c_d$ is unknown (Scheme A versus Scheme B, Figure 1).

Historical collections of leaf material

Two sets of species-specific material were used to reconstruct the historical gas exchange responses of plants to past CO$_2$ variations. The first set, of the woody dwarf shrub S. herbacea, was collected from similar altitudes in southern Sweden over the past 200 years and stored in the herbarium of the Swedish Natural History Museum, Stockholm. Leaf $\delta^{13}C$ values and stomatal density have been reported by Beerling et al. (1993a, 1993b, respectively). Leaves were sampled from flowering
shoots on the assumption that they had developed in saturating irradiance, consequently the PAR value \( I \) (Equation 6) was set at 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The maximum rates of Rubisco activity, \( V_{\text{max}} \) (Equation 4), and electron transport, \( J_{\text{max}} \) (Equation 5), for S. herbacea were assigned after Beerling and Quick (1995) for tundra vegetation. Records of atmospheric \( \text{CO}_2 \) and \( \delta^{13}c_a \) were taken from ice core studies (Friedli et al. 1986) and from the continuous monitoring record at Mauna Loa, Hawaii (Keeling and Whorf 1994). Climate inputs (temperature and humidity) used in the model (Figure 1B) were the 30-year averages reported for the site nearest the collection locality (Müller 1982).

The continuous Pleistocene sequence of leaf \( \delta^{13}C \) composition for the conifer P. flexilis reported by Van de Water et al. (1994) provides the second set of material investigated for gas exchange reconstructions. Assigned values of \( V_{\text{max}} \) and \( J_{\text{max}} \) were typical of cool/cold conifer forests (Beerling and Quick 1995). Because the irradiance each needle was exposed to over its lifetime is unknown and dependent on its position within the canopy, the value of \( I \) (Equation 6) was set at 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Site selection was made by Van de Water et al. (1994) to minimize changes in vapor pressure deficit, temperature and water availability over the past 30,000 years, and to focus attention only on the effects of changing \( \text{CO}_2 \) concentrations. This was achieved by developing the Holocene section of the 30,000-year chronology from needles recovered in middens at sites in southern Idaho, near the lower limits of P. flexilis. The modern floras and temperatures of these sites closely approximate the glacial floras and temperatures of the more southerly sites, as determined from packrat midden studies (Betancourt et al. 1990). This rationale parallels that frequently used in palaeoecology where the modern climate of one site approximates the palaeoclimate of the glacial sites. On this basis, Van de Water et al. (1994) considered that variations in precipitation and average annual temperatures were minimized. Estimates of present-day temperatures and humidity needed for the model were based on the present climate of the region (Müller 1982), and I used the ice core records of \( \text{CO}_2 \) and \( \delta^{13}c_a \) described by Beerling (1994).

**Results**

Leaf-scale photosynthetic rates, reconstructed from \( \delta^{13}C \) measurements, for the dwarf shrub S. herbacea showed a gradual increase over the past two centuries (Figure 2A), whereas stomatal conductance declined over this time. Overall, the changes in \( \text{CO}_2 \) and water vapor fluxes led to a steady increase in water use efficiency (Figure 2C).

Solving Equation 2 with the detailed Siple Station ice core record (Friedli et al. 1986) showed that the increase in photosynthetic rates of S. herbacea leaves was brought about by an increase in the average concentration of \( \text{CO}_2 \) inside the leaf \( (c_i) \) (Figure 3A). The increase in \( c_i \) was proportional to the atmospheric \( \text{CO}_2 \) increases recorded at Mauna Loa, such that the \( c_i/c_a \) ratio increased only slightly (Figure 3B). Longer-term historical gas exchange reconstructions based on fossil \( \delta^{13}C \) measurements and Equations 1–7 indicated increasing photosynthetic rates of needles of P. flexilis over the past 30,000 years (Figure 4A). Fluxes of water vapor showed a more ambiguous trend and large variations particularly between 15,000–20,000 years BP (Figure 4B), a time when the ice cores record the major \( \text{CO}_2 \) increase from glacial to interglacial concentrations (Leuenberger et al. 1992). As with S. herbacea, the responses of photosynthesis and stomatal conductance at the leaf-scale led to a steady and marked increase in water use efficiency (Figure 4C), driven primarily by the intercellular \( \text{CO}_2 \) concentration of the leaf tracking atmospheric concentrations (Figure 5A) in a nearly proportional manner (Figure 5B).

**Discussion**

Experiments in which woody plants and \( C_3 \) annuals were grown in the pre-industrial \( \text{CO}_2 \) concentration (280 ppm) demonstrated lower leaf photosynthetic rates and higher leaf stomatal conductances (Woodward 1987, 1993, Overdieck 1989,
Polley et al. 1992a, 1992b, Tissue et al. 1995) compared with plants growing at the present CO₂ concentration of 350 ppm. The historical reconstructions of leaf gas exchange responses for the woody shrub *S. herbacea* are in accordance with these experimental data, i.e., they showed an overall increase in leaf photosynthetic rates of 23% (Figure 2A), a decrease in stomatal conductance of about 6%, and a steady increase in leaf water use efficiency over the past two centuries (Figure 2C). Whether this photosynthetic stimulation will be sustained by *S. herbacea* as the CO₂ concentration continues to rise appears to depend on an increase in air temperatures enhancing nutrient supply rates from the soil (Oechel et al. 1994).

Ehleringer and Cerling (1995) suggested that the $c_i/c_a$ ratio has remained nearly constant with increasing CO₂ concentration over the past 200 years, because $c_i$ tracks atmospheric concentrations. The 200-year time series of δ¹³C measurements of *S. herbacea* support this idea (Figure 3A), although a small increase in the value of $c_i/c_a$ from 0.59 to 0.65 was evident (Figure 3B). This contrasts with the pattern obtained from an analysis of a 100-year sequence of *Prosopis alba* leaves collected from the Atacama desert in northern Chile (Ehleringer et al. 1992, Ehleringer and Cerling 1995). In this system, leaves growing in an increasing atmospheric CO₂ concentration over the 100-year period had a constant $c_i/c_a$ ratio but a decreasing $c_i$ value, suggesting constant photosynthetic rates but an increasing water use efficiency (Ehleringer and Cerling 1995). These contrasting sets of results may reflect the different climates in which the plants grew (boreal versus desert), or differences in the sensitivity of stomatal conductance and stomatal density to CO₂ increases, or both.

One possible mechanism operating to maintain a constant $c_i/c_a$ ratio in leaves in response to increasing CO₂ concentrations is a decrease in stomatal conductance (Ehleringer and Cerling 1995). In the case of *S. herbacea*, the small increase in $c_i/c_a$ (Figure 3) can be considered against a 60% decrease in leaf stomatal density over the past two centuries (Beerling et al. 1993b). Woodward (1987) reported that the stomatal density of temperate arboreal species decreased by 40% in response to CO₂ increases over the same time leading to the expectation that these temperate species would show a rising value of $c_i$ and
a constant $c_i/c_a$ ratio over this time. Extracting values of $c_i$ (Equation 2) and $c_i/c_a$ from reported $\delta^{13}C$ measurements of the same material showed a rising value of $c_i$ and a constant $c_i/c_a$ ratio over time (Figures 6A and 6B). However, despite these two sets of supporting evidence, it is clear that a decrease in stomatal density is not a prerequisite for regulating a nearly constant $c_i/c_a$ ratio. Malone et al. (1993) reported no significant decrease in stomatal density of C$_3$ crop plants over a CO$_2$ concentration gradient of 200–350 ppm, yet the isotopically derived gas exchange responses revealed a near-constant $c_i/c_a$ ratio (Polley et al. 1993a, 1993b, Tissue et al. 1995, Dippery et al. 1995), reconstructed photosynthetic rates of P. flexilis needles were lower during glacial times relative to the present day (Figure 5A). Similar observations have been reported for ponderosa pine growing across an altitudinal gradient (Callaway et al. 1994), indicating that P. flexilis may have responded similarly during the last glaciation. However, leaf stomatal conductance to water vapor showed no major decrease in response to the CO$_2$ increase from 180 to 290 ppm (Figure 5B), in agreement with a previous modeling study (Beerling 1994), as a result of the combined effect of changes in stomatal density and photosynthetic rates over the past 30,000 years. The relative responses of photosynthesis, stomatal conductance and stomatal density all contribute to a linear increase in water use efficiency with increasing CO$_2$ concentration, as has been observed in experimental studies (Polley et al. 1993a, 1993b, Tissue et al. 1995). Values of $c_i$ gradually increased over the past 30,000 years so that $c_i/c_a$ was maintained at a near-constant value (Figure 6A and 6B). Accompanying this response, the stomatal density of P. flexilis needles from the same sites and of the same ages decreased (Van de Water et al. 1994). Collectively these data provide evidence that stomatal density has had a regulatory role in maintaining a near-constant $c_i/c_a$ ratio over a much longer timescale than previously reported (Ehleringer and Cer-
Global atmospheric CO$_2$ concentrations rose rapidly during the deglaciation and apparently induced oscillatory gas exchange behavior in *P. flexilis* needles (Figures 4 and 5). Stability seems to have been achieved when the atmospheric CO$_2$ concentration reached values of about 290 ppm in the early Holocene. Fluctuations in leaf gas exchange 15,000–20,000 years BP are not readily explained by an increased number of sampling localities at this time (see Table 1 in Van de Water et al. 1994), and the extent of the oscillations is greater than the background noise in the samples. Therefore, it is possible that either the metabolism of the *P. flexilis* needles took some time to achieve homeostatic adjustment to the increase in atmospheric CO$_2$ concentration, or that the effect was associated with the switch in the global climate system from a glacial to an interglacial state (Lautenschlager and Herterich 1990). If the latter explanation is true, then these low-frequency oscillations show the coupling of cool, dry conifer forests in the western USA with changing regional atmospheric circulation patterns, representing a previously unreported phenomenon of plant responses to environmental change during the deglaciation.

The historical gas exchange reconstructions described here are presented with two important caveats. First, because $c_i$ was derived from leaf $\delta^{13}$C composition, it represents an average value over the lifetime of the leaf (Ehleringer 1993). This leads, in turn, to the reconstruction of average photosynthetic rates, rather than maximum values under optimum conditions. Furthermore, the reconstructed absolute photosynthetic rates depend on the chosen values of $V_{max}$ and $J_{max}$, and both values can be influenced by CO$_2$ and temperature. Temperature dependence of the values was included in the model and predicted on the basis of the polynomial responses given by McMurtrie and Wang (1993), whereas the direct influence of CO$_2$ on $V_{max}$ and $J_{max}$ was not explicitly modeled. The values of $V_{max}$ and $J_{max}$ used in the calculations were determined at the present ambient CO$_2$ concentration of 350 ppm (Beerling and Quick 1995). Tissue et al. (1995) found that growth of the C$_3$ annual *Abutilon theophrasti* Medic. at low glacial CO$_2$ concentrations resulted in a measurable reduction in $V_{max}$ and $J_{max}$ relative to plants grown at the present ambient CO$_2$ concentration. However, studies on trees grown in elevated CO$_2$ concentrations generally show a decrease in $V_{max}$ and $J_{max}$, although a variety of responses have been reported (Gunderson and Wullschleger 1994). Further observations are needed to provide the detail required to model the influence of CO$_2$ on $V_{max}$ and $J_{max}$ accurately.

The second caveat is that experiments designed to simulate plant growth at low glacial CO$_2$ concentrations can never fully represent how plants grew under glacial conditions because modern genotypes are unlikely to be representative of those prevailing during the last, or previous, glaciations (Beerling and Woodward 1993). However, the approach outlined here offers a basis for reconstructing the gas exchange responses of plants utilizing the genotypes that existed at that time and allows comparison with the responses of modern genotypes in current experiments.

An approach has been presented for reconstructing past rates of leaf CO$_2$ uptake and water loss of leaves from leaf $\delta^{13}$C composition and climate. The approach has been applied to leaf collections of the dwarf shrub *S. herbacea* spanning the past 200 years of CO$_2$ increase and to Pleistocene sequences of fossilized needles of *P. flexilis*. Both taxa showed historical gas exchange responses to CO$_2$ increases similar to those anticipated on the basis of experimental results. The maintenance of a near-constant $c_i/c_a$ ratio in response to increasing CO$_2$ concentrations on both time scales was achieved, at least in part, by a decrease in stomatal density. I conclude that leaf-level plant responses to future global change can be reasonably predicted in an experimental context.

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References


