

Atmospheric CO₂ and the ratio of intercellular to ambient CO₂ concentrations in plants

JAMES R. EHLERINGER¹ and THURE E. CERLING²

¹ Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA

² Department of Geology, University of Utah, Salt Lake City, Utah 84112, USA

Received October 8, 1993

Summary Much attention is focused today on predicting how plants will respond to anticipated changes in atmospheric composition and climate, and in particular to increases in CO₂ concentration. Here we review the long-term global fluctuations in atmospheric CO₂ concentration as a framework for understanding how current trends in atmospheric CO₂ concentration fit into a selective, evolutionary context. We then focus on an integrated approach for understanding how gas exchange metabolism responds to current environmental conditions, how it previously responded to glacial–interglacial conditions, and how it may respond to future changes in atmospheric CO₂ concentration.

Keywords: carbon dioxide, carbon isotope ratio, global change, intercellular carbon dioxide.

Gases released by volcanoes, including H₂S, NH₃, CH₄ and H₂O, made up much of the highly reducing early atmosphere of earth. An important mechanism for oxidizing the earth's primitive atmosphere was the leakage of molecular hydrogen (produced by UV radiation of water) to space from the stratosphere. In this process, reducing molecules, such as methane, became oxidized to form an early CO₂-rich atmosphere. Over time, the concentration of carbon dioxide decreased as it was consumed in the weathering of silicates by reactions such as: $2\text{NaAlSi}_3\text{O}_8 + 11\text{H}_2\text{O} + 2\text{CO}_2 = \text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 + 2\text{Na}^+ + 2\text{HCO}_3^- + 4\text{H}_4\text{SiO}_4$, which produce bicarbonate and cations that are eventually removed from solution by carbonate precipitation. In addition, photosynthesis removed CO₂ from the atmosphere. Burial and lithification of carbonates and organic matter to limestone, coal or keragen are the processes resulting in long-term removal of carbon from the atmosphere to the lithosphere. Although the detailed history of CO₂ is not well known for the whole of earth's history, some periods are reasonably well understood.

Detailed measurements of atmospheric CO₂ go back only a few decades. The best known is the time series started by Keeling (1986) in 1955 on Mauna Loa volcano, Hawaii, which is situated far from anthropogenic sources of CO₂ (Figure 1). This time series records a seasonal amplitude of about 10 ppmV, due to net photosynthesis in the northern hemisphere summer and net respiration in the northern hemisphere winter.

Other observations show that the seasonal effect is dampened in the southern hemisphere because it has a smaller land mass than the northern hemisphere. The Mauna Loa station recorded a CO₂ increase from 315 ppmV in 1955 to 360 ppmV in 1993.

The pre-Industrial Revolution record is clearly revealed by ice bubbles in the accumulating ice fields of Greenland and Antarctica. The data of Friedli et al. (1986) and Wahlen et al. (1991) show that atmospheric CO₂ concentration was about 270 ppmV before the Industrial Revolution (Figure 1). The value was constant for several hundred years, within the precision of the measurements at the time. Current research is underway in Greenland (Greenland Ice Sheet Project and GRIP) to see if small changes in atmospheric CO₂ concentration occurred during the Little Ice Age which extended from about 1450 to 1700 B.P.

Another important time is the glacial and interglacial record, which repeats on a time scale of about 110,000 years. Global climate was about 5 °C cooler in the last ice age which peaked about 18,000 years ago, when continental glaciers covered much of mid-continental Europe, North America, Asia and Antarctica, which is still covered with continental glaciers. Ice bubbles in the Vostok Antarctica core reveal that the CO₂ concentration in the atmosphere was reduced to about 165 ppmV during each of the two previous glacials (18,000 and 130,000 years ago), and that the previous interglacial period (130,000 to 110,000 years ago) had peak CO₂ concentrations of about 270 ppmV, which is similar to the pre-Industrial value (Figure 2). Ocean sediments show that the present glacial–interglacial oscillation is only one of many that have persisted for at least 2.5 million years.

Beyond the age of glacial ice (perhaps 2 million years for the oldest ice in Greenland or Antarctica), only indirect estimates are available for atmospheric CO₂ concentrations. Stable isotopes in soil carbonates (Cerling 1991, 1992) and marine biomarkers (Freeman and Hayes 1992), and stomatal indices (van der Burgh et al. 1993) have been proposed as indicators of atmospheric CO₂ concentration. Figure 3 shows paleo-CO₂ estimates for the last 550 million years, based on the modeling of Berner (1991) and estimates derived from the isotopic shift in soil carbonates resulting from atmospheric mixing, which has been shown to be a possible indicator of atmospheric CO₂ concentration (Cerling 1984, 1991, 1992). The geological re-

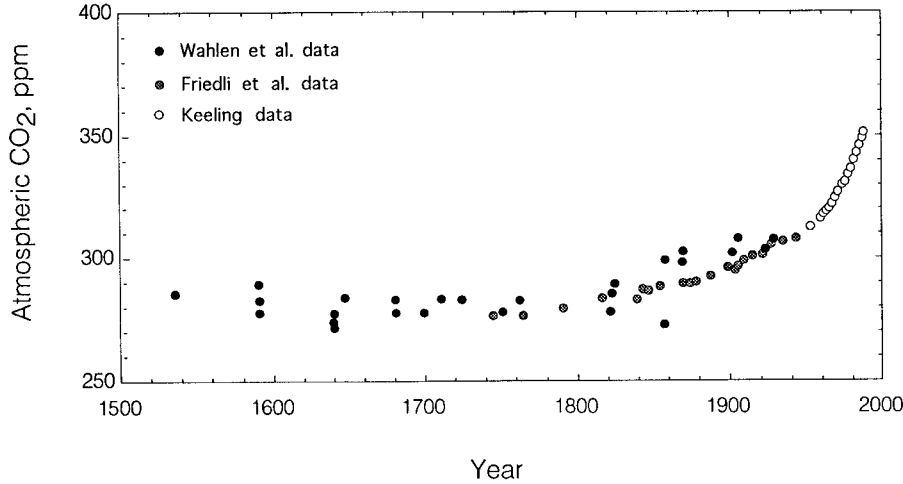


Figure 1. Recent history of the atmospheric CO₂ concentration. Open circles represent direct atmospheric measurements from Keeling (1986) at Mauna Loa in Hawaii. Closed circles represent measurements from ice core bubbles by Fiedli et al. (1985) and Wahlen et al. (1991).

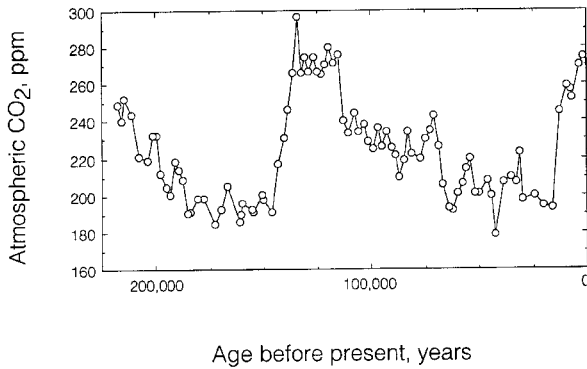


Figure 2. A history of the atmospheric CO₂ concentration during the past two major glacial periods. Data are from Barnola et al. (1987, 1991) and Jouzel et al. (1993).

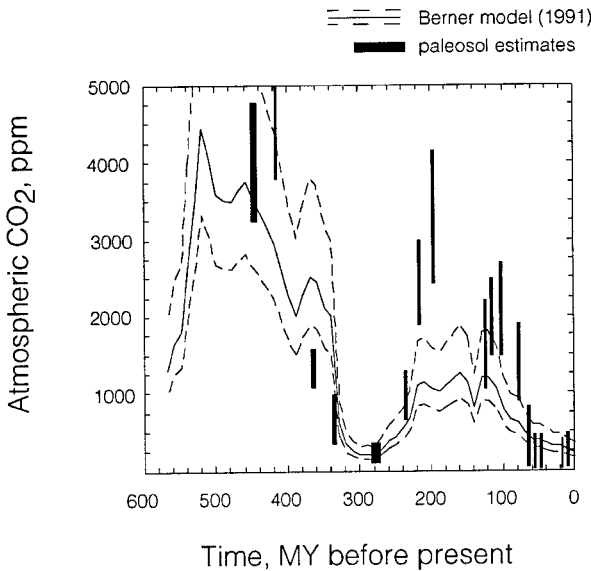


Figure 3. A history of atmospheric CO₂ concentration during the past 550 million years. Modeling data are from Berner (1991), and the paleosols estimates are from Cerling (1991, 1992, unpublished), Ferguson et al. (1991), Koch et al. (1992), Mora and Driese (1993), and Yapp and Poeths (1992).

cord of terrestrial plants includes several important events that are reflected in the CO₂ record. First, vascular plants initially appeared in the Silurian, approximately 350 million years ago. The dramatic decrease in atmospheric CO₂ at the end of the Mississippian, which seems to be documented by the paleosol record, resulted from the extensive expansion of swamp regions that were preserved as coals. Never since the Silurian period have such extensive coal deposits been formed. A later dramatic drop in atmospheric CO₂ seems to have occurred near the Cretaceous–Tertiary boundary. A rapid expansion of angiosperms occurred in the Tertiary, perhaps in response to decreased atmospheric CO₂ concentration. A third important change in the global ecosystem appears to have taken place between about 7 and 5 million years ago, when a rapid global expansion of plants with the C₄ photosynthetic pathway occurred. Ehleringer et al. (1991) and Cerling et al. (1993) suggest that this global expansion may indicate that atmospheric CO₂ concentration fell below a critical threshold, on the order of perhaps 400 to 500 ppmV, at which C₄ plants had a competitive advantage over C₃ plants in some environments. Low atmospheric CO₂ concentration in the late Tertiary and concentrations below 1000 ppmV for the last 65 million years are compatible with estimates of paleo-p(CO₂) from the marine record (Freeman and Hayes 1992) and from stomatal indices (van der Burgh et al. 1993). Therefore, it appears that the terrestrial atmosphere and biosphere have evolved together over the last 350 million years, each influencing the other. How have these changes influenced photosynthetic gas exchange?

Three gases are exchanged in substantial quantities between plants and the atmosphere: carbon dioxide, oxygen and water vapor. At the leaf level, we can consider two aspects of these gas exchange processes. First, what environmental and physiological factors result in changes in the absolute flux rates? Second, what factors set maximum potential flux rates? Changes in maximum photosynthesis (*A*) and transpiration (*E*) rates in response to abiotic parameters (including elevated CO₂) have been described in numerous studies (Sharkey 1985, Stitt 1991). Although absolute photosynthesis and transpiration rates among species may exhibit substantial variation in response to environmental change, these flux rates typically

increase as resources increase. Changes in flux rates and canopy photosynthetic area almost always show a nearly linear response to resource availability over the natural range of variation (e.g., water availability), and species vary widely in their capacity to maintain photosynthetic activity under different environmental stresses. Canopy photosynthetic area also varies in response to resource availability, subject to constraints in other components associated with growth. Because productivity depends on both photosynthetic area and photosynthetic rate, instantaneous measures of gas exchange activity at a single point in time may provide only limited insight into primary productivity and ultimate plant fitness in response to climatic change, even though the parameters are linked with each other (Figure 4).

An alternative to examining potential changes in absolute flux rates and their impact on gas exchange performance under elevated CO₂ is to examine how the integration and coordination of gas exchange activities respond to environmental change. Such an integration would include all structural and biochemical components affecting water flux and carbon reduction within the plant. Ehleringer (1993a, 1993b) proposed use of the ratio of intercellular to ambient CO₂ concentrations (c_i/c_a) as a measure of a control point or set point in gas exchange metabolism for studying how plants respond to environmental change. In this way, the c_i/c_a ratio represents a set point for the integration and coordination of gas exchange activities. Such set points may be more stable to an environmental change than absolute flux rates, thereby providing some insight into coordinated changes in different components that must occur in response to the environmental perturbation. That is, whereas flux rates will vary greatly in response to resource availabilities in the short-term or to stress levels in the long-term, changes in the integration (i.e., set point) may vary substantially less and the rankings of differences among genotypes might not vary at all. One set point illustrated in Figure 4 is the ratio of intercellular to ambient CO₂ concentration (c_i/c_a), which represents a balance between the rates of inward CO₂ diffusion (controlled by stomatal conductance) and CO₂ as-

similation (controlled by photosynthetic light/dark reactions). In principle, there are no restricted relationships between flux rate and set point. At a particular environmental condition, we can consider c_i as a set point; however, over the range of possible atmospheric CO₂ conditions it may be more appropriate to consider the c_i/c_a ratio.

Over extended time periods, the c_i/c_a ratio can be estimated by measuring the carbon isotope composition of plant material (Farquhar et al. 1989). Carbon isotope discrimination (Δ) in C₃ plants is related to photosynthetic gas exchange; because Δ is in part determined by c_i/c_a (Farquhar et al. 1982, Farquhar and Richards 1984, Farquhar et al. 1989). The c_i/c_a ratio differs among plants because of variation in stomatal conductance or because of variation in the chloroplast demand for CO₂, or both. Discrimination in leaves of C₃ plants is related to c_i/c_a as

$$\Delta = a + (b - a) \frac{c_i}{c_a}, \quad (1)$$

where a is the fractionation occurring due to diffusion in air (4.4‰), and b is the net fractionation caused by carboxylation (mainly discrimination by RuBP carboxylase, approximately 27‰). The result of these constant fractionation processes during photosynthesis is that the leaf carbon isotope composition represents the assimilation-weighted intercellular CO₂ concentration during the lifetime of the tissue (Farquhar et al. 1989, Ehleringer et al. 1993). Although leaf carbon isotope composition has been associated with estimates of water use efficiency (ratio of photosynthesis to transpiration) in C₃ plants, it is a mistake to associate the Δ value only with water use efficiency, because other water relations parameters are also directly related to the c_i value (Cowan and Farquhar 1977, Farquhar and Sharkey 1982, Jones 1985, Ehleringer et al. 1993). In this sense, the c_i/c_a value represents a holistic, integrated measure of the multitude of factors that influence both CO₂ uptake and water loss in plants. Martin et al. (1989) observed that three restriction-fragment-length polymorphism markers accounted for over 80% of the carbon isotope variation in tomatoes. More recently, Masle et al. (1993) have observed similar patterns with *Arabidopsis thaliana*. Leaf Δ exhibits strong correlations with gas exchange parameters such as leaf conductance (Condon et al. 1987), with growth parameters such as the leaf/root ratio (Virgona et al. 1990), and with hydraulic features of the xylem (Meinzer et al. 1993), implying that certain character combinations and Δ values are associated with each other.

Substantial variation in leaf Δ values occurs both among individuals within a population and between different species. Much of this variation has a genetic basis, with patterns or ranking differences among genotypes maintained through time (Ehleringer et al. 1993). Overall, there is a tendency for higher Δ values to be associated with shorter-lived species within a community (Ehleringer and Cooper 1988, Smedley et al. 1991) and with more rapidly maturing genotypes at the species level (Ehleringer et al. 1990, Richards and Condon 1993, White 1993). Schuster et al. (1992) showed that, in arid land species, the intrapopulation variation in carbon isotope discrimination

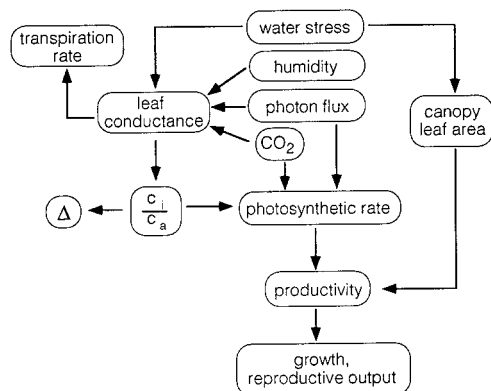


Figure 4. A conceptual model of leaf-level gas exchange and environmental influences, illustrating how intercellular CO₂ concentration reflects a balance between leaf conductance (determining supply rate of CO₂) and photosynthetic dark reactions (determining consumption rate of CO₂).

was broader in shorter-lived species than in longer-lived species. However, all of these studies have been carried out on plants growing at present-day CO₂ concentrations.

Differences in leaf carbon isotope discrimination often directly or indirectly reflect environmental influences on stomatal behavior (Comstock and Ehleringer 1992). These could arise in several ways, including stomatal sensitivity to humidity (Comstock and Ehleringer 1993), water stress effects (Guy et al., 1986), hydraulic constraints (Meinzer et al. 1993), and combinations thereof. The characteristic differences in carbon isotope composition that have been observed for herbaceous vegetation also appear to occur in tree species. There was approximately a 1.5‰ difference in mean leaf Δ values between Utah juniper (*Juniperus osteosperma*) and ponderosa pine (*Pinus ponderosa*) (Figure 5). The lower leaf Δ values in Utah juniper than in ponderosa pine indicate a lower overall intercellular CO₂ concentration, which is not surprising because the species occurs on much drier sites than ponderosa pine. Both tree species conform to the general pattern of carbon isotope variation within a population (Figure 5). A 1‰ shift in carbon isotope discrimination is equal to approximately a 15 ppmV shift in the intercellular CO₂ concentration. Because the c_i value is directly related to several aspects of gas exchange, this implies that different trees within a population will vary significantly in their photosynthesis and water relations characteristics. Although small differences in the instantaneous photosynthetic or transpiration rates on the order of 3–5% are often difficult to resolve among adjacent trees because of current technological limitations, such differences are likely to be resolved by means of carbon isotope discrimination, because it provides an integrated long-term measure of c_i . It is both the constancy of the rankings of leaf Δ values among individuals within a population and the linkages with life history (e.g., development rates, flowering, life expectancy) that favor Δ as a candidate for a set point that might reveal integrated aspects of metabolism.

If Δ values do show a constancy in response to current environmental stresses, how do leaf Δ values respond to global changes in atmospheric CO₂? If there is a pattern between

atmospheric CO₂ and Δ , what is the basis of the response? The answers to these questions are relevant to understanding the nature of integrated plant response to climate change. Existing literature provides some insight into the anticipated responses by different plants. For instance, it is known that stomata respond to CO₂, and variation in that response should relate to the potential constancy of a set point such as c_i/c_a . Perhaps the most relevant research is that of Polley et al. (1992, 1993), who investigated the responses of plants to changes in atmospheric CO₂ concentration over the range that the earth experienced during the glacial–interglacial cycles of the past several million years (see Figures 2 and 3), and of Woodward (1987, 1993) and Woodward and Bazzaz (1988), who investigated patterns between stomatal characteristics and atmospheric CO₂ concentration.

Under reduced atmospheric CO₂ concentrations (150–300 ppm CO₂), photosynthetic rates of C₃ plants, such as *Avena sativa* (oats) and *Brassica kaber* (mustard), are greatly reduced (Polley et al. 1992); photosynthetic rate is linearly related to ambient CO₂ concentrations. Over this range there is apparently little biochemical acclimation to a change in CO₂ concentration, and as a consequence, photosynthetic dependence on c_i remains constant (Polley et al. 1992). Although photosynthetic flux rates, growth rates and water use efficiencies changed in response to changes in atmospheric CO₂, the c_i/c_a value remained constant for mustard and oats (Figure 6). This could be viewed as supporting the notion of a “constant” metabolic set point in response to a global CO₂ change; however, the data available for *Triticum aestivum* (wheat) suggest that some caution is necessary (Figure 6). Over the range of 200–300 ppm atmospheric CO₂, the c_i/c_a value of wheat cultivars did not remain absolutely constant, but increased slightly from 0.65 to 0.68 in cv. Seri M82 and from 0.62 to 0.65 in cv. Yaqui. These changes in c_i/c_a values are small and imply a strong coordination of gas exchange activities over the atmospheric CO₂ ranges experienced by plants in the previous 250,000 years, but nonetheless the c_i/c_a values did not remain constant. In another recent study where herbaria samples were used to obtain materials grown under different subambient

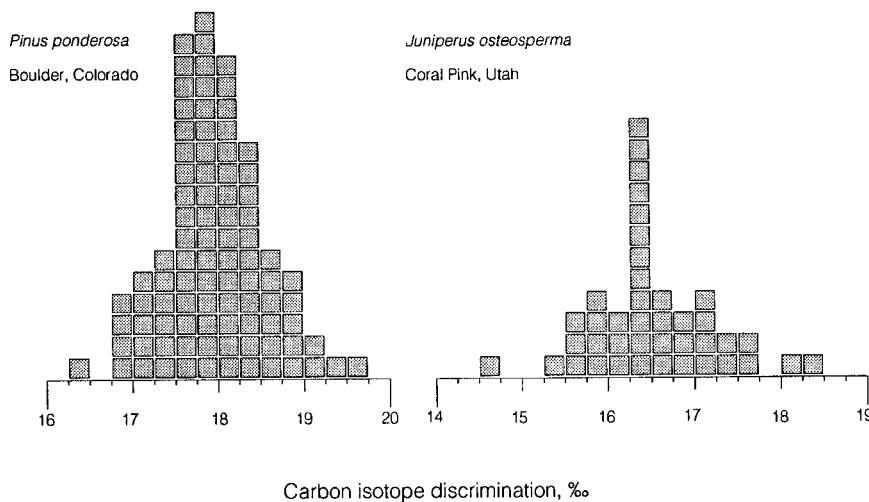


Figure 5. Population-level variation in the carbon isotope discrimination values of Utah juniper and ponderosa pine trees. The Utah juniper (*Juniperus osteosperma*) trees were from a mature stand immediately north of Coral Pink State Park in southern Utah, USA. The ponderosa pine (*Pinus ponderosa*) trees were from a mature stand immediately west of Boulder, Colorado, USA. All points represent bulked sun leaves from an individual tree; samples were analyzed on a delta S isotope ratio mass spectrometer at SIRFER.

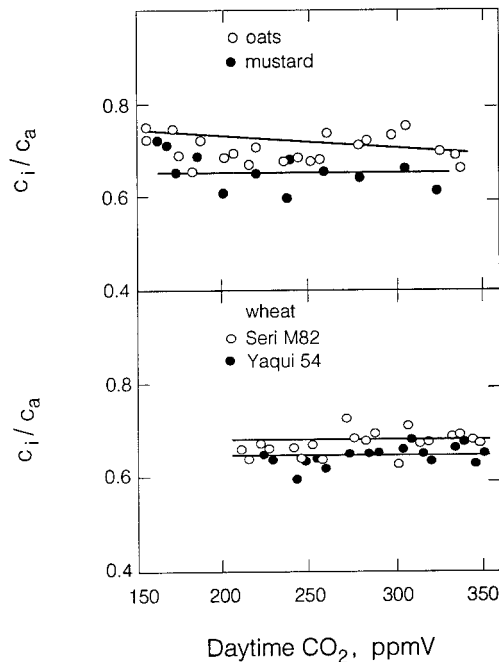


Figure 6. The ratio of intercellular to ambient CO₂ concentrations for plants grown under subambient CO₂ concentrations similar to that experienced in glacial and interglacial periods. From Polley et al. (1993).

CO₂ concentrations, Piñuelas and Azcón-Bieto (1992) observed that leaf Δ values remained constant over the atmospheric CO₂ range of 280–310 ppm.

One mechanism for regulating or maintaining a constant or near-constant c_i/c_a value as atmospheric CO₂ concentrations increase is to decrease the stomatal density. Woodward (1987) provided evidence of a relationship between stomatal density in tree species and time in herbaria samples, with the more recently collected vouchers (exposed to elevated CO₂ concentrations) having a reduced stomatal density. Since that initial observation, numerous additional studies have confirmed that many species exhibit reduced stomatal density in response to elevated CO₂ concentrations (Woodward and Bazzaz 1988, Piñuelas and Matamala 1990, Woodward 1993, Beerling and Chaloner 1992, 1993); however, this pattern is not universal for all plants (see reviews by Beerling and Chaloner 1992 and Woodward 1993).

Predicting responses to CO₂ concentrations above 290 ppm is more difficult, because plants have not been exposed to such elevated CO₂ values for possibly 7 million years or more (recall Figure 3). If there were a set point constancy, one would predict that the c_i/c_a value should remain reasonably constant, resulting in an increasing c_i value under elevated atmospheric CO₂. One possible response might then be a down regulation of photosynthetic capacity, but this is by no means a unique response.

Detailed c_i/c_a records of long-term responses by plants in elevated CO₂ conditions are limited. One such record can be extracted from *Prosopis alba* in the Atacama Desert of north-

ern Chile (Ehleringer et al. 1992). This tree grows in the rainless region of the Atacama, relying on groundwater located near the surface. Temperatures, insolation and groundwater levels have been near constant in this arid region, with atmospheric CO₂ increases being one of the primary environmental factors changing during the past century. Because litter does not decompose in this rainless region, litter profiles provide an historical record of “leaf physiology.” During the past 100 years, the c_i/c_a value has been decreasing (Figure 7). That is, as atmospheric CO₂ concentrations have increased, intercellular CO₂ concentrations have remained effectively constant. Leaf nitrogen values have also remained constant over this time period (Ehleringer et al. 1992). When combined with a constant c_i value, this implies that *Prosopis* may have maintained a constant photosynthetic rate and an increasing water use efficiency over this interval. These data suggest a response different from that observed by Polley et al. (1993). It may be that the two data sets reflect inherent differences in the responses of trees versus herbs. Naturally occurring CO₂ springs (Miglietta et al. 1993) may be additional systems for evaluating the extent to which the c_i/c_a or the c_i value remain constant as atmospheric CO₂ concentrations increase, because these sites contain both herbaceous and woody vegetation.

In a broader perspective, these data sets might be interpreted as indicating close regulation of the c_i/c_a value by plants experiencing the range of conditions over which recent evolutionary change has taken place (last several million years), and possibly less control over the c_i/c_a set point for plants now

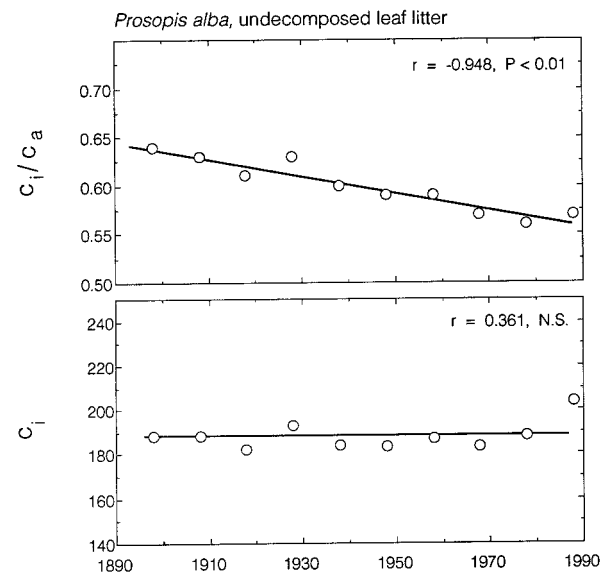


Figure 7. Calculated intercellular CO₂ concentrations and the ratio of intercellular to ambient CO₂ concentrations for leaves of *Prosopis alba* in the northern Atacama Desert, Chile. Material is as described by Ehleringer et al. (1992). Atmospheric CO₂ concentrations and $\delta^{13}\text{C}$ values necessary to calculate the ratio of intercellular to ambient CO₂ concentrations were derived from Friedli et al. (1986) and Keeling (1986). All points represent bulked leaves from a specific litter depth; samples were analyzed on a delta S isotope ratio mass spectrometer at SIRFER.

exposed to conditions outside the range under which they had evolved (i.e., atmospheric CO₂ above 290 ppmV). Fossil leaf material may shed some light on the extent of c_i/c_a regulation under elevated CO₂ concentrations such as might have existed during the Cretaceous or even the coal-forming eras. Interestingly, the carbon isotope ratios of most fossil material are approximately -25 to -26‰ (Deines 1980), suggesting that the c_i/c_a values under ancient atmospheric conditions that exceeded 1000 ppmV were not all that different from the c_i/c_a values of present-day C₃ vegetation.

It is likely that the nature of the c_i/c_a response under elevated CO₂ concentrations will remain constant in some species and vary in others. Irrespective of whether the c_i/c_a value changes slightly or remains constant in response to future increases in atmospheric CO₂ concentration, an understanding of the c_i/c_a changes should provide insights into the integrated responses by plants and of the extent to which compensatory changes must occur in one component of the gas exchange metabolism as a direct consequence of changes in other components. Given the high resolving power of carbon isotope discrimination for detecting small differences between adjacent genotypes, such an approach may be useful for evaluating the differential responses of genotypes to changes in climatic conditions.

References

- Barnola, J.M., D. Raynaud, Y.S. Korotkevich and C. Lorius. 1987. Vostok ice core provides 160,000 year record of atmospheric CO₂. *Nature* 329:408-414.
- Barnola, J.M., P. Pimienta, D. Raynaud and Y.S. Korotkevich. 1991. CO₂-Climate relationships as deduced from the Vostok ice core: a re-examination based on new measurements and on a re-evaluation of the air dating. *Tellus* 43B:83-90.
- Beerling, D.J. and W.G. Chaloner. 1992. Stomatal density as an indicator of atmospheric CO₂ concentration. *The Holocene* 2:71-78.
- Beerling, D.J. and W.G. Chaloner. 1993. The impact of atmospheric CO₂ and temperature change on stomatal density: observations from *Quercus robur* Lammas leaves. *Ann. Bot.* 71:231-235.
- Berner, R.A. 1991. A model for atmospheric CO₂ over Phanerozoic time. *Am. J. Sci.* 291:339-376.
- Cerling, T.E. 1984. The stable isotopic composition of modern soil carbonate and its relationship to climate. *Earth Planet. Lett.* 71:229-240.
- Cerling, T.E. 1991. Carbon dioxide in the atmosphere: evidence from Cenozoic and Mesozoic paleosols. *Am. J. Sci.* 291:377-400.
- Cerling, T.E. 1992. Use of carbon isotopes in paleosols as an indicator of the p(CO₂) of the paleoatmosphere. *Global Biogeochem. Cycles* 6:307-314.
- Cerling, T.E., Y. Wang and J. Quade. 1993. Expansion of C₄ ecosystems as an indicator of global ecological change in the late Miocene. *Nature* 361:344-345.
- Comstock, J.P. and J.R. Ehleringer. 1992. Correlating genetic variation in carbon isotopic composition with complex climatic gradients. *Proc. Natl. Acad. Sci.* 89:7747-7751.
- Comstock, J.P. and J.R. Ehleringer. 1993. Stomatal response to humidity in common bean (*Phaseolus vulgaris*): implications for maximum transpiration rate, water-use efficiency, and productivity. *Aust. J. Plant Physiol.* 20:669-691.
- Condon, A.G., R.A. Richards and G.D. Farquhar. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. *Crop Sci.* 27:996-1001.
- Cowan, I.R. and G.D. Farquhar. 1977. Stomatal functions in relation to leaf metabolism and environment. *In* Integration of Activity in the Higher Plant. Symposium of the Society of Experimental Biology, Vol. 31, pp 471-505.
- Deines, P. 1980. The isotopic composition of reduced organic carbon. *In* Handbook of Environmental Geochemistry. Vol. 1. Eds. P. Fritz and J.C. Fontes. Elsevier Press, Amsterdam, pp 329-406.
- Ehleringer, J.R. 1993a. Carbon and water relations in desert plants: an isotopic perspective. *In* Stable Isotopes and Plant Carbon/Water Relations. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, pp 155-172.
- Ehleringer, J.R. 1993b. Gas exchange implication of isotopic variation in arid land plants. *In* Plant Responses to Water Deficit. Eds. H. Griffiths and J. Smith. Environmental Plant Biology Series, BIOS Scientific Publ., London, pp 265-284.
- Ehleringer, J.R. and T.A. Cooper. 1988. Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia* 76:562-566.
- Ehleringer, J.R., A.E. Hall and G.D. Farquhar. 1993. Stable isotopes and plant carbon/water relations. Academic Press, San Diego, 555 p.
- Ehleringer, J.R., R.F. Sage, L.B. Flanagan and R.W. Pearcy. 1991. Climate change and the evolution of photosynthesis. *Trends Ecol. Evol.* 6:95-99.
- Ehleringer, J.R., J.W. White, D.A. Johnson and M. Brick. 1990. Carbon isotope discrimination, photosynthetic gas exchange, and water-use efficiency in common bean and range grasses. *Acta Oecol.* 11:611-625.
- Ehleringer, J.R., H.A. Mooney, P.W. Rundel, R.D. Evans, B. Palma and J. Delatorre. 1992. Lack of nitrogen cycling in the Atacama Desert. *Nature* 359:316-318.
- Farquhar, G.D. and R.A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust. J. Plant Physiol.* 11:539-552.
- Farquhar, G.D. and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol.* 33:317-345.
- Farquhar, G.D., M.H. O'Leary and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* 9:121-137.
- Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Mol. Biol.* 40:503-537.
- Ferguson, K.M., T.H. Lehman and R.T. Gregory. 1991. C- and O-isotopes of pedogenic soil nodules from two sections spanning the K-T transition in west Texas. *Geol. Soc. Am. Abstr.* 23:A302.
- Freeman, K.H. and J.M. Hayes. 1992. Fractionation of carbon isotopes by phytoplankton and estimates of ancient CO₂ levels. *Global Biogeochem. Cycles* 6:185-198.
- Friedli, H., H. Löttscher, H. Oeschger, U. Siegenthaler and B. Stauffer. 1986. Ice core record of ¹³C/¹²C ratio of atmospheric CO₂ in the past two centuries. *Nature* 324:237-238.
- Guy, R.D., D.M. Reid and H.R. Krouse. 1986. Factors affecting the ¹³C/¹²C ratios of inland halophytes. Controlled studies on growth and isotopic composition of *Puccinellia nuttalliana*. *Can. J. Bot.* 64: 2693-2699.
- Jones, H.G. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant Cell Environ.* 8:95-104.
- Jouzel, J., N.I. Barkov, J.M. Barnola, M. Bender, J. Chappellaz, C. Genthon, V.M. Kotlyakov, V. Lipendov, C. Lorius, J.R. Petit, D. Raynaud, G. Raisbeck, C. Ritz, T. Sowers, M. Stievenard, F. Yiou and P. Yiou. 1993. Extending the Vostok ice-core record of paleoclimate to the penultimate glacial period. *Nature* 364:407-412.

- Keeling, C.D. 1986. Atmospheric CO₂ concentrations—Mauna Loa Observatory, Hawaii 1958–1986. NDP-001/R1. Carbon Dioxide Information Analysis Center, Oakridge National Laboratory, Oak Ridge, Tennessee, 128 p.
- Koch, P.L., J.C. Zachos and P.D. Gingerich. 1992. Correlations between isotope records in marine and continental carbon reservoirs near the Palaeocene/Eocene boundary. *Nature* 358:319–322.
- Martin, B., J. Nienhuis, G. King and A. Schaefer. 1989. Restriction fragment length polymorphisms associated with water-use efficiency in tomato. *Science* 243:1725–1728.
- Masle, J., J.S. Shin and G.D. Farquhar. 1993. Analysis of restriction fragment length polymorphisms associated with variation of carbon isotope discrimination among ecotypes of *Arabidopsis thaliana*. *In* Stable Isotopes and Plant Carbon/Water Relations. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, pp 371–386.
- Meinzer, F.C., G. Goldstein and D.A. Grantz. 1993. Carbon isotope discrimination and gas exchange in coffee during adjustment to different moisture regimes. *In* Stable Isotopes and Plant Carbon/Water Relations. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, pp 327–345.
- Miglietta, F.A., A. Rjaschi, I. Bettarini, R. Resti and F. Selvi. 1993. Natural CO₂ springs in Italy: a resource for examining long-term response of vegetation to rising atmospheric CO₂ concentrations. *Plant Cell Environ.* 16:873–878.
- Mora, C.I. and S.G. Driese. 1993. A steep, mid-to late Paleozoic decline in atmospheric CO₂: evidence from soil carbonate CO₂ paleobarometer. *Chem. Geol.* 107:217–219.
- Peñuelas, J. and R. Matamala. 1990. Changes in N and S leaf content, stomatal density and specific leaf area of 14 plant species during the last three centuries of CO₂ increase. *J. Exp. Bot.* 41:1119–1124.
- Peñuelas, J. and J. Azcón-Bieto. 1992. Changes in leaf $\Delta^{13}\text{C}$ of herbaceous plant species during the past 3 centuries of CO₂ increase. *Plant Cell Environ.* 15:485–489.
- Polley, H.W., H.B. Johnson and H.S. Mayeux. 1992. Growth and gas exchange of oats (*Avena sativa*) and wild mustard (*Brassica kaber*) at subambient CO₂ concentrations. *Int. J. Plant Sci.* 153:453–461.
- Polley, H.W., H.B. Johnson, B.D. Marino and H.S. Mayeux. 1993. Increase in C₃ plant water-use efficiency and biomass over Glacial to present CO₂ concentrations. *Nature* 361:61–64.
- Richards, R.A. and A.G. Condon. 1993. Challenges ahead in using carbon isotope discrimination in plant-breeding programs. *In* Stable Isotopes and Plant Carbon/Water Relations. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, pp 451–462.
- Schuster, W.S.F., D.R. Sandquist, S.L. Phillips and J.R. Ehleringer. 1992. Comparisons of carbon isotope discrimination in populations of arid land plant species differing in life span. *Oecologia* 91:332–337.
- Sharkey, T.D. 1985. Photosynthesis in intact leaves of C₃ plants: physics, physiology and rate limitations. *Bot. Rev.* 51:53–105.
- Smedley, M.P., T.E. Dawson, J.P. Comstock, L.A. Donovan, D.E. Sherrill, C.S. Cook and J.R. Ehleringer. 1991. Seasonal carbon isotopic discrimination in a grassland community. *Oecologia* 85:314–320.
- Stitt, M. 1991. Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant Cell Environ.* 14:741–762.
- van der Burgh, J., H. Vissher, D.L. Dilcher and W.M. Kürschner. 1993. Paleatmospheric signatures in Neogene fossil leaves. *Nature* 260:1788–1790.
- Virgona, J.M., K.T. Hubick, H.M. Rawson, G.D. Farquhar and R.W. Downes. 1990. Genotypic variation in transpiration efficiency, carbon isotope discrimination and carbon allocation during early growth in sunflower. *Aust. J. Plant Physiol.* 17:207–214.
- Wahlen, M., D. Allen, B. Deck and A. Herchenroder. 1991. Initial measurements of CO₂ concentrations (1530–1940 AD) in air occluded in the GISP 2 ice core from central Greenland. *Geophys. Res. Lett.* 18:1457–1460.
- White, J.W. 1993. Implications of carbon isotope discrimination studies for breeding common bean under water deficits. *In* Stable Isotopes and Plant Carbon/Water Relations. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, pp 387–398.
- Woodward, F.I. 1987. Stomatal numbers are sensitive to increases in CO₂ from pre-Industrial levels. *Nature* 327:617–618.
- Woodward, F.I. 1993. Plant responses to past CO₂ concentrations. *Vegetatio* 104:145–155.
- Woodward, F.I. and F.A. Bazzaz. 1988. The responses of stomatal density to CO₂ partial pressure. *J. Exp. Bot.* 39:1771–1781.
- Yapp, C.J. and H. Poths. 1992. Ancient atmospheric CO₂ pressures inferred from natural geothites. *Nature* 355:342–347.