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

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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:

\[ 2\text{NaAlSi}_3\text{O}_8 + 11\text{H}_2\text{O} + 2\text{CO}_2 = \text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 + 2\text{Na}^+ + 2\text{HCO}_3^- + 4\text{H}_2\text{SiO}_4^- \]

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-continent 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-
cord of terrestrial plants includes several important events that are reflected in the CO$_2$ record. First, vascular plants initially appeared in the Silurian, approximately 350 million years ago. The dramatic decrease in atmospheric CO$_2$ 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 CO$_2$ seems to have occurred near the Cretaceous--Tertiary boundary. A rapid expansion of angiosperms occurred in the Tertiary, perhaps in response to decreased atmospheric CO$_2$ 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 C$_4$ photosynthetic pathway occurred. Ehleringer et al. (1991) and Cerling et al. (1993) suggest that this global expansion may indicate that atmospheric CO$_2$ concentration fell below a critical threshold, on the order of perhaps 400 to 500 ppmV, at which C$_4$ plants had a competitive advantage over C$_3$ plants in some environments.

Low atmospheric CO$_2$ concentration in the late Tertiary and concentrations below 1000 ppmV for the last 65 million years are compatible with estimates of paleo-p(CO$_2$) 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 CO$_2$) 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 CO$_2$ 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 CO$_2$ 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 CO$_2$ concentration ($c_i/c_a$), which represents a balance between the rates of inward CO$_2$ diffusion (controlled by stomatal conductance) and CO$_2$ assimilation (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 CO$_2$ 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 ($\Delta$) in C$_3$ plants is related to photosynthetic gas exchange; because $\Delta$ 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 CO$_2$, or both. Discrimination in leaves of C$_3$ plants is related to $c_i/c_a$ as

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

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 CO$_2$ 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 C$_3$ plants, it is a mistake to associate the $\Delta$ 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 CO$_2$ 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 $\Delta$ 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 $\Delta$ values are associated with each other.

Substantial variation in leaf $\Delta$ 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 $\Delta$ 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 CO$_2$ concentration reflects a balance between leaf conductance (determining supply rate of CO$_2$) and photosynthetic dark reactions (determining consumption rate of CO$_2$).](image-url)
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 CO\textsubscript{2} 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 CO\textsubscript{2} 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 CO\textsubscript{2} concentration. Because the c\textsubscript{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\textsubscript{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 CO\textsubscript{2}? If there is a pattern between atmospheric CO\textsubscript{2} 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 CO\textsubscript{2}, and variation in that response should relate to the potential constancy of a set point such as c\textsubscript{i}/c\textsubscript{a}. Perhaps the most relevant research is that of Polley et al. (1992, 1993), who investigated the responses of plants to changes in atmospheric CO\textsubscript{2} 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 CO\textsubscript{2} concentration.

Under reduced atmospheric CO\textsubscript{2} concentrations (150–300 ppm CO\textsubscript{2}), photosynthetic rates of C\textsubscript{3} plants, such as Avena sativa (oats) and Brassica kaber (mustard), are greatly reduced (Polley et al. 1992); photosynthetic rate is linearly related to ambient CO\textsubscript{2} concentrations. Over this range there is apparently little biochemical acclimation to a change in CO\textsubscript{2} concentration, and as a consequence, photosynthetic dependence on c\textsubscript{i} remains constant (Polley et al. 1992). Although photosynthetic flux rates, growth rates and water use efficiencies changed in response to changes in atmospheric CO\textsubscript{2}, the c\textsubscript{i}/c\textsubscript{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 CO\textsubscript{2} 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 CO\textsubscript{2}, the c\textsubscript{i}/c\textsubscript{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\textsubscript{i}/c\textsubscript{a} values are small and imply a strong coordination of gas exchange activities over the atmospheric CO\textsubscript{2} ranges experienced by plants in the previous 250,000 years, but nonetheless the c\textsubscript{i}/c\textsubscript{a} values did not remain constant. In another recent study where herbaria samples were used to obtain materials grown under different subambient...
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, Peñuelas 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 northern 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...
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/έa 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/έa values under ancient atmospheric conditions that exceeded 1000 ppmV were not all that different from the c/έa values of present-day C₃ vegetation.

It is likely that the nature of the c/έa response under elevated CO₂ concentrations will remain constant in some species and vary in others. Irrespective of whether the c/έa value changes slightly or remains constant in response to future increases in atmospheric CO₂ concentration, an understanding of the c/έa changes should provide insights into the integrated responses of 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


