



Tansley review

Plant responses to low [CO₂] of the past

Author for correspondence:
Joy K. Ward
Tel: +1 785 8645218
Email: joyward@ku.edu

Laci M. Gerhart and Joy K. Ward

Department of Ecology & Evolutionary Biology, University of Kansas, Lawrence, KS 66045, USA

Received: 3 June 2010
Accepted: 5 July 2010

Contents

Summary	1	VII. Interactions of low [CO ₂] with other factors	14
I. Introduction	2	VIII. Low-[CO ₂] effects on community composition	16
II. The case for low-[CO ₂] studies	2	IX. Low-[CO ₂] effects on the ecosystem	16
III. Experimental approaches for reducing [CO ₂]	4	X. Low-[CO ₂] effects on early human societies	17
IV. Early low-[CO ₂] studies	6	XI. Conclusions	18
V. Low-[CO ₂] effects on the individual plant	7	Acknowledgements	19
VI. Low [CO ₂] and plant evolution	10	References	19

Summary

New Phytologist (2010)
doi: 10.1111/j.1469-8137.2010.03441.x

Key words: glacial plants, Last Glacial Maximum, low CO₂, photosynthesis, plant evolution, Pleistocene, selection experiments, subambient CO₂.

During the Last Glacial Maximum (LGM; 18 000–20 000 yr ago) and previous glacial periods, atmospheric [CO₂] dropped to 180–190 ppm, which is among the lowest concentrations that occurred during the evolution of land plants. Modern atmospheric CO₂ concentrations ([CO₂]) are more than twice those of the LGM and 45% higher than pre-industrial concentrations. Since CO₂ is the carbon source for photosynthesis, lower carbon availability during glacial periods likely had a major impact on plant productivity and evolution. From the studies highlighted here, it is clear that the influence of low [CO₂] transcends several scales, ranging from physiological effects on individual plants to changes in ecosystem functioning, and may have even influenced the development of early human cultures (via the timing of agriculture). Through low-[CO₂] studies, we have determined a baseline for plant response to minimal [CO₂] that occurred during the evolution of land plants. Moreover, an increased understanding of plant responses to low [CO₂] contributes to our knowledge of how natural global change factors in the past may continue to influence plant responses to future anthropogenic changes. Future work, however, should focus more on the evolutionary responses of plants to changing [CO₂] in order to account for the potentially large effects of genetic change.

I. Introduction

Rising atmospheric [CO₂] (CO₂ concentration) is expected to increase global temperatures and has been deemed a major threat to climate, economic development, and human health (IPCC, 2007a). It is perhaps ironic to be considering plant responses to low [CO₂] during an era in which most research has focused on rising [CO₂]. Here we will emphasize that a strong foundation in understanding plant responses to low [CO₂] is critical for predicting the implications of rising [CO₂], explaining plant evolutionary patterns over geologic time scales, and estimating past and future levels of net primary productivity (NPP). In addition, low-[CO₂] studies provide a baseline for defining plant response to minimum [CO₂] of the geologic past, such that changes in plant functioning in response to rising [CO₂] can be assessed within a broader temporal context.

Atmospheric CO₂ is the main source of carbon for photosynthesis and serves as a fundamental substrate for plant growth. CO₂ assimilation during photosynthesis represents a critical exchange of carbon between the atmosphere and the biosphere within the global carbon cycle (Schlesinger, 1997). CO₂ is unique in that plants assimilate this resource from the atmosphere, whereas other resources such as water and nutrients are acquired from the soil. Furthermore, CO₂ is evenly distributed throughout the Earth's atmosphere, and therefore CO₂ availability to plants is similar across all terrestrial ecosystems (although there can be local gradients with altitude and within canopies). This is unlike other plant resources such as light, water, and nutrients that vary across ecosystems, as well as at much smaller spatial scales. Although similar within a time period, [CO₂] has varied throughout geologic time, and during some periods may have been so low as to greatly limit plant growth and reproduction (Ward, 2005). Owing to the inability to substitute 'space for time', our knowledge of the evolutionary responses of plants to low [CO₂] is rather limited compared with our understanding of evolutionary responses to other resource limitations, although advances have been made in this area and are reviewed here.

Beginning with the rise of vascular land plants through modern times, atmospheric [CO₂] reached maximum values of 3300–3600 ppm during the early Devonian (Berner, 2006), possibly dropped as low as 150 ppm during the late Pliocene (Tripathi *et al.*, 2009), and consistently ranged between 170 and 190 ppm during glacial maxima of the past million yr (Petit *et al.*, 1999; EPICA, 2004; Brook, 2005; Tripathi *et al.*, 2009; Fig. 1). Since CO₂ is a major substrate for photosynthesis, such extreme changes in the availability of this resource likely had profound effects on plant productivity, community structure, and evolution through time. The LGM that occurred 18 000–20 000 yr ago represents a fascinating time when low [CO₂] likely constrained the physiological functioning of C₃ plants

(Polley *et al.*, 1993a; Dippery *et al.*, 1995; Sage & Coleman, 2001; Ward *et al.*, 2005; Lewis *et al.*, 2010). During that period, [CO₂] dropped to 180–190 ppm (Petit *et al.*, 1999; EPICA, 2004), which is among the lowest concentrations predicted to have occurred during the evolution of land plants (Berner, 2003, 2006; Tripathi *et al.*, 2009). Following the LGM, [CO₂] gradually increased to 270 ppm just before the Industrial Revolution, and has been rising rapidly in recent decades as a result of expanding industrialization (IPCC, 2007b). Currently, [CO₂] is 392 ppm (recorded at Mauna Loa Observatory, Hawaii; <http://www.CO2now.org>), a value that may not have occurred since the mid-Miocene (14–16 million yr ago; Tripathi *et al.*, 2009). Thus, modern [CO₂] values are more than twice the minimum concentrations that occurred during the LGM and *c.* 45% higher than pre-industrial values. [CO₂] is expected to continue rising in the future, potentially reaching 1000 ppm by 2100, depending on the carbon emissions scenario that actually occurs (IPCC, 2007b).

II. The case for low-[CO₂] studies

To date, most CO₂ studies have focused on elevated [CO₂] that is predicted to occur within the next 50–100 yr as a result of anthropogenic carbon emissions (McLeod & Long, 1999; Ainsworth & Long, 2005; Körner, 2006; Miyagi *et al.*, 2007; Springer & Ward, 2007; Lloyd & Farquhar, 2008). The motivation for many of these studies has been to determine the degree to which plants will serve as sinks to offset carbon emissions. Far fewer studies have focused on plant responses to low [CO₂] of the past, even though this work is crucial for understanding long-term responses of plants to changing [CO₂] over geologic and evolutionary time scales. From the studies that have been conducted, it is clear that modern C₃ plant genotypes grown at low [CO₂] (180–200 ppm) exhibit severe reductions in photosynthesis, survival, growth, and reproduction, suggesting that reduced [CO₂] during glacial periods may have induced carbon limitations that would have been highly stressful on C₃ plants (Polley *et al.*, 1993a; Dippery *et al.*, 1995; Sage, 1995; Tissue *et al.*, 1995; Sage & Coleman, 2001; Ward & Kelly, 2004; Tonsor & Scheiner, 2007; see Fig. 2 for a photo of plants from Dippery *et al.*, 1995). In addition, carbon limitations at low [CO₂] may have altered plant tolerance ranges to other stressors such as drought, heat, and herbivory (Sage & Cowling, 1999). Furthermore, most studies focusing on the full range of plant responses to past through future [CO₂] report much greater physiological and growth enhancements in response to increases in [CO₂] below modern concentrations than to increases above modern concentrations (Sage & Reid, 1992; Polley *et al.*, 1993b; Dippery *et al.*, 1995; Ward & Strain, 1997). Thus, plants may have already exhausted much of their

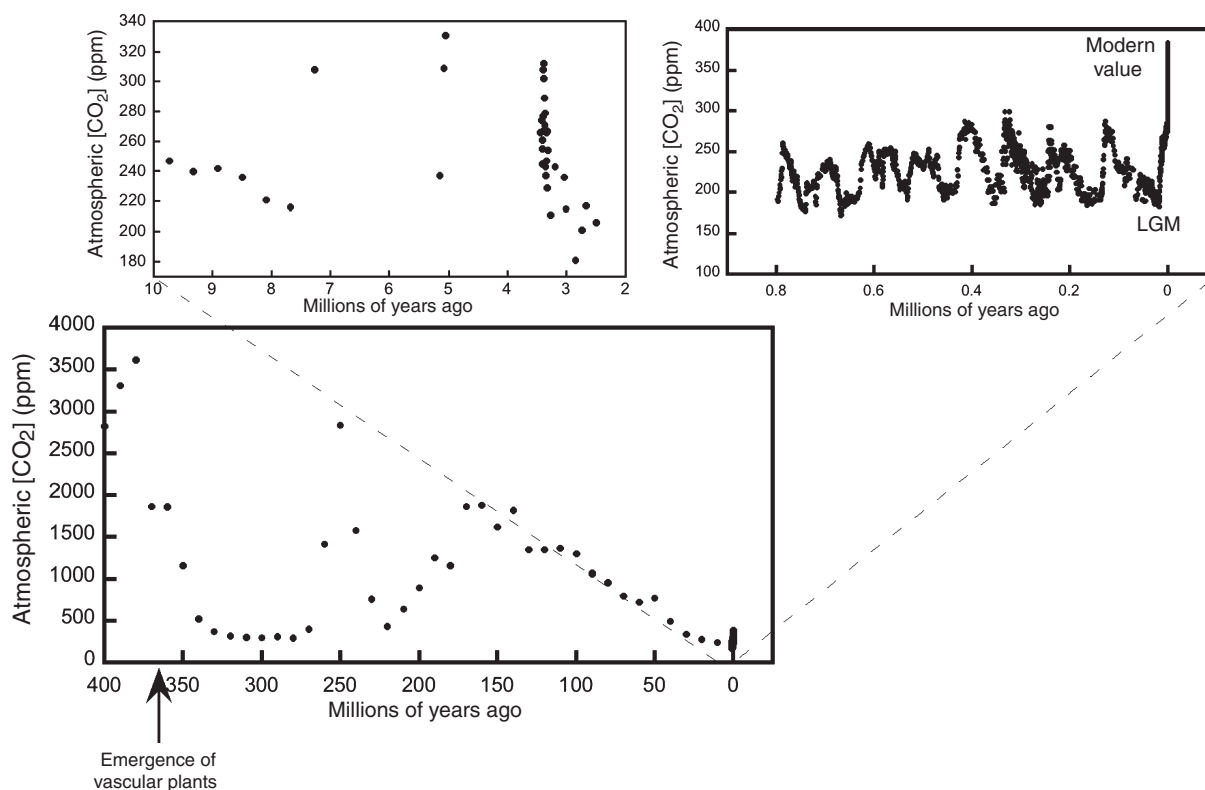


Fig. 1 Changes in atmospheric $[\text{CO}_2]$ throughout the evolution of vascular land plants. The upper right insert shows the past million yr expanded in order to show low $[\text{CO}_2]$ during glacial periods. The upper left insert is expanded to show low $[\text{CO}_2]$ periods over the last 10 million yr (data are from Petit *et al.*, 1999; Monnin *et al.*, 2001; Siegenthaler *et al.*, 2005; Berner, 2006; Lüthi *et al.*, 2008; Keeling *et al.*, 2005; Tripathi *et al.*, 2009).

potential to respond to rising $[\text{CO}_2]$, unless, for example, major evolutionary changes occur in the future. From these findings, it is clear that assessing the full continuum of plant response to changes in atmospheric $[\text{CO}_2]$ through geologic time is essential for making accurate predictions regarding the functioning of both past and future ecosystems.

Studies addressing the effects of low $[\text{CO}_2]$ on plants are also fundamental for understanding plant evolution in response to changes in resource availability through time – primarily since changing $[\text{CO}_2]$ has been shown to have major implications for plant fitness (Ward *et al.*, 2000). Modern plants grown at low $[\text{CO}_2]$ (150–200 ppm) exhibit highly compromised survival (Ward & Kelly, 2004) and reproduction (Dippery *et al.*, 1995) at conditions that occurred only 18 000–20 000 yr ago. Such findings beg the question of how glacial plants survived during low- $[\text{CO}_2]$ periods, especially considering the lack of evidence for plant extinctions during these times. Furthermore, past work has demonstrated that low $[\text{CO}_2]$ has the potential to act as a strong selective agent on plants, and therefore evolutionary responses may have ameliorated some of the negative effects of low $[\text{CO}_2]$ in the past (Ward *et al.*, 2000). However, the full suite of mechanisms accounting for these adaptive responses is currently unknown, as well as how

adaptive processes may have been influenced by other interactions with climate change (for a discussion of possibilities see Sage, 1994; Sage & Cowling, 1999; Ward *et al.*, 2000; Beerling, 2005). Furthermore, it is also important to consider that any genetic changes that occurred in the recent geologic past as a result of low $[\text{CO}_2]$ may continue to affect the responses of plants to rising $[\text{CO}_2]$ throughout the next century (Strain, 1991; Sage & Cowling, 1999).

Overall, low- $[\text{CO}_2]$ studies are critical for understanding plant responses to past environments when carbon resources were most limiting, evaluating physiological and growth constraints for response to rising $[\text{CO}_2]$, determining the full continuum of plant responses to changes in $[\text{CO}_2]$ over evolutionary time scales, assessing the impacts of low $[\text{CO}_2]$ on plant community composition and ecosystem functioning, and understanding the influence that low $[\text{CO}_2]$ may have had on early human cultures via influences on the development of agriculture. Moreover, studying plant responses to low $[\text{CO}_2]$ provides information about past ecosystem functioning, such as estimates of glacial NPP (Prentice & Harrison, 2009), as well as insights into the availability of food resources for animals (Coltraine *et al.*, 2004) and early humans (Sage, 1995; Richerson *et al.*, 2001).

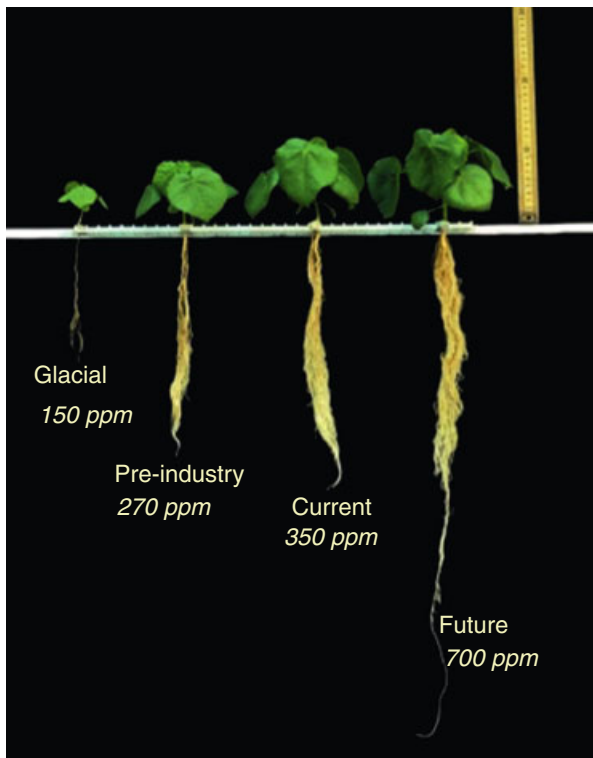


Fig. 2 Representative plants of *Abutilon theophrasti* (C_3) grown at glacial through future $[CO_2]$. All plants were 14 d of age and were grown under similar water, light, and nutrient conditions. These plants were photographed during a study by Dippery *et al.* (1995). (Photograph is courtesy of Anne Hartley, Florida Gulf Coast University.)

In this review, we mainly focus on the effects of low $[CO_2]$ that occurred during glacial periods, with an emphasis on plants possessing the C_3 photosynthetic pathway (85–90% of all species), since they tend to be most responsive to low $[CO_2]$ (Ehleringer *et al.*, 1991, 1997). For our purposes, ‘low’ or ‘glacial’ CO_2 corresponds to values of 170–200 ppm, ‘pre-industrial’ values correspond to 250–300 ppm, ‘modern’ values range between 350 and 400 ppm, and ‘elevated’ values correspond to $[CO_2]$ at or > 500 ppm. Although numerous environmental factors have changed between glacial and interglacial periods (e.g. precipitation, temperature), few studies have focused on the interactive effects of low $[CO_2]$ with other variables. We incorporate interactive effects with low $[CO_2]$ when possible, recognizing that our understanding in this area is limited.

We emphasize that the majority of low- $[CO_2]$ studies involve the use of modern plants grown at simulated conditions of the past. Such studies allow us to characterize the effects of limiting $[CO_2]$ on physiological, growth, and reproductive processes. Unfortunately, however, because these studies generally report on only a single generation of growth at low $[CO_2]$, they limit the scope of our

understanding of evolutionary processes. Therefore, we also discuss the results of studies that incorporate fossil material from glacial plants that were more fully adapted to low $[CO_2]$, as well as studies focusing on the evolutionary responses of plants to low $[CO_2]$ over multiple generations of artificial selection. We also fit low- $[CO_2]$ research into the larger context of global change studies, discuss technologies for reducing $[CO_2]$ during experimentation, and provide recommendations for future research directions in the field.

III. Experimental approaches for reducing $[CO_2]$

Methods for elevating $[CO_2]$ above ambient concentrations are often achieved via external sources of CO_2 and have been applied to growth chambers, open-top chambers, and free-air CO_2 enrichment (FACE) sites, providing multiple scales for elevated $[CO_2]$ research. Reducing $[CO_2]$ below the modern value is much more difficult, since CO_2 gas must be scrubbed from the atmosphere rather than added to it. This presents a major challenge when there is a large volume of air that must be scrubbed of CO_2 in a controlled and rapid manner. Several approaches have been developed to address this issue, and the benefits and limitations of each are discussed in the following.

The earliest methods for reducing $[CO_2]$ took advantage of the finding that C_4 plants can outcompete C_3 plants under limiting $[CO_2]$. In order to reduce $[CO_2]$, researchers enclosed C_4 plants side by side with experimental C_3 plants in small chambers, effectively lowering $[CO_2]$ below the C_3 compensation point ($[CO_2]$ where carbon gain through photosynthesis equals carbon loss from respiration; e.g. Sharma *et al.*, 1979; Fig. 3a). This approach did not allow for tight control of $[CO_2]$, and resulted in rapid onset of carbon starvation in plants, but was a clever way to lower $[CO_2]$ before the introduction of more sophisticated methods.

In the mid-1990s, a later phase of technology was introduced at the Grassland, Soil, and Water Research Laboratory (USDA-Agricultural Research Service) that involves an outdoor tunnel system, whereby plants are grown across a continuum of modern to low $[CO_2]$ (Mayeux *et al.*, 1993; Fig. 3b,c). During the day, air of known $[CO_2]$ is pumped into one end of the tunnel where plants experience relatively high $[CO_2]$. Plants near the end of the tunnel, on the other hand, experience low $[CO_2]$ (similar to glacial values) as a result of photosynthetic removal of CO_2 from air as it moves progressively through the tunnel. At night, airflow is reversed while plants are solely respiring, and this serves to equalize $[CO_2]$ throughout the tunnel. The desired $[CO_2]$ is maintained by varying the rate at which air flows through the chamber in response to changes in photosynthetic and respiration rates. A major strength of this system is that these chambers are housed

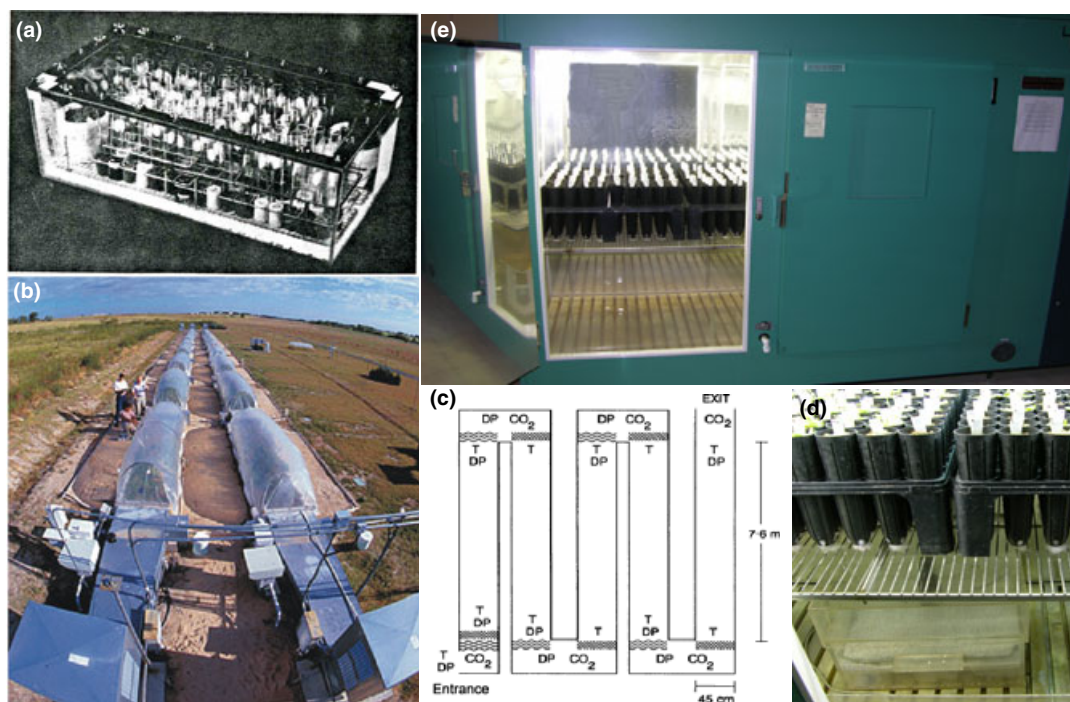


Fig. 3 Experimental approaches for achieving low-[CO₂] treatments. (a) Small growth chamber with C₄ plants grown side by side with experimental C₃ plants, used by Sharma *et al.* (1979, Fig. 1); (b) tunnel chambers for reducing [CO₂] along a continuum, described by Mayeux *et al.* (1993) (photograph courtesy of Wayne Polley, Grassland, Soil, and Water Research Laboratory); (c) diagram of the tunnel chambers indicating where [CO₂] (CO₂), dew point (DP), and air temperature (T) are sampled. Wavy lines represent chilled water cooling coils and cross-hatched areas represent electrical resistance heaters (from Mayeux *et al.*, 1993, Fig. 2); (d) experimental growth chamber with soda lime box underneath plants to reduce [CO₂] within the chamber (photograph courtesy of Joy K. Ward, University of Kansas); (e) reach-in experimental growth chamber with low-[CO₂] control (Conviron BDR-16; photograph courtesy of Joy K. Ward, University of Kansas).

outdoors and are exposed to full, natural lighting conditions with tight temperature control. In addition, plant data acquired from these experiments can be analyzed across a [CO₂] gradient, as opposed to using discrete CO₂ treatments. A disadvantage, however, is that the [CO₂] gradient cannot be maintained during dark hours, and sample size within a specific, targeted [CO₂] may be limited.

A different approach to lowering [CO₂] is through the use of controlled growth chambers, which can range in size from small reach-in varieties (Ward *et al.*, 2000; Mohan *et al.*, 2004; Sage & Reid, 1992; Fig. 3e) to large walk-in chambers commonly housed in experimental phytotrons (Ward *et al.*, 2008). In these chambers, plants can be grown with a variety of options, such as computer-controlled temperature, humidity, light and CO₂ conditions. Most commonly, low CO₂ treatments are obtained by scrubbing CO₂ from the atmosphere, either by forcing air through 'scrub boxes' containing soda lime (contents may include calcium hydroxide, sodium hydroxide, and/or potassium hydroxide depending on the manufacturer; Fig. 3d) or by passing compressed air across soda lime filters. Even in these small and highly controlled systems, the maintenance of [CO₂] can be inherently difficult. We have found that the best results occur when the scrubbing of CO₂ within scrub boxes

(Fig. 3d) is coupled with CO₂ additions from an external tank with a computer-generated switch, allowing for more constant [CO₂] control within the chamber (i.e. competing additions and scrubbing of CO₂ gas tend to stabilize [CO₂]; JK Ward unpublished). In addition, other researchers have utilized a system where CO₂-free air is emitted into enclosed chambers in order to lower [CO₂]. These units at the University of Florida (Gainesville, FL, USA) were made of glass and were housed outdoors, allowing for natural, full sun conditions (Baker *et al.*, 1990; Allen *et al.*, 1998). Overall, the tight control offered by the experimental systems described earlier is accompanied by the loss of realistic field conditions, and therefore these approaches may be less informative for strict ecological questions. These systems, however, can be highly useful for uncovering basic genetic, physiological, and growth mechanisms that may be driving individual plant responses to low [CO₂].

In order to advance low-[CO₂] research, it is hoped that future approaches will combine technologies for reducing [CO₂], maintenance of environmental control, as well as employing conditions that more closely simulate natural, field conditions. Such plans are currently under consideration and are being discussed among the scientific community at large.

IV. Early low-[CO₂] studies

The earliest studies focusing on plant responses to low [CO₂] began in the early 1960s and were prompted by the development of new technologies for measuring plant gas exchange. These studies focused on determining the CO₂ compensation point of plants, as well as comparing respiration and photosynthetic rates among species and genotypes. For example, Moss (1962) grew a variety of economically important crops (corn, sugar cane, orchard grass, tobacco, geranium, tomato, and Norway maple) in a closed system and allowed plants to draw down CO₂ over time. The author found that corn and sugar cane (now known to be C₄ plants) could draw down [CO₂] below 10 ppm, whereas the other species (now known to be C₃ plants) could only draw down [CO₂] between 60 and 145 ppm. Interestingly, this low-[CO₂] study distinguished plants with the C₄ vs C₃ photosynthetic pathways several years before the formal discovery of C₄ photosynthesis. At the time, the author was unable to provide a specific mechanism to account for these differences, but realized he was working with two unique physiological systems. It was later shown that C₄ plants have a CO₂ compensation point that is close to 0 ppm, whereas that of C₃ plants is in the vicinity of 50–60 ppm between 25 and 30°C.

During the early 1960s, Billings *et al.* (1961) also dealt with plant responses to low [CO₂], but from a more ecological perspective. The authors compared gas exchange rates of two populations of *Oxyria digyna* – one from high altitude (Logan Pass, MT, USA) and the other from sea level (mouth of the Pitmegea River, AL, USA). Seeds from these populations were grown under common conditions and photosynthetic rates were measured across a wide range of [CO₂]. The authors found that photosynthetic rates were higher and CO₂ compensation points were lower in offspring that originated from alpine populations, and suggested that this may reflect adaptations to lower CO₂ partial pressure at high altitude. The authors also concluded that low CO₂ partial pressure, and not temperature, limited the upward distribution of the *Oxyria* populations in this case. A subset of more recent studies have supported these findings, while others have pointed out that there may be alternative explanations for these results. Similar to the findings of Billings *et al.* (1961), Körner & Diemer (1994) showed that species of the genera *Ranunculus* and *Geum* exhibited higher carbon gain when grown at elevated [CO₂] compared with lowland species of the same genera. In addition, Ward & Strain (1997) found that *Arabidopsis* genotypes from high elevations produced greater seed numbers (indicating higher fitness) when grown at low [CO₂] (200 ppm) compared with genotypes from lower elevations (where CO₂ partial pressure was higher). Other studies, however, have noted additional factors with increasing altitude that would serve to enhance photosynthesis, thus

overcoming some, if not all, of the negative effects of low CO₂ partial pressure (Gale, 1972; Sage & Reid, 1992; Terashima *et al.*, 1995). These include increased diffusivity of CO₂ at high elevation that facilitates the movement of CO₂ from air to intercellular spaces, as well as reductions in O₂ partial pressure with elevation (proportionally similar to CO₂) that would reduce photorespiration. These factors can potentially offset the drop in CO₂ partial pressure up to 2500 m, after which stromal [CO₂] may be reduced by 20% (although here low temperatures may begin to limit carbon gain over CO₂; Sage & Reid, 1992). Taken together, these considerations suggest that high-altitude plants may not serve as a viable model for understanding adaptive responses of plants to global reductions in [CO₂] of the past (Terashima *et al.*, 1995).

A new era of low-[CO₂] studies began during the late 1960s, with the objective of identifying plants with high photosynthetic efficiency and low photorespiration rates, with the overall goal of improving crop productivity. This research occurred in conjunction with the green revolution that emphasized initiatives for increasing food production. In several studies (Cannell *et al.*, 1969; Nelson *et al.*, 1975; Sharma *et al.*, 1979), both crop and model plants were grown at extremely low [CO₂] that was near or below the CO₂ compensation point of C₃ plants (achieved by growing newly identified C₄ plants alongside experimental C₃ plants). Subsequently, genotypes were screened for survival at these extremely limiting carbon conditions with the assumption that surviving genotypes would exhibit superior photosynthetic efficiency. While mostly unsuccessful with respect to the original goal, these studies provided valuable insights into the degree of physiological stress induced by low [CO₂]. For example, Cannell *et al.* (1969) screened 2458 genotypes of soybean (*Glycine max*, C₃) and found that none could survive at low [CO₂] near the compensation point (ranging between 10 and 50 ppm CO₂). A decade later, Sharma *et al.* (1979) conducted studies with 33 *Arabidopsis thaliana* (C₃) genotypes grown below the CO₂ compensation point. They found considerable variation, whereby some genotypes survived only 1 wk at these conditions, whereas others could survive longer than 2 wk (relatively large variation for such a short-lived species). They also noted that the crossing of parents with extreme differences in survival time near the CO₂ compensation point resulted in a highly variable F₂ population (with some genotypes being superior to both parents), suggesting that there is considerable natural genetic variation for survival at limiting [CO₂]. In more recent advances (described in Delgado *et al.*, 1994; Medrano *et al.*, 1995), haploid tobacco (*Nicotiana tabacum*, C₃) lines derived from mutagen-treated anthers were selected for survival near the CO₂ compensation point (60–70 ppm). Approximately 5% of the lines survived, and, of those, plants had greater total leaf area, maintained higher photosynthetic rates specifically in

mature and older leaves, and had lower respiration rates (on a leaf mass basis, but not an area basis) relative to parental plants. This study, however, did not find alterations in photorespiration rates and properties of Rubisco in selected plants, as originally hoped.

In the 1980s, the publication of ice core data characterized the CO₂ composition of the atmosphere during the late Pleistocene, Holocene, and pre-industrial periods. Consequently, studies began focusing on the effects of low [CO₂] on plants from a geological and historical perspective. One of the first of these studies was conducted by Overdieck *et al.* (1988), who found that C₃ herbaceous annuals (*Vigna unguiculata* L., cowpea; *Abelmoschus esculentus* (L.) Moench, okra; *Raphanus sativus* L., radish) exhibited an average 8% reduction in growth at the pre-industrial value of 270 ppm CO₂ relative to the modern value that was 350 ppm at the time. These results suggested that anthropogenic increases in [CO₂] over contemporary time scales may have already modified plant functioning and productivity within modern ecosystems. Using *Lyonia maritima*, Overdieck (1989) was among the first to show that plants grown at pre-industrial [CO₂] exhibited greater water loss as a result of higher stomatal conductance (*g*) (by as much as 16%) compared with plants grown at modern [CO₂]. More recent studies have generally confirmed this result with instantaneous gas exchange measurements, but few studies have allowed for full physiological acclimation to pre-industrial [CO₂] over a full generation as in the Overdieck (1989) study. In a more recent study, Dippery *et al.* (1995) found that the biomass production of *Abutilon theophrasti* was reduced by 24% when grown at pre-industrial (270 ppm) vs modern (350 ppm) CO₂ (see Fig. 2 for a photograph), although specific effects of [CO₂] on physiology and other leaf properties could not be identified between these treatments (Tissue *et al.*, 1995).

In summary, these early studies laid the groundwork for more recent work by showing that plants with different photosynthetic pathways exhibit differential responses to low [CO₂], modern C₃ plants can become highly stressed when grown at low [CO₂], and plants exhibit genetic variation in response to low [CO₂] (whether natural or induced) that is often explained by shifts in biomass allocation or developmental timing rather than through direct alterations in photosynthesis/photorespiration rates or photosynthetic enzyme characteristics.

In the remaining sections, we describe the results of more recent low-[CO₂] studies that investigate a variety of scales ranging from physiological to ecosystem-scale processes. We also address the potential for plant evolutionary responses to low [CO₂] by reviewing studies that focus on plant fossils that occurred during glacial periods, as well as modern plants that were selected for high fitness over multiple generations at low [CO₂]. We also discuss the current status of a hypothesis by Sage (1995), suggesting that low

[CO₂] may have influenced the timing of agriculture among early humans. Although we are unable to describe all low-[CO₂] studies in detail, we highlight a wide range of work that reflects our current understanding of plant responses to low [CO₂].

V. Low-[CO₂] effects on the individual plant

1. Physiological responses

In C₃ plants, low [CO₂] affects net photosynthetic rates by reducing the rate of carboxylation of Rubisco resulting from substrate limitations and through higher photorespiration rates. Photorespiration is increased at low [CO₂] because both CO₂ and O₂ compete for the same active site of Rubisco. A reduction in [CO₂]/[O₂] enhances oxygenation, resulting in carbon loss to the plant. Note that unlike [CO₂], [O₂] has remained unchanged in the atmosphere for at least the last several million yr (Berner *et al.*, 2007), and thus [O₂] changes are not relevant to the time periods covered in this review.

Studies that measure plant responses to low [CO₂] most commonly involve growing plants at modern [CO₂], followed by instantaneous gas exchange measurements on a small portion of leaf area across a large range of [CO₂] (typically ranging between 0 and 1000 ppm). Referred to as *A*-*c*_i curves (photosynthetic assimilation vs leaf intercellular [CO₂]), these measurements encompass low [CO₂] that was characteristic of glacial periods (Fig. 4). *A*-*c*_i curves generally show steep linear increases in net photosynthetic rates (*A*) between *c*_i values of 0 and 250 ppm CO₂, with continued positive slopes through *c*_i values as high as

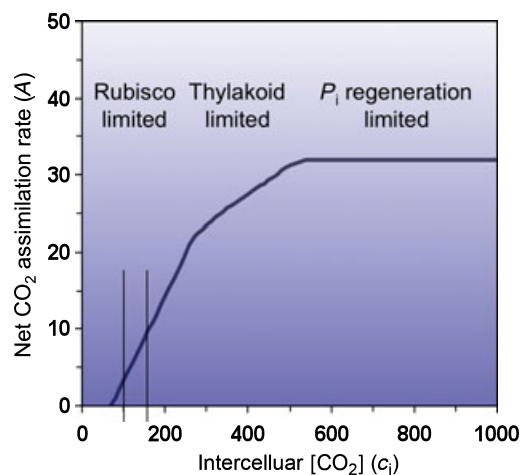


Fig. 4 Theoretical *A*-*c*_i curve showing the relationship between photosynthetic assimilation of CO₂ (*A*) and leaf intercellular [CO₂] (*c*_i). The region within the vertical lines represents approximate *c*_i values experienced by glacial plants (at corresponding atmospheric [CO₂] of 170–200 ppm and based on *c*_i values from glacial plants; Van de Water *et al.*, 1994; Ward *et al.*, 2005).

1000 ppm (Sage & Coleman, 2001). These curves indicate that reductions in $[\text{CO}_2]$ during glacial periods produced Rubisco-limited photosynthetic rates (Sage & Coleman, 2001). A survey of $A-c_i$ curves from a variety of C_3 species indicate that net photosynthetic rates are reduced by 40–60% at low (180 ppm) vs modern (350–380 ppm) CO_2 (c_a values), with *c.* 30% of this reduction being induced by higher photorespiration rates when temperatures are optimal (Sage & Cowling, 1999).

$A-c_i$ curves are highly informative for understanding the biochemical properties of Rubisco in response to changing $[\text{CO}_2]$. Because this enzyme has been highly conserved across evolutionary time scales (Griffiths, 2006), such measurements may have the potential to be extrapolated to glacial plants. It should be noted, however, that the extent of physiological acclimation at each $[\text{CO}_2]$ measurement is short-term in nature, and thus full acclimation responses are rarely assessed with $A-c_i$ curves. To further highlight this point, changes in $A-c_i$ curves have been observed in C_3 plants grown at modern $[\text{CO}_2]$ and then transferred to low $[\text{CO}_2]$ (200 ppm) for several wk, and the direction of change was generally unpredictable (Sage, 1994; also see Overdieck, 1989; Sage & Reid, 1992). In addition, such measurements do not provide insights into whole-plant responses to low $[\text{CO}_2]$ over the full life cycle, such as modifications in both relative and total leaf area that can influence whole-plant carbon uptake. Another point worth noting is that the $A-c_i$ curves of plants that are fully adapted to low $[\text{CO}_2]$ (over many generations of genetic change) may potentially deviate from that of modern plants, thus limiting the potential for physiological extrapolation to the past. Because we know essentially nothing about this issue, future work should focus on the extent to which modern physiological responses can be extrapolated to the past, with the inclusion of full acclimatory and adaptive responses.

In addition to having direct effects on photosynthetic rates and carbon gain, changes in $[\text{CO}_2]$ can also have large effects on g that can influence transpiration rates and water-use efficiency (WUE; carbon assimilation/stomatal conductance). In the vast majority of C_3 plants, g increases with reductions in $[\text{CO}_2]$ by 35–50% (depending on the species) between modern (350–380 ppm) and glacial values (180–200 ppm). This response is observed during short-term gas exchange measurements (Lloyd *et al.*, 1992; Flexas *et al.*, 2007), as well as long-term exposure to low $[\text{CO}_2]$ over a full generation (Polley *et al.*, 1993b; Ward *et al.*, 1999; Tonsor & Scheiner, 2007). Higher g serves to enhance CO_2 uptake by reducing the diffusional resistance of CO_2 into the leaf interior, but with the cost of higher water loss. Furthermore, Polley *et al.* (2002) found that higher g within a C_3/C_4 grass assemblage grown at low $[\text{CO}_2]$ translated into greater depletion of soil water than seen in similar assemblages grown at modern $[\text{CO}_2]$ (Polley *et al.*, 2002),

suggesting that stomatal responses to low $[\text{CO}_2]$ can have implications for water availability at the ecosystem level.

Using carbon isotopes ratios, Polley *et al.* (1995) estimated WUE in a variety of C_3 plants (*Triticum aestivum*, *Bromus tectorum*, *Prosopis glandulosa*) and found reduced values at low vs modern $[\text{CO}_2]$. Interestingly, reductions in WUE were directly proportional to reductions in $[\text{CO}_2]$ in all species, suggesting that the ability to scale this response may be relatively straightforward. In the same study, nitrogen-use efficiency (NUE, biomass produced/plant N) decreased in *B. tectorum* and *P. glandulosa* at low $[\text{CO}_2]$, but this response was not as pronounced or consistent as changes in WUE, suggesting that low $[\text{CO}_2]$ may have increased the requirements for water to a greater extent than N in C_3 species.

2. Biomass production

In order to determine how physiological responses to low $[\text{CO}_2]$ manifest themselves at the whole-plant level, plants must be grown for a full generation at low $[\text{CO}_2]$. Such studies have shown that the average biomass production of modern C_3 plants is reduced by *c.* 50% when grown at low (180–220 ppm) vs modern (350–380 ppm) $[\text{CO}_2]$, when other conditions are optimal (Sage & Coleman, 2001; Fig. 5). There is, however, variation in this response among C_3 species (Fig. 5), as well as within C_3 species, whereby reductions in biomass may vary by 40–70% among genotypes (Ward & Strain, 1997; Hovenden & Schimanski, 2000; Mohan *et al.*, 2004). In addition, as $[\text{CO}_2]$ declines to 150 ppm, biomass production may be reduced by as much as 92%, as was observed in *A. theophrasti* (Dippery *et al.*, 1995; Figs 2, 5).

It is also generally found that the same absolute change in $[\text{CO}_2]$ below the modern value has a much greater effect on biomass production (and A) than the same absolute change in $[\text{CO}_2]$ above the modern value (Allen *et al.*, 1991; Polley *et al.*, 1992; Dippery *et al.*, 1995; Tissue *et al.*, 1995; Ward & Strain, 1997; Ward, 2005; Fig. 5). Thus, even small changes in $[\text{CO}_2]$ during glacial periods may have had large effects on plant productivity. It is possible, however, that modern plant responses to past $[\text{CO}_2]$ may be accentuated relative to glacial plants that were more fully adapted to low $[\text{CO}_2]$. Moreover, the lack of evidence for plant extinctions during glacial periods indicates the likelihood that plants underwent adaptive changes in response to low $[\text{CO}_2]$ that may have altered biomass production, and these responses may no longer be evident in some modern genotypes (Ward *et al.*, 2000; Ward & Kelly, 2004; Tonsor & Scheiner, 2007).

When comparing biomass production and net photosynthetic rates (A) of C_3 plants, there is often a stronger correlation between these measurements at low $[\text{CO}_2]$ than at modern or elevated $[\text{CO}_2]$ (Tissue *et al.*, 1995; Sage &

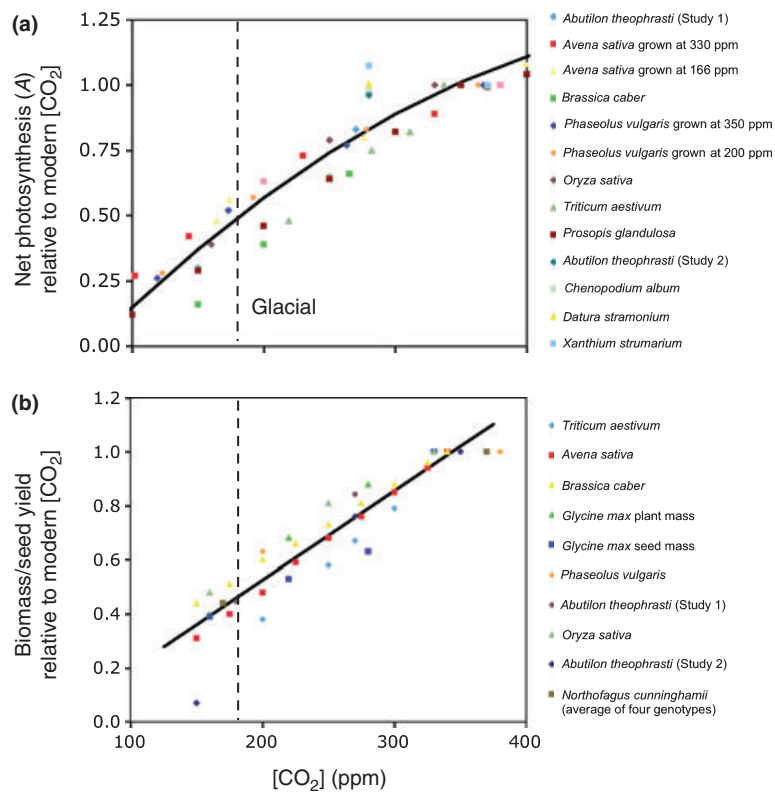


Fig. 5 Relative net photosynthesis (a) and biomass production (or seed yield) (b) for a variety of modern plants grown at [CO₂] spanning from glacial to modern times. Data in the y-axis are responses at a given [CO₂] relative to modern [CO₂] (350–380 ppm), with the line representing responses for plants grown at the glacial [CO₂] minimum (adapted from Sage & Coleman, 2001, Fig. 3, with updated data from Cowling & Sage, 1998; Ward *et al.*, 1999; Hovenden & Schimanski, 2000; Bunce, 2001).

Coleman, 2001; but also see Cowling & Sage, 1998 where this correlation becomes decoupled at very high temperature). This finding indicates that photosynthate is more directly converted into biomass at low [CO₂], and this is often accompanied by lower starch and sugar accumulation within leaves (Sage, 1995; Allen *et al.*, 1998). Furthermore, lower photosynthetic rates at low [CO₂] often persist throughout a full generation, contributing to sustained reductions in biomass production. This is the case even when plants undergo acclimation responses to low [CO₂] (reviewed in Sage & Coleman, 2001), such as higher concentrations of Calvin–Benson cycle enzymes (mainly Rubisco; Tissue *et al.*, 1995; but also see Gesch *et al.*, 2000 for an example of partial recovery), higher *g* (Polley *et al.*, 1993b; Ward *et al.*, 1999), increased activity of carbonic anhydrase that facilitates CO₂ diffusion into the chloroplasts (Coleman, 2000), and greater leaf area ratio (leaf area/total plant mass, LAR) that may enhance whole-plant carbon uptake (Dipperry *et al.*, 1995).

3. Biomass partitioning

It has been hypothesized that plants grown at low [CO₂] would partition a higher proportion of biomass to above-ground than to below-ground structures; this response would increase LAR and enhance overall investment in carbon assimilation under limiting [CO₂] (Sage & Coleman, 2001). A variety of studies have found support

for this idea, including Dipperry *et al.* (1995) who showed that *A. theophrasti* partitioned a higher proportion of biomass to shoots relative to roots at 150 than at 350 ppm CO₂ (root : shoot mass = 0.17 vs 0.34, respectively); this finding was additionally confirmed by allometric analysis that accounted for ontogenetic shifts (J. K. Ward, unpublished). Surprisingly, this response ended up being counter-productive, since reduced investment in roots resulted in reduced N uptake and lower Rubisco production, further compounding the negative effects of low [CO₂] on carbon uptake (Tissue *et al.*, 1995). Interestingly, Cowling & Sage (1998) observed that *Phaseolus vulgaris* also had reduced partitioning of biomass to roots at low [CO₂], but this response was associated with increased stem biomass as opposed to leaf biomass. Such a finding may be associated with direct effects of [CO₂] on biomass partitioning, or may be the result of the indirect effects of shifting plant ontogeny in response to differences in [CO₂]. With respect to contemporary time scales, neither Dipperry *et al.* (1995) nor Bunce (2001) found differences in LAR in *A. theophrasti* and other C₃ annuals grown at pre-industrial (270 ppm) and modern (350–370 ppm) [CO₂]. Also, Ghannoum *et al.* (2010) found no differences in root allocation in two *Eucalyptus* species grown at pre-industrial (290 ppm) and current (400 ppm) CO₂ at ambient temperature. These findings suggest that increases in [CO₂] over the past 100 yr may not have had a large influence on plant biomass partitioning (even though absolute biomass is often affected).

4. Developmental timing

Elevated $[\text{CO}_2]$ is known to affect plant developmental timing and such responses have been shown to influence plant fitness (Ward & Kelly, 2004; Springer & Ward, 2007; Springer *et al.*, 2008). Unfortunately, very little is known about the effects of low $[\text{CO}_2]$ on the developmental timing of C_3 plants. Sage & Coleman (2001) hypothesized that increasing carbon storage within roots before reproduction would enhance fitness at low $[\text{CO}_2]$ (assuming there was sufficient time remaining in the growing season to reproduce), and this could be achieved by delaying developmental milestones such as flowering. In support of this idea, Ward & Strain (1997) found that field-collected genotypes of *A. thaliana* required on average 9 d more to initiate flowering when grown at 200 vs 350 ppm CO_2 . This represented a proportionally large shift in developmental timing considering that this species only has a 40–60 d life cycle. Unlike this finding, Polley *et al.* (1993b) did not find modifications in developmental timing in two cultivars of wheat grown from 200 to 350 ppm CO_2 . These studies suggest that at this point, there is no predictable response pattern to describe the effects of low $[\text{CO}_2]$ on the developmental timing of C_3 annuals and indicate that additional work is needed in this area. Furthermore, little is known about the effects of low $[\text{CO}_2]$ on the developmental timing of perennials. It is predicted that they would have had an advantage over annuals at low $[\text{CO}_2]$ in that reproduction could be delayed until adequate resources were acquired for successful reproduction (Cowling, 2001; Ward, 2005).

5. Reproduction and survival (fitness components)

Of the few studies measuring the survival and reproduction of C_3 plants grown at low $[\text{CO}_2]$, all have reported large effects on these measurements. Dippery *et al.* (1995) found the most extreme response, where low $[\text{CO}_2]$ (150 ppm) prevented reproduction in the modern C_3 annual, *A. theophrasti*, as a result of the abortion of all flower buds that drove the fitness response to zero. This finding suggested that 150 ppm CO_2 may be near the threshold for successful completion of the life cycle in some C_3 species. Campbell *et al.* (2005) found that tobacco was able to successfully reproduce at both 100 and 150 ppm CO_2 , although after a very large amount of time (16 wk), and the germination percentage of offspring was compromised at 100 relative to 150 ppm CO_2 . Both the Dippery *et al.* (1995) and Campbell *et al.* (2005) studies provide a strong reminder that the whole-plant CO_2 compensation point allowing for full completion of the plant life cycle may be substantially higher than the leaf CO_2 compensation point. In addition, much more time may be required for successful reproduction at low $[\text{CO}_2]$ since sufficient carbon must be accumulated and stored in order for reproduction to be successful.

Such considerations must be kept in mind when predicting the full effects of low $[\text{CO}_2]$ on plant reproduction and fitness.

Tonsor & Scheiner (2007) investigated the effects of CO_2 availability (ranging between 250 and 710 ppm) on patterns of trait integration among 35 genotypes of *A. thaliana*. They found that at low $[\text{CO}_2]$, whole-plant carbon assimilation was the main determinant of reproductive mass, although this relationship was not maintained at higher $[\text{CO}_2]$. This study echoes the findings with *A* and biomass production (see Section V.2), but now additionally shows that *A* and fitness can also be closely correlated at low $[\text{CO}_2]$. In addition, Ward & Kelly (2004) also worked with *A. thaliana* and found that six field-collected genotypes exhibited reduced survival, ranging between 20 and 49%, and reduced seed production ranging between 38 and 81% when grown at 200 ppm vs 350 ppm CO_2 . As a result, reductions in estimated fitness (percentage survival \times total seed production) ranged between 59 and 87% among the genotypes, indicating substantial genetic variation for low- $[\text{CO}_2]$ response (significant $\text{CO}_2 \times$ genotype interaction; Fig. 6). Furthermore, the reductions in reproductive output among the *Arabidopsis* genotypes were a result of overall reduced plant size, as opposed to changes in the partitioning of biomass to reproduction (Ward & Kelly, 2004). Taken together, these studies, as well as others (also see Mohan *et al.*, 2004 with maple), indicate that there is ample genetic variation to account for potential evolutionary responses to low $[\text{CO}_2]$.

In a different type of study using ^{13}C as a label, Lehmeier *et al.* (2005) found that low $[\text{CO}_2]$ (200 ppm) did not alter the mobilization rate of seed-derived carbon within newly developing sunflower seedlings; such a response may have influenced survival (not measured in this study), since rapid movement of carbon reserves would be essential for maintaining a positive carbon balance in seedlings grown at low $[\text{CO}_2]$. In addition, seed size may play a major role in determining survival at low $[\text{CO}_2]$, since greater carbon reserves would enhance the production of leaf area within seedlings, reducing the chances of destructive negative carbon budgets during rapid and early growth stages (Ward & Kelly, 2004; Ward, 2005; also see Metz *et al.*, 2010 for experimental and theoretical considerations of seed size and survival). Likewise, the small seed size of *A. thaliana* may have contributed to the high mortality rates that were observed at low $[\text{CO}_2]$ in the Ward & Kelly (2004) study discussed earlier (Fig. 6).

VI. Low $[\text{CO}_2]$ and plant evolution

In past work, researchers have speculated about possible evolutionary responses of plants to low $[\text{CO}_2]$ during different geologic time periods (Beerling, 2005). For instance, Beerling *et al.* (2001) pointed out an association between

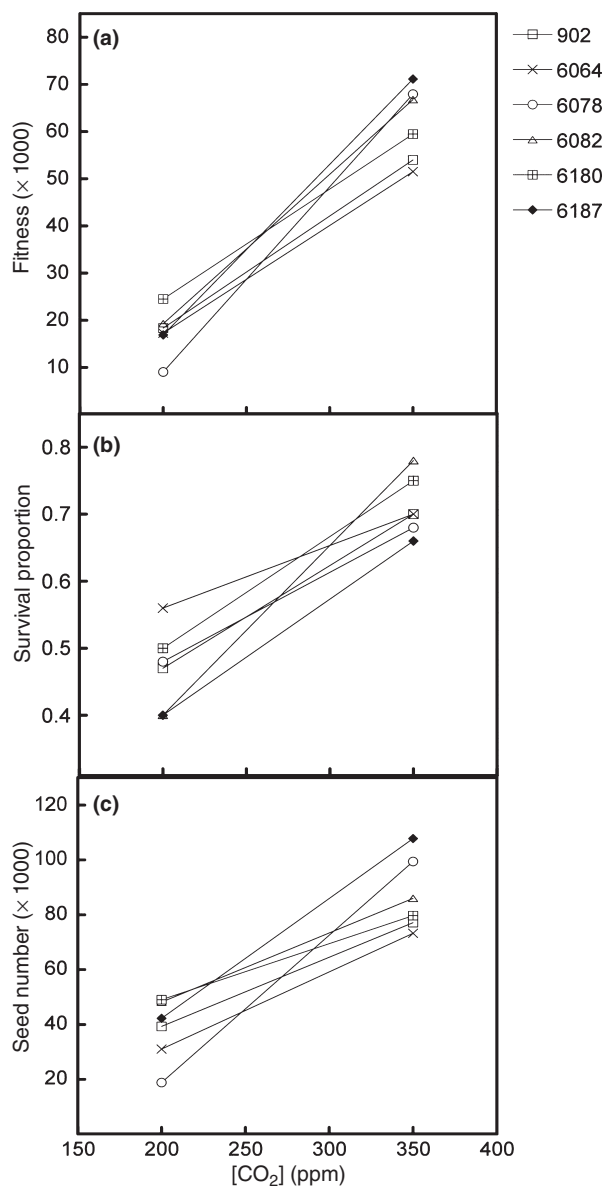


Fig. 6 Responses of six field-collected genotypes of *Arabidopsis thaliana* grown at 200 and 350 ppm CO₂ and measured for estimated total fitness (a), proportion of survival (b), and total seed number (c). Genotype numbers are from The Arabidopsis Biological Resource Center, Ohio State University (adapted from Ward & Kelly, 2004, Fig. 6).

decreasing [CO₂] during the late Devonian and the evolution of megaphyll leaves, which had higher stomatal densities, higher transpiration rates, and greater capacity for cooling (based on modeling) compared with more primitive leaves. Furthermore, McElwain *et al.* (2005) suggested linkages between declining [CO₂] during the Cretaceous and an increase in the relative abundance and radiation of the angiosperms.

The rise of C₄ photosynthesis is also a major evolutionary event that has been attributed to low [CO₂] (initially by

Ehleringer *et al.*, 1991; also in Ehleringer *et al.*, 1997; Cerling *et al.*, 1998; reviewed by Sage, 2004). Early work suggested that [CO₂] decline during the late Miocene contributed to the evolution of C₄ species, since these plants can concentrate CO₂ in bundle sheath cells, even when atmospheric [CO₂] is low. More recent work, however, has postulated that C₄ photosynthesis originated much earlier, most likely during the Oligocene (20 million yr earlier), and this idea has yielded phylogenetic support (Sage, 2004). Similar to the initial idea, however, C₄ evolution has still been linked with reductions in [CO₂], although much earlier in time, which became possible as the geologic record of atmospheric [CO₂] improved. In more recent geologic periods, it is now recognized that there was a major expansion of C₄-dominated grasslands during the late Miocene-early Pliocene that would have resulted in dramatic shifts in plant productivity and ecosystem functioning (Sage, 2004; Tipple & Pagani, 2007; Edwards *et al.*, 2010). This transition occurred during an abrupt [CO₂] decline, as recently shown by Tripathi *et al.* (2009) using boron : calcium ratios in foraminifera. Their results showed that atmospheric [CO₂] dropped as low as 200–300 ppm between 5 and 10 million yr ago, suggesting a likely role for low [CO₂] in this C₄ expansion. However, Tipple & Pagani (2007), and more recently Edwards *et al.* (2010), noted other factors that may have played a role in C₄ expansion, including aridity, a more seasonal climate, fire disturbance, and monsoon. These papers emphasized that this C₄ expansion should be evaluated on a more regional scale, and with attention to multiple C₄ adaptations (in addition to low [CO₂] tolerance), since C₄ expansion does not appear to be globally synchronous. In more recent geologic time, there is strong evidence that C₄ plants continued to expand their range in response to low [CO₂] during glacial periods of the past million yr, particularly in the tropics where warmer temperatures would have additionally favored C₄ photosynthesis (see Section VIII for more details). In addition, this has also been deemed a period of recent C₄ evolution, mainly through the rise of a variety of C₄ dicot lineages (Ehleringer *et al.*, 1997; Sage, 2004).

Even with this recent geologic expansion of C₄ species, C₃ plants still persisted in many ecosystems during glacial periods (e.g. Coltrain *et al.*, 2004; southern California), and it is not yet fully understood how they functioned and adapted to low [CO₂] during that time. It is clear, however, that modern C₃ plants are products of an ancestry that had undergone at least 2 million yr of glacial–interglacial cycles, with corresponding changes in climate and [CO₂] (Ward & Kelly, 2004). Thus, glacial plants must have had the genetic capacity to adapt to changing [CO₂], and mechanisms accounting for this process may still be detectable in some modern species/genotypes. Furthermore, a better understanding of these responses will provide important insights into the capacity for plants to evolve in response to

rising $[\text{CO}_2]$ in the future, which is a critical issue at this juncture.

While studies conducted for an entire generation at low $[\text{CO}_2]$ provide critical information on whole-plant responses with full acclimation (Section V), these studies do not incorporate adaptive changes at low $[\text{CO}_2]$ that occur over multiple generations with genetic change. The process of understanding plant evolution at low $[\text{CO}_2]$ has been addressed through two main approaches. In the first, modern plants are selected at low $[\text{CO}_2]$ (180–200 ppm) for high growth or reproduction, allowing for an understanding of how genetic change may drive adaptive processes in living plants. These studies are conducted over multiple generations, and differ from the screening methods described in earlier studies, where genotypes were selected for survival during only one generation of exposure to extremely low $[\text{CO}_2]$ (e.g. Sharma *et al.*, 1979). A second approach involves studying fossilized plant material that actually occurred during glacial periods; such fossils are highly useful for determining evolutionary responses to low $[\text{CO}_2]$ since these plants had thousands of yr to adapt to these conditions over multiple generations. A relatively large number of plant specimens dating to the LGM have been naturally preserved in tar pits, bogs, and caves, and these may serve as critical resources for further addressing this issue (Wells & Jorgensen, 1964; Stock & Harris, 2001). Unfortunately, however, measurements on these fossils are generally restricted to stable isotopes, morphology, DNA sequencing (in some cases), and modeling approaches (because they are nonliving). By studying these specimens, along with modern plants that have been adapted to low $[\text{CO}_2]$ over multiple generations, we may better understand how plants functioned across a wider range of $[\text{CO}_2]$, and we may be able to pinpoint the mechanisms that enabled C_3 plants to survive during the most limiting $[\text{CO}_2]$ periods of the past.

To investigate evolutionary processes, Ward *et al.* (2000) conducted a selection experiment with *A. thaliana* (derived from out-crossings) for high seed number over five generations at low $[\text{CO}_2]$ (200 ppm; control plants were also grown side by side with selected plants, but were randomly selected). At the fifth and final generation of selection, plants from selected populations produced 25% more seeds on average (with no changes in quality) than nonselected control plants when both were grown at low $[\text{CO}_2]$ (Fig. 7). In conjunction with this, selection plants delayed flowering by *c.* 4 d, had an extended life cycle, and produced 34% more total biomass than control plants (Fig. 7). Initially, no differences in physiology were detected between selection and control plants (Ward *et al.*, 2000). However, in a more recent study with the same populations (and improved methods), González-Meler *et al.* (2009) found that respiration rates were lower in *Arabidopsis* plants selected at 200 ppm CO_2 than in control plants (without reductions in N tissue content), with a large portion of this

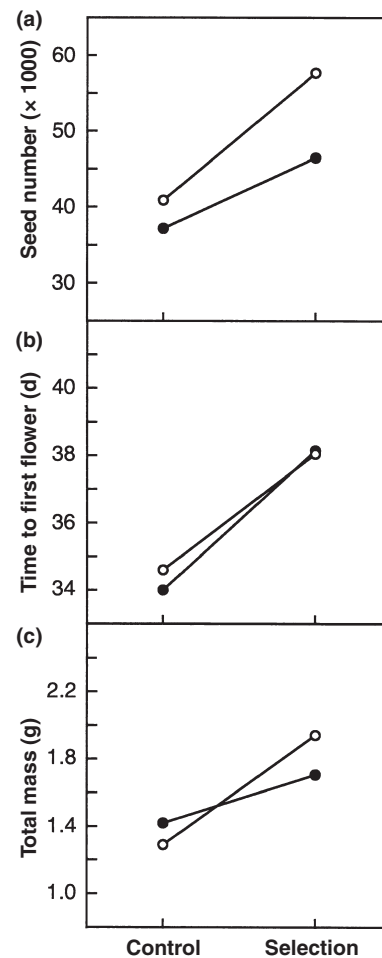


Fig. 7 Mean values for seed number (a), time (d) to first flower (b), and total biomass (c) for selected and control (nonselected) plants of *Arabidopsis thaliana* at the fifth and final generation of selection for high seed number at 200 ppm. All plants were grown under the same conditions at 200 ppm CO_2 . Lines connect mean values for replicate control and selection populations, and open or closed circles designate different growth chambers. Note that low $[\text{CO}_2]$ was found to be the selective agent acting on these plants via subsequent reciprocal transplant experiments (see Ward *et al.*, 2000) (adapted from Ward & Kelly, 2004, Fig. 3(a); Ward *et al.*, 2000).

response occurring from reduced activity of the alternative pathway. Reduced respiration rates improved the carbon budgets of the low- $[\text{CO}_2]$ -selected plants, representing a potentially important adaptive response to low $[\text{CO}_2]$. Also with respect to the Ward *et al.* (2000) study, Bone & Farres (2001) surveyed the literature and found that the rate of selection at low $[\text{CO}_2]$ was among the fastest rates reported for a novel global change factor, based on both rates of change over time (yr) and generation number. Taken together, these studies show that low $[\text{CO}_2]$ can act as a strong selective agent on C_3 plants, and suggests that single-generation studies may not be fully representative of the responses of glacial plants to low $[\text{CO}_2]$.

Using fossil plants from different regions, a variety of studies have shown that ancient plants exhibited higher stomatal density/index during low-[CO₂] periods of the past (reviewed in Royer, 2001; also see Beerling & Chaloner, 1993; Beerling & Woodward, 1993; Wooller & Agnew, 2002; Roth-Nebelsick, 2005). A number of groups have stated that this may be an adaptive response to low [CO₂] that reduced the resistance for entry of CO₂ into the interior of leaves when [CO₂] was most limiting, but potentially at the cost of higher water loss. Other groups, however, have not found a correlation between stomatal density/index and [CO₂] and have argued against this possibility (see Körner, 1988; Bettarini *et al.*, 1998; Maherali *et al.*, 2002; and mixed responses reported by Knapp *et al.*, 1994). We will briefly describe the data supporting both sides of this issue, and make some suggestions for possibly resolving this debate.

In support of this idea, Beerling *et al.* (1993a) reported higher stomatal density and index of European *Salix herbacea* L. that occurred during low [CO₂] of the LGM relative to modern plants (Fig. 8). In addition, using specimens from packrat middens collected from the Great Basin, Van de Water *et al.* (1994) found that stomatal density was 17% higher in *Pinus flexilis* needles dating to the LGM relative to the Holocene; it is important to note, however, that in this study, higher stomatal density did not translate into higher leaf $c_i : c_a$ (lower, in fact), possibly because of reduced g or changes in photosynthetic capacity. In addition to these findings, the relationship between stomatal density/index and [CO₂] has been supported by reductions in stomatal density/index during the contemporary rise in [CO₂], and such responses may serve to conserve water as CO₂ becomes less limiting (Woodward, 1987; Beerling *et al.*, 1993a). More specifically, Beerling (2005) found that UK populations of *Selaginella selagenoides* and *Selaginella kraussiana* exhibited a *c.* 30% decrease in stomatal index between the period when [CO₂] rose from 280 to 360 ppm. Interestingly, Wagner *et al.* (1996) looked at this issue from an acclimation perspective, and showed that stomatal density decreased within the lifetime of a single birch tree (*Betula pendula*) during the contemporary rise in atmospheric [CO₂] (stomatal density declined -0.6% for every 1 ppm increase in [CO₂]). This group considered this a reflection of evolved plasticity that may have emerged from changing [CO₂] over geologic cycles.

Although many studies find evidence for higher stomatal density (or index) at low [CO₂], *c.* 12% of studies find the completely opposite trend (Royer, 2001), and this may be an underestimate since negative results are usually more difficult to publish. For example, in a study of modern C₃ grasses (*Solanum dimidiatum*, *Bromus japonicus*) grown across a [CO₂] gradient (200–550 ppm), plants exhibited lower stomatal density at low [CO₂] (Maherali *et al.*, 2002), and instead had larger stomatal pore size, suggesting

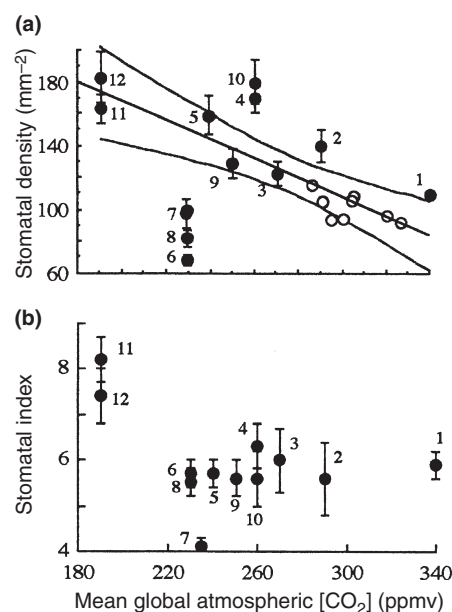


Fig. 8 Stomatal density (a) and stomatal index (b) of fossil *Salix herbacea* leaves in response to changes in atmospheric [CO₂] from the Last Glacial Maximum to the present (taken from Beerling *et al.*, 1993a, Fig. 2).

an alternative response to limiting [CO₂]. In addition, Körner (1988) found no relationship between stomatal density and [CO₂] for 200 plant species from central Europe that grew during the pre-industrial period (270 ppm CO₂) vs modern times (340 ppm CO₂), including both alpine and lowland species. Royer (2001) also pointed out that most studies supporting the notion of higher stomatal density or index at low [CO₂] occur with fossil plants (88 and 94% of studies, respectively), with fewer studies showing this response in modern plants grown for short periods at low [CO₂] (36 and 40% of studies, respectively). This may be indicative of an evolved response that occurs only after many generations at low [CO₂], as suggested by Royer (2001), and/or may reflect the fact that other growth conditions (water, light, temperature) are less controlled in fossil studies than in modern experimental work. In opposition to Royer's idea, Bettarini *et al.* (1998) studied 17 plant species growing near high [CO₂] springs in central Italy where [CO₂] was twice the modern value for at least two centuries, and found that stomatal density was unaffected by elevated [CO₂] in the majority of species.

Despite these inconsistencies, the issue of stomatal index/density response to [CO₂] of the past has increased interest and investment in the understanding of plant response to low [CO₂]. Roth-Nebelsick (2005) laid out the challenges to this field, including high variance in stomatal measurements, especially in fossil samples, large interspecific differences, nonlinear response to [CO₂] increase, and differences between long-term and short-term studies. To date, a specific developmental mechanism linking stomatal

index/density to $[\text{CO}_2]$ has not been determined, although the *Arabidopsis* HIC (high carbon dioxide) gene may play a role in this response (Gray *et al.*, 2000). The authors suggest that these challenges may be addressed by striving to identify fossil material that is growing under more tightly controlled conditions (i.e. limiting differences in factors other than $[\text{CO}_2]$), as well as advancing the understanding of molecular mechanisms tied to this response (Bergmann & Sack, 2007). One approach may be to conduct a thorough quantitative trait locus (QTL) analysis with model plants to determine the chromosomal regions (and eventually genes) that influence stomatal density/index across a range of $[\text{CO}_2]$ growth conditions. A more thorough understanding of the primary mechanisms driving stomatal density/index responses to $[\text{CO}_2]$ may eventually allow us to resolve the reasons for the inconsistencies reported in this field.

In addition to studying leaf morphology, researchers have also analyzed the carbon isotope ratios of fossil material to determine if there were evolutionary shifts in leaf physiology during low- $[\text{CO}_2]$ periods (Beerling *et al.*, 1993b; Van de Water *et al.*, 1994; Beerling, 1996; Pedicino *et al.*, 2002; Ward *et al.*, 2005). For example, Van de Water *et al.* (1994) calculated $c_i : c_a$ from carbon discrimination values (that account for changes in carbon isotope ratios of source air through time) of *P. flexilis* leaves that occurred between the LGM and the Holocene. They found lower $c_i : c_a$ during the last glacial period (0.55) relative to the Holocene (0.64), which was the result of either lower g or higher photosynthetic capacity. Most interesting here was the finding that neither $c_i : c_a$ nor $c_a - c_i$ (representing the $[\text{CO}_2]$ gradient) were maintained through time, and c_i values dropped as low as 109.7 during the last glacial period. Ward *et al.* (2005) also calculated $c_i : c_a$ from carbon discrimination measurements in *Juniperus* trees from the Rancho La Brea tar pits in southern California. The authors found that $c_i : c_a$ ratios were actively maintained over 60 000 yr (as hypothesized by Ehleringer & Cerling, 1993), and, as a result, glacial trees experienced c_i values as low as 113 ppm CO_2 . If such reductions in c_i were to occur in modern vegetation, plant growth would be reduced by half, according to studies with modern plants (Polley *et al.*, 1993a; Van de Water *et al.*, 1994; Ward *et al.*, 2005). As a whole, these studies support the notion that trees were potentially carbon-starved during low- $[\text{CO}_2]$ periods because of glacial c_i values that are, for the most part, unprecedented in modern vegetation.

VII. Interactions of low $[\text{CO}_2]$ with other factors

A variety of studies have examined the interactive effects of low $[\text{CO}_2]$ with other environmental factors, such as temperature, water, and nutrients. It is expected that low $[\text{CO}_2]$ would accentuate the effects of other stressors, mainly

because plants would already be compromised by low carbon availability (Cowling & Sage, 1998; Cowling & Sykes, 1999; Ward, 2005). Certainly the interactions of low $[\text{CO}_2]$ with other environmental factors may have had synergistic effects on physiology, plant productivity, and evolution. To address this issue, recent studies incorporating the interactive effects of low $[\text{CO}_2]$ with temperature, water, and nutrients are described in the following sections.

1. Temperature

Sage & Cowling (1999) studied the interactive effects of low $[\text{CO}_2]$ and high temperature on several C_3 species (*P. vulgaris*, wheat, and tobacco). They found that at high temperatures (day : night, 35 : 29°C), biomass production was reduced by 75–95% at 200 ppm CO_2 relative to 380 ppm CO_2 , whereas at low temperatures (day : night, 25 : 20°C) biomass production was only reduced by 40–60%. Interestingly, the effects of high temperature on the low- $[\text{CO}_2]$ response could not be attributed to lower A (or, indirectly, higher photorespiration), but rather to reduced leaf area (relative to total mass) that resulted from lower leaf expansion and less leaf initiation (Sage & Cowling, 1999). From this and other similar studies, altered leaf development has been implicated as the primary response to high-temperature stress at low $[\text{CO}_2]$. Moreover, these results suggest that C_3 plants that occurred in warm regions, such as in the tropics, may have been most negatively affected by low $[\text{CO}_2]$ during glacial periods.

In a more recent study, Ward *et al.* (2008) grew *A. theophrasti* at low $[\text{CO}_2]$ (200 ppm), with both modern (30/24°C) and glacial temperatures (22/16°C). The authors discovered that any beneficial effects of low temperature on the C_3 species, such as reduced photorespiration, did not overcome the very negative effects of low $[\text{CO}_2]$ on growth. Also, the authors could not detect an effect of temperature on physiology (A , respiration, g), biomass production, or total leaf area at low $[\text{CO}_2]$. This may be explained by earlier empirical work (using *Nerium oleander*) showing that the sensitivity of C_3 photosynthesis to increasing leaf temperature is diminished at low $[\text{CO}_2]$ (Berry & Björkman, 1980; see Fig. 4 therein). In the same study, Ward *et al.* (2008) found that C_4 plants (*Amaranthus retroflexus*) were greatly inhibited by the lower glacial temperatures, but still maintained a large absolute growth advantage over the C_3 species in response to low $[\text{CO}_2]$.

2. Water

It has been predicted that low $[\text{CO}_2]$ during glacial periods would have increased the water consumption of C_3 plants, and this has been supported experimentally, mainly through single-generation studies (Baker *et al.*, 1990; Polley *et al.*, 1992, 1993a,b; Beerling & Woodward, 1993; Sage, 1995;

Ward *et al.*, 1999; Tonsor & Scheiner, 2007). Higher water loss is often a result of greater leaf biomass and/or area relative to total plant mass (proposed by Sage & Coleman, 2001; shown in Dippery *et al.*, 1995; Ward *et al.*, 1999 in well-watered plants; also see Medrano *et al.*, 1995 where this response occurred during selection at low [CO₂]), higher *g*, and greater stomatal density that enhance CO₂ uptake on a whole-plant basis, but produce greater water loss. It is also worth noting that despite evidence indicating greater water demand of plants at low [CO₂], precipitation was much lower during glacial periods on a global basis (Yung *et al.*, 1996; Lambert *et al.*, 2008). Such a combination of low water availability and low [CO₂] may have been extremely stressful on plants and may have interacted to greatly reduce productivity during glacial periods.

In one example of work investigating this issue, Ward *et al.* (1999) grew both a C₃ (*A. theophrasti*) and C₄ (*A. retroflexus*) species at 180 (glacial), 270 (pre-industrial), 350 (modern), and 700 (elevated) ppm CO₂ with severe drought treatments. In this case, the authors found that the C₃ species responded to drought by dropping a large number of leaves, and retaining high water potential in remaining leaves at all CO₂ treatments. At 180 ppm CO₂, however, C₃ plants retained relatively greater leaf area (by dropping fewer leaves) and delayed the lowering of *g* following the induction of drought relative to plants grown at 350 ppm CO₂. The combined effects of these responses contributed to the maintenance of a positive carbon budget in the C₃ plants grown at 180 ppm CO₂. Surprisingly, the response of the C₃ species produced similar degrees of relative recovery from drought at low [CO₂] (assessed as biomass before and after drought) as in the C₄ species. Thus, developmental and growth responses to low [CO₂] and drought superseded the effects of physiological responses in this case, producing similar relative recovery of C₃ and C₄ plants that would not have been predicted from theoretical expectations. Note, however, that in this study (Ward *et al.*, 1999) the C₄ species still had much higher biomass on an absolute basis at low [CO₂] compared with the C₃ species.

In the same study (Ward *et al.*, 1999), the C₄ species (*A. retroflexus*) grown under well-watered conditions responded physiologically to changes in [CO₂] with respect to water use. With increasing [CO₂] between 180 and 700 ppm CO₂, the C₄ species showed gradual increases in *A* and decreases in *g* and transpiration that somewhat mimicked the responses of the C₃ species. These physiological responses, however, did not result in enhanced leaf area and biomass production, but did serve to conserve water. *Amaranthus* was previously shown to reach CO₂ saturation at a *c*_i value of 200 ppm CO₂ (Tissue *et al.*, 1995), indicating that this species may not have been CO₂-saturated at the low-CO₂ treatment (180 ppm), explaining why it may have responded physiologically to increasing [CO₂]. Similar to these findings, Polley *et al.* (1994) observed that

Schizachyrium scoparium (C₄) exhibited decreased *g* and had higher *A* between 200 and 340 ppm CO₂, but plant growth remained unaffected. In addition, lower *g* and higher *A* have been observed in *A. retroflexus* (C₄) and *Setaria faberii* (C₄) in response to CO₂ enrichment (Garbutt *et al.*, 1990). Also, when grown in competition, elevated [CO₂] stimulated the biomass production of *A. retroflexus* (C₄) to a greater extent than *A. theophrasti* (C₃) (Bazzaz *et al.*, 1989). These results, as well as others (Cunniff *et al.*, 2008), demonstrate that some C₄ species can be quite physiologically responsive to changes in [CO₂], suggesting that, like C₃ plants, C₄ species may have also had higher water demands during the LGM than in modern times.

3. Nutrients

Very little work has focused on the interactive effects of low [CO₂] with nutrients. As mentioned earlier (Section V.1.), Polley *et al.* (1995) found that across a [CO₂] gradient (200–360 ppm; without N treatments), WUE was decreased much more consistently at low [CO₂] (200 ppm) than was NUE among C₃ annual grasses and a woody perennial. This indicated that the requirements for water and nitrogen may have shifted between the LGM and modern times, with higher water demand appearing to be the more dominant and consistent factor. In a newer study with *Populus deltoids* (cottonwood), Lewis *et al.* (2010) showed that low [CO₂] (200 ppm) inhibited the responses of *A* and biomass production to increases in phosphorus (P), whereas positive responses to P additions were more prominent at modern (350 ppm) and elevated [CO₂] (700 ppm). Even more interesting was the finding that at high P concentrations (0.5 mM), biomass was less enhanced with increases in [CO₂] below the modern value relative to increases above the modern value, reversing the trend for previous studies that did not include P treatments (see Section V.2.). In limiting P scenarios, Campbell & Sage (2006) found that white lupin (*Lupinus albus* L.) was restricted in its ability to respond to increasing [CO₂] above 200 ppm with respect to *A*. As a result of these findings, the authors emphasized that nutrient availability must be factored into models predicting responses of ancient plants to low [CO₂], as well as plant responses to increases in [CO₂] between pre-industrial and modern times.

Sage (1995) predicted that symbiotic relationships (mycorrhizal fungi, N-fixing bacteria) may have been less prevalent during glacial periods in response to low [CO₂]. The high investment of photosynthate required to support symbionts may not have been as beneficial during a period when carbon was potentially more limiting than nutrients. In support of this idea, Polley *et al.* (1994) found that N fixation was reduced for plants (*P. glandulosa*) grown at low [CO₂] compared with modern [CO₂] with the same availability of soil N. Again, this speaks to the potential for

major changes in ecosystem functioning as a result of shifts in limiting resources during low-[CO₂] periods.

VIII. Low-[CO₂] effects on community composition

Past work has incorporated low-[CO₂] effects on community composition, with a focus on shifts in plants possessing the C₃ vs C₄ photosynthetic pathways. From stable carbon isotope ratios, it is clear that low-[CO₂] periods were characterized by major shifts from C₃ to C₄ dominance in some regions (Ehleringer *et al.*, 1991; Cerling *et al.*, 1997, 1998; Street-Perrott *et al.*, 1997; Flores *et al.*, 2009). Other studies, however, have concluded that climate (temperature, precipitation) may have been a stronger force behind C₃–C₄ shifts relative to low [CO₂] (Morgan *et al.*, 1994; Latorre *et al.*, 1997; Pagani *et al.*, 1999; Huang *et al.*, 2001, 2006; Keeley & Rundel, 2005; Flores *et al.*, 2009; Edwards *et al.*, 2010). Many of these discrepancies may be explained by the regional conditions of each study, and whether or not water availability and temperature further enhanced or negated the transition to C₄ dominance.

Past studies have also investigated ancient shifts in plant communities in response to low [CO₂] via empirical studies with pollen records, as well as modeling efforts. Using a process-based vegetation model, Jolly & Haxeltine (1997) predicted a dominance of xerophytic scrub in East Africa during the LGM compared with the current montane forest. In this case, modeled results showed that lowering [CO₂] to the LGM value of 190 ppm produced a shift to the LGM scrub community, even under modern temperature and precipitation values (and may explain the enhanced African scrub belt from LGM pollen records). For this reason, the authors concluded that paleoclimate reconstructions based on present climate analogs may be unreliable if they do not incorporate the direct effects of low [CO₂] on vegetation.

In a different study, Levis *et al.* (1999) predicted that low [CO₂] of the past 21 000 yr favored grasslands and tundra over the forests currently located at temperate and boreal latitudes. These changes would have greatly affected evapotranspiration and possibly the entire water cycle of the region. This result suggested that large-scale changes in the characteristics of canopy cover (e.g. leaf area index, LAI) in response to low [CO₂] may have had a more overwhelming effect on ecosystem functioning than smaller-scale physiological changes within individual plants (Levis *et al.*, 1999). Furthermore, Cowling *et al.* (2008) modeled vegetation distributions in central Africa during the LGM and predicted that even though tropical broadleaf forests were not displaced during the LGM (with the exception of grassland encroachment on the edges), structural changes in forest canopy characteristics (e.g. lower LAI, tree height, and density) may have greatly altered ecosystem functioning. This

change may have further impacted the migration patterns of numerous plant and animal species, including *Homo sapiens*. Taken together, these studies show that low [CO₂] can influence the characteristics of community structure, ranging from alterations in canopy characteristics, shifts in C₃ vs C₄ dominance, to changes in biome structure.

IX. Low-[CO₂] effects on the ecosystem

In order to better understand the effects of low [CO₂] on whole ecosystems, global climate and biosphere models have been used to predict glacial values of NPP, terrestrial carbon storage, and isoprene emissions. The following section summarizes the results of these modeling efforts and discusses the benefits and limitations of scaling low-[CO₂] effects on individual plants to the ecosystem level.

Using a mass-balance carbon isotope approach, Bird *et al.* (1994) constrained the increase in terrestrial carbon storage between the LGM and pre-industrial period to 310–355 Gt (or 270–720 Gt when oceanic δ¹³C uncertainties were incorporated), which supported previous estimates made by others using different approaches (e.g. Friedlingstein *et al.*, 1992; Prentice *et al.*, 1993; Van Campo *et al.*, 1993). Later, François *et al.* (1998) coupled the CARAIB vegetation model to a general circulation model (ECHAM) in order to understand changes in terrestrial NPP and carbon storage across glacial–interglacial boundaries. Their modeling efforts predicted that NPP values increased from 38 Gt C yr⁻¹ during the LGM to 53 Gt C yr⁻¹ during the pre-industrial period; in addition, their estimates of changes in carbon storage overlapped that of Bird *et al.* (1994). François *et al.* (1998) attributed the possible low NPP during the LGM to changes in land area, cooler temperatures, and effects of low [CO₂] on vegetation. In a subsequent analysis, they predicted that low-[CO₂] effects on vegetation were the dominant factor in reducing NPP during the LGM, and a later sensitivity analysis supported this finding (Otto *et al.*, 2002; François *et al.*, 2006). In addition, Turcq *et al.* (2002) predicted from modeling efforts that carbon storage in Amazonia ranged between 44 and 94% of modern amounts, and this reduction was attributed to the effects of low [CO₂] on vegetation, as well as cooler and drier conditions.

Harrison & Prentice (2003; using BIOME4) predicted that the effects of low [CO₂] on plants may have reduced tropical forest cover by 44–69% during the LGM. Before this, Levis *et al.* (1999) also predicted that increases in C₄ grasslands during the LGM, which occurred at the expense of forests, produced positive feedbacks associated with albedo that resulted in warmer and drier ecosystems, particularly in the tropics. Using a global-vegetation model, Crucifix *et al.* (2004) also found indications of major changes in vegetation distribution between the LGM and the pre-industrial period, with specific disappearance of the

Siberian boreal forest, greater shrub cover in Europe, and an expansion of subtropical desert land area. Much of this change was attributed to differential effects of low $[\text{CO}_2]$ on plant physiology and water use, where grasses and shrubs had lower water requirements during glacial conditions than trees. By comparing modeled estimates to empirical continent-scale palaeoclimate data, Jolly & Haxeltine (1997) stated that efforts to determine paleo-temperatures based on tree-line depression may be faulty, and may overestimate reductions in temperature if low- $[\text{CO}_2]$ effects on vegetation are ignored. Taken together, these studies support the recent plea by Prentice & Harrison (2009) to incorporate direct physiological effects of low $[\text{CO}_2]$ on plants more widely when modeling past climates and ecosystem functioning, because failure to do so may result in erroneous conclusions.

Global vegetation models have also been used to better understand how changes in $[\text{CO}_2]$ may have affected post-fire recovery rates of trees and grasses from the LGM through modern times. In South African grasslands, Bond *et al.* (2003) predicted from modeling efforts that low $[\text{CO}_2]$ of the LGM reduced sapling growth to the point where young trees could not reach a fire-proof size between fire events, resulting in the elimination of trees in favor of C_4 grasses. Furthermore, increased $[\text{CO}_2]$ during the Holocene may have allowed for the reappearance of savanna trees (Bond *et al.*, 2003). Moreover, this study predicts that the interactive effects of low $[\text{CO}_2]$ and disturbance factors may have altered plant establishment patterns, which is an issue that had not been previously appreciated.

Plant emissions of trace gases can be influenced by changes in $[\text{CO}_2]$, climate, and other environmental factors, and can also feed back to influence climate change through the production of organic aerosols (Possell *et al.*, 2005). In particular, isoprene emissions constitute approximately half of the biogenic volatile organic compounds released by the terrestrial biosphere, and therefore should be incorporated into the glacial–interglacial transition. To address this issue, Possell *et al.* (2005) grew the known isoprene-emitters *Mucuna pruriens* and *Arundo donax* in growth chambers at glacial (180 ppm), pre-industrial (280 ppm), and modern (366 ppm) $[\text{CO}_2]$. On a leaf area basis, the lowest CO_2 treatment produced a threefold increase in isoprene production relative to modern $[\text{CO}_2]$. However, low $[\text{CO}_2]$ -induced reductions in above-ground biomass and LAI offset this increase, such that total canopy isoprene emissions showed no significant change between low and modern $[\text{CO}_2]$. When cooler conditions of the LGM were incorporated, isoprene emissions were significantly reduced at the canopy level and on a leaf mass basis, but remained similar across all CO_2 treatments. In a more recent study, Wilkinson *et al.* (2009) showed that Eucalyptus trees had significantly higher isoprene emissions at 240 ppm than at 380 ppm, while sweetgum showed no significant change

across this gradient. It is still not clear why plants exhibit differential isoprene emissions in response to low $[\text{CO}_2]$ or even how best to represent these changes in modeling efforts. This emphasizes the need to better understand the interactive effects of climate and $[\text{CO}_2]$ on trace gas emissions of the past.

On a different note, little is known about how reduced $[\text{CO}_2]$ may have influenced the availability and quality (e.g. C : N) of food sources to herbivores and how shifts in vegetation may have affected animal diets during glacial periods (Ehleringer *et al.*, 2002). The majority of studies dealing with these issues are mainly focused on other time periods such as the Cenozoic (MacFadden, 2005) and late Miocene–Pliocene boundary (Cerling *et al.*, 1997). Hopefully in the future, more attention can be focused on the effects of low $[\text{CO}_2]$ on glacial animals.

In summary, a variety of modeling studies show strong evidence for reductions in NPP and carbon storage during glacial periods, with low $[\text{CO}_2]$ playing a major role in these changes. Much of this modeling work relies on empirical and theoretical changes in A and g with $[\text{CO}_2]$, mainly from Farquhar *et al.* (1980). As mentioned earlier, there tends to be a close correlation between A and growth (and possibly reproductive output) at low $[\text{CO}_2]$. Therefore, the sole use of physiological data for estimating glacial NPP and carbon storage has validity, and is probably accurately representing our understanding of single-generation responses of modern plants to past $[\text{CO}_2]$. It is quite possible, however, that as we gain further insight into our understanding of plant evolutionary responses to low $[\text{CO}_2]$, these large-scale estimates may need to be revised. As illustrated earlier (Section VI), Ward *et al.* (2000) found a 34% increase in plant biomass at 200 ppm CO_2 following only five generations of selection for high fitness. Unfortunately, very few estimates of this kind exist. Thus, as we learn more about potential adaptive responses to low $[\text{CO}_2]$, these should be incorporated into future modeling efforts.

X. Low- $[\text{CO}_2]$ effects on early human societies

The emergence of agriculture among early human societies appeared throughout the world between 5000 and 10 000 yr ago, and this represents a rather short time span with respect to evolutionary change, particularly for crop plants. Sage (1995) proposed that such synchronous origins may have been the result of a common global factor, more specifically the rise in $[\text{CO}_2]$ from 200 to 270 ppm that occurred between 15 000 and 10 000 yr ago (Fig. 9). In this section, we discuss the role that low $[\text{CO}_2]$ may have played in the emergence of agriculture.

In the past, anthropological literature had mostly attributed the onset of agriculture to changes in human social and cultural structure and complexity (Bar-Yosef, 1998; Mannion, 1999). Although this has been a long-standing

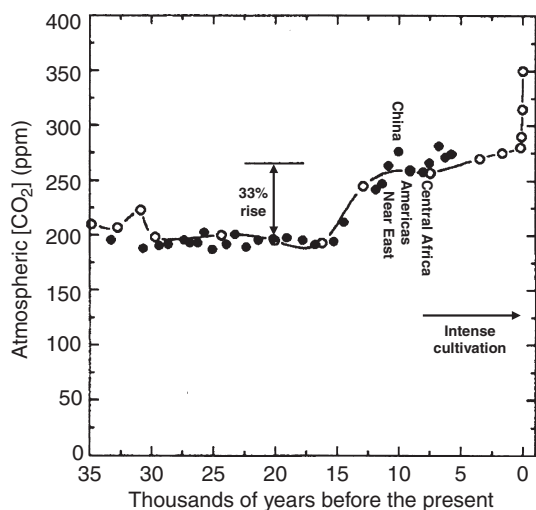


Fig. 9 The timing for the emergence of agriculture among different human societies and changes in atmospheric $[\text{CO}_2]$ through time. Geographic names indicate the period when domesticated plants first appear in the archeological record for that region (from Sage, 1995, Fig. 1).

idea, cultural mechanisms were unlikely to have occurred simultaneously throughout all societies, and therefore this idea does not fully explain the synchronous emergence of agriculture (Cunniff *et al.*, 2008). Other ideas have incorporated the effects of changing palaeoclimates, such as increasing precipitation and temperature (Bar-Yosef, 1998), although often, these are in the context of how climate impacted human society and not the crops themselves (Bar-Yosef, 1998). Although changes in climate are influential in their impact on human culture, a sole focus on this aspect of environmental change ignores the potentially large and direct effects of $[\text{CO}_2]$ on crops.

A plethora of past work strongly suggests that the increase in $[\text{CO}_2]$ that occurred between 15 000 and 10 000 yr ago may have been large enough to have had a profound impact on crop productivity, and hence on human subsistence patterns (Sage, 1995; Fig. 9). In general, glacial conditions would have been a hostile environment for C_3 crops because of low $[\text{CO}_2]$, as well as drier soils and higher seasonal variation (Richerson *et al.*, 2001). As the interglacial period commenced, the onset of rising $[\text{CO}_2]$ and other climatic changes would have removed an environmental limitation to the development of agriculture (Sage, 1995). Increasing $[\text{CO}_2]$ during the interglacial period may have directly enhanced plant productivity and may have reduced the effects of interactive stressors, such that crop production could be sustained year after year within human societies (Sage, 1995).

Anthropologists are beginning to incorporate the importance of CO_2 into their ideas on agricultural development. For example, Bettinger *et al.* (2009) attributed the development of agriculture to the combined effects of climatic and

cultural changes, including increasing $[\text{CO}_2]$. He commented that once the external environmental constraints were removed, social innovation and more organized human communities became the predominant driver for the development of agriculture (Bettinger *et al.*, 2009).

The importance of $[\text{CO}_2]$ in agricultural origins has met with some debate, mainly since some early crops were C_4 (although these were in the minority), and it was initially thought that they would not have responded to increasing $[\text{CO}_2]$ (Cunniff *et al.*, 2008). By contrast, however, a number of studies have shown that C_4 species experience up to a 40% increase in biomass production between glacial and interglacial $[\text{CO}_2]$, as well as a 35% reduction in g_s , indicating that C_4 species may have been strong responders to this rise in $[\text{CO}_2]$ (Cunniff *et al.*, 2008). In addition, increasing WUE and A with rising $[\text{CO}_2]$ would have been most pronounced in the hot climates where development of C_4 crops first occurred (e.g. millets in north China, sugarcane in New Guinea). Future research may be able to tease apart the impacts of $[\text{CO}_2]$, climate change, and cultural development on the emergence of agriculture among different societies. Such work will be enhanced by stronger collaboration among anthropologists, plant physiologists, and ecologists.

XI. Conclusions

From the studies highlighted in this review, as well as others, we now know that the influence of low $[\text{CO}_2]$ during glacial periods transcends many levels, ranging from physiological effects on individual plants to changes in ecosystem functioning, and may have even influenced early agricultural development. Results of these studies provide a baseline for plant response to minimal $[\text{CO}_2]$ that occurred during the evolution of land plants. Within a single generation of exposure to low $[\text{CO}_2]$, modern C_3 plants show an average reduction in photosynthesis and biomass production of 50% (although this can range from 40 to 70% among species/genotypes). When scaled up in space and time, these physiological responses imply large reductions in NPP and carbon storage during glacial periods relative to the present, as well as changes in vegetation distributions, post-fire recovery, and trace gas emissions.

Despite these advances, we still have much to learn with respect to plant evolution at low $[\text{CO}_2]$. Although numerous environmental factors were changing between glacial and interglacial periods, it is clear that CO_2 alone can act as a strong selective agent on plants. For example, productivity increased by 34% within a C_3 model system (*Arabidopsis*) after only five generations of selection for high fitness at low $[\text{CO}_2]$ (Ward *et al.*, 2000). The potential for rapid genetic change in response to low $[\text{CO}_2]$ is great and carries implications for our understanding of ancient plant productivity and ecosystem functioning. For these reasons, an improved understanding of plant adaptation to changes in $[\text{CO}_2]$ is

necessary to accurately predict plant function under both past and future conditions, and such information must eventually be incorporated into modeling efforts.

Overall, a better understanding of plant responses to low [CO₂] will help us to understand how plants acclimated and adapted to changing carbon resources over geologic time scales. In order to advance our understanding in this area, it will be necessary to develop larger and more realistic growth environments, include more disciplines in order to move this field forward, extend experimental durations to allow for full acclimation and adaptation of plants, and continue studying the interactions between low [CO₂] other environmental factors. Moreover, this will allow us to better predict future plant responses to anthropogenic increases in [CO₂], since changes in plants that were a result of low-[CO₂] periods in the past may still influence the potential for plants to fully utilize increasing carbon resources in the future.

Acknowledgements

The authors would like to thank the National Science foundation (CAREER award: 0746822), as well as the Wohlgemuth Faculty Scholar Award (with an endowment made possible by Ms Dorothy Lynch), and the Department of Ecology & Evolutionary Biology at the University of Kansas (KU) for support during the writing of this manuscript. In addition, LMG was supported by the C-CHANGE IGERT fellowship from the National Science Foundation (0801522) and from the Madison and Lila Self Graduate Fellowship at KU.

References

- Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties, and plant production to rising CO₂. *New Phytologist* 165: 351–372.
- Allen LH Jr, Bisbal EC, Boote KJ. 1998. Nonstructural carbohydrates of soybean plants grown in subambient and superambient levels of CO₂. *Photosynthesis Research* 56: 143–155.
- Allen LH Jr, Bisbal EC, Boote KJ, Jones PH. 1991. Soybean dry matter allocation under subambient and superambient levels of carbon dioxide. *Agronomy Journal* 83: 875–883.
- Baker JT, Allen LH Jr, Boote KJ, Jones PH, Jones JW. 1990. Rice photosynthesis and evapotranspiration in subambient, ambient, and superambient carbon dioxide concentrations. *Agronomy Journal* 82: 834–840.
- Bar-Yosef O. 1998. The Natufian culture in the Levant, threshold to the origins of agriculture. *Evolutionary Anthropology* 6: 159–177.
- Bazzaz FA, Garbutt K, Reekie EG, Williams WE. 1989. Using growth analysis to interpret competition between a C₃ and C₄ annual under ambient and elevated CO₂. *Oecologia* 79: 223–235.
- Beerling DJ. 1996. Ecophysiological responses of woody plants to past CO₂ concentrations. *Tree Physiology* 16: 389–396.
- Beerling DJ. 2005. Evolutionary responses of land plants to atmospheric CO₂. In: Ehleringer JR, Cerling TE, Dearing MD, eds. *A history of atmospheric CO₂ and its effects on plants, animals and ecosystems*. New York, NY, USA: Springer, 114–132.
- Beerling DJ, Chaloner WG. 1993. Evolutionary responses of stomatal density to global CO₂ change. *Biological Journal of the Linnean Society* 48: 343–353.
- Beerling DJ, Chaloner WG, Huntley B, Pearson JA, Tooley MJ. 1993a. Stomatal density responds to the glacial cycle of environmental change. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 251: 133–138.
- Beerling DJ, Matthey DP, Chaloner WG. 1993b. Shifts in the d¹³C composition of *Salix herbacea* L. leaves in response to spatial and temporal gradients of atmospheric CO₂ concentration. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 253: 53–60.
- Beerling DJ, Osborne CP, Chaloner WG. 2001. Evolution of leaf form in land plants linked to atmospheric CO₂ decline in the late Palaeozoic Era. *Nature* 410: 352–354.
- Beerling DJ, Woodward FI. 1993. Ecophysiological responses of plants to global environmental change since the Last Glacial Maximum. *New Phytologist* 125: 641–648.
- Bergmann DC, Sack FD. 2007. Stomatal development. *Annual Review of Plant Biology* 58: 163–181.
- Berner RA. 2003. The long-term carbon cycle, fossil fuels and atmospheric composition. *Nature* 426: 323–326.
- Berner RA. 2006. GEOCARBSULF: a combined model for Phanerozoic atmospheric O₂ and CO₂. *Geochimica et Cosmochimica Acta* 70: 5653–5664.
- Berner RA, Van den Brooks M, Ward PD. 2007. Oxygen and evolution. *Science* 27: 557–558.
- Berry JA, Björkman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology* 31: 491–543.
- Bettarini I, Vaccari FP, Miglietta F. 1998. Elevated CO₂ concentrations and stomatal density: observations from 17 plant species growing in a CO₂ spring in central Italy. *Global Change Biology* 4: 17–22.
- Bettinger RL, Richerson PJ, Boyd R. 2009. Constraints on the development of agriculture. *Current Anthropology* 50: 627–631.
- Billings WD, Clebsch EEC, Mooney HA. 1961. Effect of low concentrations of carbon dioxide on photosynthesis rates of two races of *Oxyria*. *Science* 133: 1834.
- Bird MI, Lloyd J, Farquhar GD. 1994. Terrestrial carbon storage at the LGM. *Nature* 371: 566.
- Bond WJ, Midgley FF, Woodward FI. 2003. The importance of low atmospheric CO₂ and fire in promoting the spread of grasslands and savannas. *Global Change Biology* 9: 973–982.
- Bone E, Farres A. 2001. Trends and rates of microevolution in plants. *Genetica* 112: 165–182.
- Brook EJ. 2005. Tiny bubbles tell all. *Science* 310: 1285–1287.
- Bunce JA. 2001. Are annual plants adapted to the current atmospheric concentration of carbon dioxide? *International Journal of Plant Science* 162: 1261–1266.
- Campbell CD, Sage RF. 2006. Interactions between the effects of atmospheric CO₂ content and P nutrition on photosynthesis in white lupin (*Lupinus albus* L.). *Plant, Cell & Environment* 29: 844–853.
- Campbell CD, Sage RF, Kocacinar F, Way DA. 2005. Estimation of the whole-plant CO₂ compensation point of tobacco (*Nicotiana tabacum* L.). *Global Change Biology* 11: 1956–1967.
- Cannell RA, Brun WA, Moss DN. 1969. A search for high net photosynthetic rate among soybean genotypes. *Crop Science* 9: 840–841.
- Cerling TE, Ehleringer JR, Harris JM. 1998. Carbon dioxide starvation, the development of C₄ ecosystems and mammalian evolution. *Philosophical Transactions of the Royal Society B-Biological Sciences* 353: 159–171.

- Cerling TE, Harris JM, MacFadden BJ, Leacey MG, Quade J, Eisenmann V, Ehleringer JR. 1997. Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389: 153–158.
- Coleman JR. 2000. Carbon anhydrase and its role in photosynthesis. In: Sharkey TD, Leegood RC, von Caemmerer S, eds. *Photosynthesis: physiology and metabolism*. New York, NY, USA: Springer, 353–367.
- Coltraine JB, Harris JM, Cerling TE, Ehleringer JR, Dearing MD, Ward JK, Allen J. 2004. Rancho La Brea stable isotope biogeochemistry and its implications for the palaeoecology of late Pleistocene, coastal southern California. *Palaeogeography, Palaeoclimatology, Palaeoecology* 205: 199–219.
- Cowling SA. 2001. Plant carbon balance, evolutionary innovation and extinction in land plants. *Global Change Biology* 7: 231–240.
- Cowling SA, Cox PM, Jones CD, Maslin MA, Peros M, Spall SA. 2008. Simulated glacial and interglacial vegetation across Africa: implications for species phylogenies and trans-African migration of plants and animals. *Global Change Biology* 14: 827–840.
- Cowling SA, Sage RF. 1998. Interactive effects of low atmospheric CO₂ and elevated temperature on growth, photosynthesis and respiration in *Phaseolus vulgaris*. *Plant, Cell & Environment* 21: 427–435.
- Cowling SA, Sykes MT. 1999. Physiological significance of low atmospheric CO₂ for plant-climate interactions. *Quaternary Research* 52: 237–242.
- Crucifix M, Betts RA, Hewitt CD. 2004. Pre-industrial-potential and Last Glacial Maximum global vegetation simulated with a coupled climate-biosphere model: diagnosis of bioclimatic relationships. *Global and Planetary Change* 45: 295–312.
- Cunniff J, Osborne CP, Ripley BS, Charles M, Jones G. 2008. Response of wild C₄ crop progenitors to subambient CO₂ highlights a possible role in the origin of agriculture. *Global Change Biology* 14: 576–587.
- Delgado E, Vadell J, Medrano H. 1994. Photosynthesis during leaf ontogeny in field-grown *Nicotiana tabacum* L. lines selected for survival at low CO₂ concentrations. *Journal of Experimental Botany* 45: 547–552.
- Dippery JK, Tissue DT, Thomas RB, Strain BR. 1995. Effects of low and elevated CO₂ on C₃ and C₄ annuals. I. Growth and biomass allocation. *Oecologia* 101: 13–20.
- Edwards EJ, Osborne CP, Strömberg CAE, Smith SA, C₄ Grasses Consortium. 2010. The origins of C₄ grasslands: integrating evolutionary and ecosystem science. *Science* 328: 587–591.
- Ehleringer JR, Cerling TE. 1993. Atmospheric CO₂ and the ratio of intercellular to ambient CO₂ concentrations in plants. *Tree Physiology* 15: 105–111.
- Ehleringer JR, Cerling TE, Dearing MD. 2002. Atmospheric CO₂ as a global change driver influencing plant-animal interactions. *Integrative and Comparative Biology* 42: 424–430.
- Ehleringer JR, Cerling TE, Helliker BR. 1997. C₄ photosynthesis, atmospheric CO₂, and climate. *Oecologia* 112: 285–299.
- Ehleringer JR, Sage RF, Flanagan LB, Pearcy RW. 1991. Climate change and the evolution of C₄ photosynthesis. *Trends in Ecology and Evolution* 6: 95–99.
- EPICA. 2004. Eight glacial cycles from an Antarctic ice core. *Nature* 429: 623–628.
- Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149: 78–90.
- Flexas J, Ortúño MF, Ribas-Carbó M, Diaz-Espejo A, Flórez-Sarasa ID, Medrano H. 2007. Mesophyll conductance to CO₂ in *Arabidopsis thaliana*. *New Phytologist* 175: 501–511.
- Flores O, Gritti ES, Jolly D. 2009. Climate and CO₂ modulate the C₃-C₄ balance and δ¹³C signal in simulated vegetation. *Climate of the Past Discussions* 5: 1187–1213.
- François LM, Delire C, Warnant P, Munhoven G. 1998. Modeling the glacial-interglacial changes in the continental biosphere. *Global and Planetary Change* 16–17: 37–52.
- François LM, Ghislain M, Otto D, Micheels A. 2006. Late Miocene vegetation reconstruction with the CARAIB model. *Palaeogeography, Palaeoclimatology, Palaeoecology* 238: 302–320.
- Friedlingstein P, Delire C, Müller JF, Gerard JC. 1992. The climate-induced variation of the continental biosphere: a model simulation of the Last Glacial Maximum. *Geophysical Research Letters* 19: 897–900.
- Gale J. 1972. Availability of carbon dioxide for photosynthesis at high altitudes: theoretical considerations. *Ecology* 53: 494–497.
- Garbutt K, Williams WE, Bazzaz FA. 1990. Analysis of the differential response of five annuals to elevated CO₂ during growth. *Ecology* 71: 1185–1194.
- Gesch RW, Vu JCV, Boote KJ, Allen LH, Bowes G. 2000. Subambient growth at CO₂ leads to increased Rubisco small subunit gene expression in developing rice leaves. *Journal of Plant Physiology* 157: 235–238.
- Ghannoum O, Phillips NG, Conroy JP, Smith RA, Attard RD, Woodfield R, Logan BA, Lewis JD, Tissue DT. 2010. Exposure to preindustrial, current and future atmospheric CO₂ and temperature differentially affects growth and photosynthesis in *Eucalyptus*. *Global Change Biology* 16: 303–319.
- González-Meler MA, Blanc-Betes E, Flower CE, Ward JK, Gomez-Casanovas N. 2009. Plastic and adaptive responses of plant respiration to changes in atmospheric CO₂ concentration. *Physiologia Plantarum* 137: 473–484.
- Gray JE, Holroyd GH, van der Lee FM, Bahrami AR, Sijmons PC, Woodward FI, Schuch W, Hetherington AM. 2000. The HIC signalling pathway links CO₂ perception to stomatal development. *Nature* 408: 713–716.
- Griffiths H. 2006. Designs on Rubisco. *Nature* 441: 940–941.
- Harrison SP, Prentice IC. 2003. Climate and CO₂ controls on global vegetation distribution at the last glacial maximum: analysis based on palaeovegetation data, biome modeling and palaeoclimate simulations. *Global Change Biology* 9: 983–1004.
- Hovenden MJ, Schimanski LJ. 2000. Genotypic differences in growth and stomatal morphology of Southern Beech, *Northofagus cunninghamii*, exposed to depleted CO₂ concentrations. *Australian Journal of Plant Physiology* 27: 281–287.
- Huang Y, Shuman B, Wang Y, Webb T III, Grimm EC, Jacobson GL Jr. 2006. Climatic and environmental controls on the variation of C₃ and C₄ plant abundances in central Florida for the past 62,000 years. *Palaeogeography, Palaeoclimatology, Palaeoecology* 237: 428–435.
- Huang Y, Street-Perrott FA, Metcalfe SE, Brenner M, Moreland M, Freeman KH. 2001. Climate change as the dominant control on glacial-interglacial variations in C₃ and C₄ plant abundance. *Science* 293: 1647–1651.
- IPCC. 2007a. Synthesis report. In: **Core Writing Team**, Pachauri RK, Reisinger A, eds. *Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge, UK and NY, USA: Cambridge University Press: 23–73.
- IPCC. 2007b. *Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge, UK and NY, USA: Cambridge University Press.
- Jolly D, Haxeltine A. 1997. Effect of low glacial atmospheric CO₂ on tropical African montane vegetation. *Science* 276: 786–788.
- Keeley JE, Rundel PW. 2005. Fire and the Miocene expansion of C₄ grasslands. *Ecology Letters* 8: 683–690.
- Keeling CD, Piper SC, Bacastow RB, Wahlen M, Whorf TP, Heimann M, Meijer HA. 2005. Atmospheric CO₂ and ¹³CO₂ exchange with the terrestrial biosphere and oceans from 1978 to 2000: observations and carbon cycle implications. In: Ehleringer JR, Cerling TE, Dearing MD, eds. *A history of atmospheric CO₂ and its effects on plants, animals, and ecosystems*. New York, NY, USA: Springer, 83–113.
- Knapp AK, Cocks M, Hamerlynck EP, Owensby CE. 1994. Effect of elevated CO₂ on stomatal density and distribution in a C₄ grass and a C₃ forb under field conditions. *Annals of Botany* 74: 595–599.

- Körner C. 1988. Does global increase of CO₂ alter stomatal density? *Flora* 181: 252–257.
- Körner C. 2006. Plant CO₂ responses: an issue of definition, time and resource supply. *New Phytologist* 172: 393–411.
- Körner C, Diemer M. 1994. Evidence that plants from high altitudes retain their greater photosynthetic efficiency under elevated CO₂. *Functional Ecology* 8: 58–68.
- Lambert F, Delmonte B, Petit JR, Bigler M, Kaufmann PR, Hutterli MA, Stocker TF, Ruth U, Steffensen JP, Maggi V. 2008. Dust-climate couplings over the past 800,000 years from the EPICA Dome C ice core. *Nature* 452: 616–619.
- Latorre C, Quade J, McIntosh WC. 1997. The expansion of C₄ grasses and global change in the late Miocene: stable isotope evidence from the Americas. *Earth and Planetary Science Letters* 146: 83–96.
- Lehmeier CA, Schauffele R, Schnyder H. 2005. Allocation of reserve-derived and currently assimilated carbon and nitrogen in seedlings of *Helianthus annuus* under subambient and elevated CO₂ growth conditions. *New Phytologist* 168: 613–621.
- Lewis S, Foley JA, Pollard D. 1999. CO₂, climate, and vegetation feedbacks at the Last Glacial Maximum. *Journal of Geophysical Research* 104: 31191–31198.
- Lewis JD, Ward JK, Tissue DT. 2010. Phosphorus supply drives nonlinear responses of cottonwood (*Populus deltoides*) to glacial through future [CO₂]. *New Phytologist* 187: 438–448.
- Lloyd J, Farquhar GD. 2008. Effects of rising temperatures and [CO₂] on the physiology of tropical forest trees. *Philosophical Transactions of the Royal Society B-Biological Sciences* 363: 1811–1817.
- Lloyd J, Syversten JP, Kriedemann PE, Farquhar GD. 1992. Low conductances for CO₂ diffusion from stomata to the sites of carboxylation in leaves of woody species. *Plant, Cell & Environment* 15: 873–899.
- Lüthi D, Le Floch M, Bereiter B, Blunier T, Barnola J-M, Siegenthaler U, Raynaud D, Jouzel J, Fischer H, Kawamura K *et al.* 2008. High-resolution carbon dioxide concentration record 650,000–800,000 years before present. *Nature* 453: 379–382.
- MacFadden BJ. 2005. Terrestrial mammalian herbivore response to declining levels of atmospheric CO₂ during the Cenozoic: evidence from North American fossil horses (family Equidae). In: Ehleringer JR, Cerling TE, Dearing MD, eds. *A history of atmospheric CO₂ and its effects on plants, animals and ecosystems*. New York, NY, USA: Springer, 273–292.
- Maherali H, Reid CD, Polley HW, Johnson HB, Jackson RB. 2002. Stomatal acclimation over a subambient to elevated CO₂ gradient in a C₃/C₄ grassland. *Plant, Cell & Environment* 25: 557–566.
- Mannion AM. 1999. Domestication and the origins of agriculture: an appraisal. *Progress in Physical Geography* 23: 37–56.
- Mayeux HS, Johnson HB, Polley HW, Dumesnil MJ, Spanel GA. 1993. A controlled environment chamber for growing plants across a subambient CO₂ gradient. *Functional Ecology* 7: 125–133.
- McElwain JC, Willis KJ, Lupia R. 2005. Cretaceous CO₂ decline and the radiation and diversification of angiosperms. In: Ehleringer JR, Cerling TE, Dearing MD, eds. *A history of atmospheric CO₂ and its effects on plants, animals, and ecosystems*. New York, NY, USA: Springer, 133–165.
- McLeod AR, Long SP. 1999. Free-air carbon dioxide enrichment (FACE) in global change research: a review. *Advances in Ecological Research* 28: 1–56.
- Medrano H, Keys AJ, Lawlor DW, Parry MAJ, Azcón-Bieto J, Delgado E. 1995. Improving plant production by selection for survival at low CO₂ concentrations. *Journal of Experimental Botany* 46: 1389–1396.
- Metz J, Liancourt P, Kigel J, Harel D, Sternberg M, Tielborger K. 2010. Plant survival in relation to seed size along environmental gradients: a long-term study from semi-arid and Mediterranean annual plant communities. *Journal of Ecology* 98: 697–704.
- Miyagi KM, Kinugasa T, Hikosaka K. 2007. Elevated CO₂ concentration, nitrogen use, and seed production in annual plants. *Global Change Biology* 13: 2161–2170.
- Mohan JE, Clark JS, Schlesinger WH. 2004. Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO₂. *Global Change Biology* 10: 233–247.
- Monnin E, Indermühle A, Dällenbach A, Flückiger J, Stauffer B, Stocker TF, Raynaud D, Barnola J-M. 2001. Atmospheric CO₂ concentrations over the Last Glacial Termination. *Science* 291: 112–114.
- Morgan ME, Kingston JD, Marino BD. 1994. Carbon isotopic evidence for the emergence of C₄ plants in the Neogene from Pakistan and Kenya. *Nature* 367: 162–165.
- Moss DN. 1962. The limiting carbon dioxide concentration for photosynthesis. *Nature* 193: 587.
- Nelson CJ, Asay KH, Patton LD. 1975. Photosynthetic responses of tall fescue to selection for longevity below the CO₂ compensation point. *Crop Science* 15: 629–633.
- Otto D, Rasse D, Kaplan J, Warnant P, François LM. 2002. Biospheric carbon stocks reconstructed at the Last Glacial Maximum: comparison between general circulation models using prescribed and computed sea surface temperatures. *Global and Planetary Change* 33: 117–138.
- Overdieck D. 1989. The effects of preindustrial and predicted future atmospheric CO₂ concentration on *Lyonia mariana* L.D. Don. *Functional Ecology* 3: 569–576.
- Overdieck D, Reid CD, Strain BR. 1988. The effects of preindustrial and future CO₂ concentrations on growth, dry matter production and C/N relationship in plants at low nutrient supply: *Vigna unguiculata* (cowpea), *Abgelmochus esculentus* (okra) and *Raphanus sativus* (radish). *Angewandte Botanik* 62: 119–134.
- Pagani M, Freeman KH, Arthur MA. 1999. Late Miocene atmospheric CO₂ concentrations and the expansion of C₄ grasses. *Science* 285: 876–879.
- Pedicino LC, Leavitt SW, Betancourt JL, Van de Water PK. 2002. Historical variations in δ¹³C_{leaf} of herbarium specimens in the southwestern U.S. *Western North American Naturalist* 62: 348–359.
- Petit JR, Raynaud D, Barkov NI, Barnola JM, Basile I, Bender M, Chappellaz J, Davis M, Delaygue G, Delmotte M *et al.* 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399: 429–436.
- Polley HW, Johnson HB, Derner JD. 2002. Soil- and plant-water dynamics in a C₃/C₄ grassland exposed to a subambient to superambient CO₂ gradient. *Global Change Biology* 8: 1118–1129.
- Polley HW, Johnson HB, Marino BD, Mayeux HS. 1993a. Increase in C₃ plant water-use efficiency and biomass over glacial to present CO₂ concentrations. *Nature* 361: 61–64.
- Polley HW, Johnson HB, Mayeux HS. 1992. Carbon dioxide and water fluxes of C₃ annuals and C₃ and C₄ perennials at subambient CO₂ concentrations. *Functional Ecology* 6: 693–703.
- Polley HW, Johnson HB, Mayeux HS. 1994. Increasing CO₂: comparative responses of the C₄ grass *Schizachyrium* and grassland invader *Prosopis*. *Ecology* 75: 976–988.
- Polley HW, Johnson HB, Mayeux HS. 1995. Nitrogen and water requirements of C₃ plants grown at glacial to present carbon dioxide concentrations. *Functional Ecology* 9: 86–96.
- Polley HW, Johnson HB, Mayeux HS, Malone SR. 1993b. Physiology and growth of wheat across a subambient carbon dioxide gradient. *Annals of Botany* 71: 347–356.
- Possell M, Hewitt CN, Beerling DJ. 2005. The effects of glacial atmospheric CO₂ concentrations and climate on isoprene emissions by vascular plants. *Global Change Biology* 11: 60–69.
- Prentice IC, Harrison SP. 2009. Ecosystem effects of CO₂ concentration: evidence from past climates. *Climate of the Past* 5: 297–307.

- Prentice IC, Sykes MT, Lautenschlager M, Harrison SP, Denissenko O, Barlein PJ. 1993. Modeling global vegetation patterns and terrestrial carbon storage at the last glacial maximum. *Global Ecology and Biogeography Letters* 3: 67–76.
- Richerson PJ, Boyd R, Bettinger RL. 2001. Was agriculture impossible during the Pleistocene but mandatory during the Holocene? A climate change hypothesis. *American Antiquity* 66: 387–411.
- Roth-Nebelsick A. 2005. Reconstructing atmospheric carbon dioxide with stomata: possibilities and limitations of a botanical pCO₂-sensor. *Trees-Structure and Function* 19: 251–265.
- Royer DL. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration. *Review of Palaeobotany and Palynology* 114: 1–28.
- Sage RF. 1994. Acclimation of photosynthesis to increasing atmospheric CO₂: the gas exchange perspective. *Photosynthesis Research* 39: 351–368.
- Sage RF. 1995. Was low atmospheric CO₂ during the Pleistocene a limiting factor for the origin of agriculture? *Global Change Biology* 1: 93–106.
- Sage RF. 2004. The evolution of C₄ photosynthesis. *New Phytologist* 161: 341–370.
- Sage RF, Coleman JR. 2001. Effects of low atmospheric CO₂ on plants: more than a thing of the past. *Trends in Plant Science* 6: 18–24.
- Sage RF, Cowling SA. 1999. Implications of stress in low CO₂ atmospheres of the past: Are today's plants too conservative for a high CO₂ world? In: Luo Y, Mooney HA, eds. *Carbon dioxide and environmental stress*. New York, NY, USA: Academic Press, 289–305.
- Sage RF, Reid CD. 1992. Photosynthetic acclimation to sub-ambient CO₂ (20 Pa) in the C₃ annual *Phaseolus vulgaris* L. *Photosynthetica* 27: 605–617.
- Schlesinger WH. 1997. *Biogeochemistry: an analysis of global change*. San Diego, CA, USA: Academic Press.
- Sharma RK, Griffing B, Scholl RL. 1979. Variations among races of *Arabidopsis thaliana* (L.) Heynh for survival in limited carbon dioxide. *Theoretical and Applied Genetics* 54: 11–15.
- Siegenthaler U, Stocker TF, Monnin E, Lüthi D, Schwander J, Stauffer B, Raynaud D, Barnola J-M, Fischer H, Masson-Delmotte V et al. 2005. Stable carbon cycle-climate relationship during the late Pleistocene. *Science* 310: 1313–1317.
- Springer CJ, Orozco RA, Kelly JK, Ward JK. 2008. Elevated CO₂ influences the expression of floral-initiation genes in *Arabidopsis thaliana*. *New Phytologist* 178: 63–67.
- Springer CJ, Ward JK. 2007. Flowering time and elevated atmospheric CO₂. *New Phytologist* 176: 243–255.
- Stock C, Harris JM. 2001. *Rancho La Brea: a record of Pleistocene Life in California*. Los Angeles, CA, USA: Natural History Museum of Los Angeles County, Los Angeles.
- Strain BR. 1991. Possible genetic effects of continually increasing atmospheric CO₂. In: Taylor FE Jr, Pitelka LF, Clegg MT, eds. *Ecological genetics and air pollution*. New York, NY, USA: Springer-Verlag, 237–244.
- Street-Perrott FA, Huang Y, Perrott RA, Eglinton G, Barker P, Khelifa LB, Harkness DD, Olago DO. 1997. Impact of lower atmospheric carbon dioxide on tropical mountain ecosystems. *Science* 278: 1422–1426.
- Terashima I, Masuzawa T, Ohba H, Yokoi Y. 1995. Is photosynthesis suppressed at higher elevations due to low CO₂ pressure? *Ecology* 76: 2663–2668.
- Tipple BJ, Pagani M. 2007. The early origins of terrestrial C₄ photosynthesis. *Annual Review of Earth and Planetary Sciences* 35: 435–461.
- Tissue DT, Griffin KL, Thomas RB, Strain BR. 1995. Effects of low and elevated CO₂ on C₃ and C₄ annuals. II. Photosynthesis and leaf biochemistry. *Oecologia* 101: 21–28.
- Tonsor SJ, Scheiner SM. 2007. Plastic trait integration across a CO₂ gradient in *Arabidopsis thaliana*. *American Naturalist* 169: E119–E140.
- Tripati AK, Roberts CD, Eagle RA. 2009. Coupling of CO₂ and ice sheet stability over major climate transitions of the last 20 million years. *Science* 326: 1394–1397.
- Turcq B, Cordeiro RC, Sifeddine A, Simões Filho FFL, Albuquerque ALS, Abrão JJ. 2002. Carbon storage in Amazonia during the Last Glacial Maximum: secondary data and uncertainties. *Chemosphere* 49: 821–835.
- Van Campo E, Guiot J, Peng C. 1993. A data-based re-appraisal of the terrestrial carbon budget at the last glacial maximum. *Global and Planetary Change* 8: 189–201.
- Van de Water PK, Leavitt SW, Betancourt JL. 1994. Trends in stomatal density and ¹³C/¹²C ratios of *Pinus flexilis* needles during last glacial-interglacial cycle. *Science* 264: 239–243.
- Wagner F, Below R, DeKlerk P, Dilcher DL, Joosten H, Kürschner WM, Visscher H. 1996. A natural experiment on plant acclimation: lifetime stomatal frequency response of an individual tree to annual atmospheric CO₂ increase. *Proceedings of the National Academy of Sciences, USA* 93: 11705–11708.
- Ward JK. 2005. Evolution and growth of plants in a low CO₂ World. In: Ehleringer JR, Cerling TE, Dearing MD, eds. *A history of atmospheric CO₂ and its effects on plants, animals, and ecosystems*. New York, NY, USA: Springer: 232–257.
- Ward JK, Antonovics J, Thomas RB, Strain BR. 2000. Is atmospheric CO₂ a selective agent on model C₃ annuals? *Oecologia* 123: 330–341.
- Ward JK, Harris JM, Cerling TE, Wiedenhoef A, Lott MJ, Dearing MD, Coltrain JB, Ehleringer JR. 2005. Carbon starvation in glacial trees recovered from the La Brea tar pits. *Proceedings of the National Academy of Sciences, USA* 102: 690–694.
- Ward JK, Kelly JK. 2004. Scaling up evolutionary responses to elevated CO₂: lessons from *Arabidopsis*. *Ecology Letters* 7: 427–440.
- Ward JK, Myers DA, Thomas RB. 2008. Physiological and growth responses of C₃ and C₄ plants to reduced temperature when grown at low CO₂ of the last ice age. *Journal of Integrative Plant Biology* 50: 1388–1395.
- Ward JK, Strain BR. 1997. Effects of low and elevated CO₂ partial pressure on growth and reproduction of *Arabidopsis thaliana* from different elevations. *Plant, Cell & Environment* 20: 254–260.
- Ward JK, Tissue DT, Thomas RB, Strain BR. 1999. Comparative responses of model C₃ and C₄ plants to drought in low and elevated CO₂. *Global Change Biology* 5: 857–867.
- Wells PV, Jorgensen CD. 1964. Pleistocene wood rat middens and climatic change in Mohave Desert: a record of juniper woodlands. *Science* 143: 1171–1174.
- Wilkinson MJ, Monson RK, Trahan N, Lee S, Brown E, Jackson RB, Polley HW, Fay PA, Fall R. 2009. Leaf isoprene emission rate as a function of atmospheric CO₂ concentration. *Global Change Biology* 15: 1189–1200.
- Woodward FI. 1987. Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature* 327: 617–618.
- Wooller MJ, Agnew ADQ. 2002. Changes in graminoid stomatal morphology over the last glacial-interglacial transition: evidence from Mount Kenya, East Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology* 177: 123–136.
- Yung L, Lee T, Wang C, Shieh Y. 1996. Dust: a diagnostic of the hydrologic cycle during the last glacial maximum. *Science* 27: 962–963.