Commentary

Learning from the past: how low [CO$_2$] studies inform plant and ecosystem response to future climate change

Atmospheric [CO$_2$] over the past 800 000 yr has varied generally as a function of glacial periods, with minima (*c.* 170–200 ppm) during glacial periods and maxima (*c.* 280–300 ppm) during inter-glacial periods (Luthi *et al.*, 2008). During the Last Glacial Maximum (LGM; 18 000–20 000 yr ago), atmospheric [CO$_2$] ranged from 180 to 200 ppm, which is approximately half the current [CO$_2$] (392 ppm), and among the lowest [CO$_2$] observed during the evolution of vascular land plants over the past 350 million yr. While it has been observed that low atmospheric [CO$_2$] directly limits photosynthesis (Tissue & Lewis, 2010), with subsequent reductions in biomass production (Lewis *et al.*, 2010), reproduction (Dippery *et al.*, 1995), and survival (Ward & Kelly, 2004), these studies have primarily been conducted on modern plants grown for a single generation in low [CO$_2$] (see review by Gerhart & Ward, 2010). Subsequently, they do not address the potential evolutionary adaptive responses to low [CO$_2$] which would only become evident in plants growing for long-time periods and many generations under these environmental conditions.

> *'… glacial plants were severely carbon limited over a very long time period, until atmospheric [CO$_2$] began rising during the glacial–interglacial transition.'*

In a fascinating study, in this issue of *New Phytologist*, Gerhart *et al.* (pp. 63–69) compared stable carbon isotope ratios found in the annual rings of glacial *Juniperus* wood preserved in the La Brea tar pits in southern California with modern *Juniperus* wood in the nearby mountains, and used them to calculate c_i/c_a over the 50 000-yr period spanning the last glacial period to modern times. The c_i/c_a ratio reflects both the degree of coordination of CO$_2$ supply (stomatal conductance) and demand (site of carboxylation) functions, and shifts in physiology due to changing resource availability (e.g. water, nutrients, temperature). Interestingly, they found that mean c_i/c_a was constant over the 50 000-yr time period and attributed it to higher stomatal conductance and greater chloroplast demand for CO$_2$ during the glacial period when plants would likely have adjusted physiological responses to increase carbon assimilation under low atmospheric [CO$_2$]. As a consequence of constant c_i/c_a, mean c_i was much lower in glacial trees (106 ppm) than in modern trees (168 ppm); in fact, modern trees rarely exhibited the low c_i values routinely found in glacial trees. Overall, this study provided direct evidence that glacial plants were severely carbon limited over a very long time period, until atmospheric [CO$_2$] began rising during the glacial–interglacial transition.

Perhaps one of the most important points raised by Gerhart *et al.* was that inter-annual variation in c_i/c_a was low in glacial trees relative to modern trees even though climate was generally more variable during glacial periods. In modern trees, c_i/c_a is highly variable and often dependent upon soil water availability and vapour pressure deficit (Gerhart *et al.*). Given that c_i was very low (minimum of 90 ppm) in glacial trees and inter-annual variation in c_i/c_a was low even in a highly variable climate, this would suggest that tree physiology during glacial periods was predominantly limited by low [CO$_2$] and not other environmental factors. Therefore, plants growing in very low [CO$_2$] could not utilize higher soil water availability or nutrients, thereby reducing the impact of these variables on physiology or growth. In some respects this is reassuring, in that the results of this field-study over evolutionary time are similar to short-term, controlled environment studies with modern plants grown in glacial [CO$_2$] showing significant carbon limitations on plant physiology even when other resources were generally not limiting (Dippery *et al.*, 1995; Tissue *et al.*, 1995). Overall, a major conclusion of Gerhart *et al.* was that the environmental factors that regulate photosynthesis, and indirectly plant growth, may vary across geologic time.

Adaptation to low [CO$_2$] and consequences for plant responses to climate change

Low [CO$_2$] has been proposed as a strong evolutionary selective agent, including contributing to the origin of agriculture (Sage, 1995) and the evolution of C$_4$ plants in association with high temperature and drought (Osborne & Sack, 2012). More specifically, low [CO$_2$] has generated substantial changes in leaf traits associated with CO$_2$ and water exchange, such as reduced stomatal density, greater vein density and megaphyll leaves (see review by Leakey & Lau, 2012). Given the duration of very low [CO$_2$] over geologic time and the relatively recent rise in [CO$_2$] over the past 20 000 yr, selection pressure must have been strongly exerted by low [CO$_2$]. For example, Ward *et al.* (2000) found that biomass production in *Arabidopsis* was increased 35% after only five generations of selection in low [CO_2], but not at high [CO_2], suggesting rapid and strong selective effects in low [CO_2]. It is therefore, reasonable to assume that plants are still adapted to low [CO_2], which may constrain responses to rising [CO_2] predicted to occur over the next century (Sage & Coleman, 2001).

In a future warmer, high [CO_2] world, the primary resource limiting plant function will continue to transition from [CO_2] to other resources, such as temperature, nutrients and water availability. In controlled environment studies to date, there is little evidence that adaptive evolutionary responses to elevated [CO_2] have occurred, even over many generations, despite changes in plant phenotypes (Leakey & Lau, 2012). Longer term exposure (thousands of years) to elevated [CO_2] at natural CO_2 springs also generally find minimal adaptive change despite some alterations in photosynthetic performance and biochemistry (e.g. Cook *et al.*, 1998). Interestingly, even the evolution of Rubisco appears constrained, with Rubisco specificity optimal for light-saturated photosynthesis at *c.* 200 ppm [CO_2] (Zhu *et al.*, 2004), which is the mean [CO_2] over the last 400 000 yr (Luthi *et al.*, 2008). A potential explanation for the general lack of evidence for adaptive responses to elevated [CO_2] is that few studies have adequately addressed the interactive effects of elevated [CO_2] and abiotic stress (e.g. nutrient, water, temperature) over multiple generations (Leakey & Lau, 2012). Given that these environmental conditions co-vary, and that selection is strongest under stressful conditions, this research direction should be pursued in the near future.

Reduced terrestrial carbon storage, net primary production and forest cover during glacial periods, which are characterized by very low atmospheric [CO_2], may be more accurately predicted when the impact of low [CO_2] on physiological processes is included in palaeoclimate models (Prentice & Harrison, 2009). Utilizing findings from studies that address the impact of low [CO_2] on physiological performance in C_3 and C_4 plants, it has been demonstrated that physiological effects may scale up to the ecosystem level (Prentice & Harrison, 2009). For example, changes in [CO_2] and their resultant effect on plant photosynthesis and water use efficiency in low [CO_2] have been used to accurately explain changes in the composition of plant communities (C_3 vs C_4) over the LGM, as well as account for changes in the woody component in savannas, relative forest cover, and most recently tree–grass competition during the transition from LGM to pre-industrial Holocene (Prentice *et al.*, 2011). Overall, we should utilize our improved understanding of plant adaptation and response to low and variable [CO_2] over historic time periods to better predict ecosystem response to rising [CO_2] and future climate change.

Future research directions

We suggest several directions for future research to better understand how plant adaptation to low [CO_2] may constrain future responses to rising [CO_2]. Although Gerhart *et al.* attributed a constant mean c_i/c_a to both higher stomatal conductance and greater chloroplast demand for [CO_2] we still do not have direct evidence of the relative control exerted by these factors. Therefore, greater exploration of the relative roles of these two factors in regulating c_i/c_a under low [CO_2] is required to determine which traits were more likely to have exhibited adaptive evolutionary responses to low [CO_2]. Perhaps more importantly, we are severely lacking multiple generation studies on plant responses to low and elevated [CO_2]. Accordingly, we are currently unable to develop significant conclusions regarding past constraints imposed by low [CO_2] on the relative rate of plant adaptation to rising [CO_2] and associated abiotic stresses.

While it has been well established that low [CO_2] has significantly affected leaf traits, development of different photosynthetic pathways, and human societies through impacts on agriculture, we do not know the relative role of [CO_2] on plant performance in the future. To date, studies suggest that as [CO_2] rises from glacial to future levels, the limitation imposed by [CO_2] on growth and physiology becomes secondary to other environmental factors, such as temperature and drought. For example, growth of cottonwood was limited at glacial [CO_2] despite nonlimiting temperature, soil moisture and soil [P], but at high [CO_2] the limitation to growth was largely imposed by soil [P] (Lewis *et al.*, 2010). Given the importance of the interactive effects of other environmental factors on plant response to [CO_2], we suggest that future research focus on multi-factor (e.g. low and elevated [CO_2], temperature, water, nutrients) experiments across different plant functional groups, in an effort to ultimately determine ecosystem response to future climate change.

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