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## CO<sub>2</sub> studies remain key to understanding a future world

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### Summary

Characterizing plant responses to past, present, and future changes in atmospheric [CO<sub>2</sub>] is critical for understanding and predicting the consequences of global change over evolutionary and ecological timescales. Previous CO<sub>2</sub> studies have provided great insights into the effects of rising [CO<sub>2</sub>] on leaf-level gas exchange, carbohydrate dynamics, and plant growth. However, scaling [CO<sub>2</sub>] effects across biological levels, especially in field settings, has proved challenging. Moreover, many questions remain about the fundamental molecular mechanisms driving plant responses to [CO<sub>2</sub>] and other global change factors. Here we discuss three examples of topics in which significant questions in CO<sub>2</sub> research remain unresolved: (1) mechanisms of [CO<sub>2</sub>] effects on plant developmental transitions; (2) implications of rising [CO<sub>2</sub>] for integrated plant–water dynamics and drought tolerance; and (3) [CO<sub>2</sub>] effects on symbiotic interactions and eco-evolutionary feedbacks. Addressing these and other key questions in CO<sub>2</sub> research will require collaborations across scientific disciplines and new approaches that link molecular mechanisms to complex physiological and biological interactions across spatiotemporal scales.

### Keywords

atmospheric carbon dioxide; climate change; eco-evolutionary feedbacks; flowering time; leaf gas exchange; plant hydraulics; plant; microbe interactions

## I. Introduction

CO<sub>2</sub> studies have comprised an important component of plant biology research for decades, as CO<sub>2</sub> is the primary carbon source for photosynthesis and a major driver of climate change. Only 20000 yr ago, atmospheric [CO<sub>2</sub>] were among the lowest levels that occurred during the evolution of land plants (180–200 ppm). Since then, [CO<sub>2</sub>] has more than doubled to 401 ppm due to greenhouse gas emissions, levels that plants have not encountered for several million years (Tripathi *et al.*, 2009). Consequently, [CO<sub>2</sub>] may be rising faster than the rate at which some plant species can evolve to function optimally (Lau *et al.*, 2007; but see Ward *et al.* 2000). In addition, current levels of plasticity for functional traits may be

inadequate to maintain optimal functioning in novel future environments (Franks *et al.*, 2014; Anderson & Gezon, 2015).

Over the past several decades, CO<sub>2</sub> studies have met with great success in characterizing the effects of elevated [CO<sub>2</sub>] on leaf-level gas exchange, carbohydrate dynamics, and plant growth (Ainsworth & Long, 2005; Franks *et al.*, 2013). Large-scale studies (particularly free-air CO<sub>2</sub> enrichment studies, FACE) have further provided insight into the effects of elevated [CO<sub>2</sub>] on plant community dynamics and productivity (Norby *et al.*, 2016). Nonetheless, many questions remain about plant responses to rising [CO<sub>2</sub>], as well as interactions with other changing environmental factors, particularly at the most fundamental mechanistic levels. Specifically, molecular and whole plant responses to [CO<sub>2</sub>] scale to influence higher order processes; however, such scaling factors are far from resolved and are highly variable across ecosystems. Additionally, increases in [CO<sub>2</sub>] are strongly coupled with rising temperatures and drought, and the interactive effects of these drivers on plant functioning are unclear at almost every level. The new generation of FACE studies can help address these challenges by facilitating cross-site analysis of plant through ecosystem responses to experimental [CO<sub>2</sub>] manipulations (Norby *et al.*, 2016). Studies spanning preindustrial through future [CO<sub>2</sub>] can further provide a baseline for plant functioning before human intervention (Medeiros & Ward, 2013; Becklin *et al.*, 2016). Finally, multigenerational and molecular-level studies can elucidate potential evolutionary responses to [CO<sub>2</sub>] (Ward *et al.*, 2000; Watson-Lazowski *et al.*, 2016).

Later we discuss three topics in CO<sub>2</sub> research with immediate consequences for global carbon cycling, food security, and ecosystem services (Fig. 1). How does [CO<sub>2</sub>] affect plant developmental transitions? What are the implications of rising [CO<sub>2</sub>] for plant–water dynamics? And how does [CO<sub>2</sub>] impact feedbacks in plant–microbe symbioses? Incorporating the interactive effects of [CO<sub>2</sub>] and climate as well as questions of scale will be important for understanding each of these topics. Although this is by no means an exhaustive list, we use these examples to emphasize why CO<sub>2</sub> research is more critical than ever, and will remain so long into the future.

## II. CO<sub>2</sub> effects on plant development and phenology

Numerous studies show that elevated [CO<sub>2</sub>] can impact plant developmental processes, particularly flowering time (FLT). However, we are just beginning to characterize the mechanisms driving these developmental shifts and their implications for individual plants, communities, and ecosystems. Shifts in FLT can alter the course of evolution, influence community competition, disrupt plant–pollinator interactions, and affect crop/food production (Bartomeus *et al.*, 2011; Rafferty & Ives, 2012). In a literature survey, 57% of wild species and 62% of crop species exhibited altered FLT when grown at elevated [CO<sub>2</sub>], with extreme FLT responses ranging from accelerations of 60 d to delays of 16 d depending on the species (Springer & Ward, 2007). The effects of elevated [CO<sub>2</sub>] on FLT can also vary within species, such as in *Arabidopsis thaliana* ecotypes and soybean lines, which exhibited both delayed and accelerated FLT at elevated [CO<sub>2</sub>] (Ward & Kelly, 2004; Bunce & Hilacondo, 2016). Additionally, plants do not always flower at the same size in novel environments, including elevated [CO<sub>2</sub>] (Springer *et al.*, 2008; Springate & Kover, 2014).

This may be due to [CO<sub>2</sub>] effects on signaling mechanisms that act independently of plant size or growth rate. Thus, rising [CO<sub>2</sub>] can alter plant size at key life cycle milestones, including flowering (Fig. 2), with possible downstream effects on fitness and carbon cycling.

Although molecular work in this area is still developing, elevated [CO<sub>2</sub>] has been shown to affect the expression of flowering genes and regulatory pathways. Specifically, the floral repressor, *FLC* (*FLOWERING LOCUS C*), was shown to play a key role in influencing *FLT* at elevated [CO<sub>2</sub>] in *Arabidopsis*. In this case, sustained expression of *FLC* caused a previously selected genotype to flower much later, with much higher total biomass, and many more leaves at elevated vs current [CO<sub>2</sub>] (Fig. 2) (Springer *et al.*, 2008). Changes in [CO<sub>2</sub>] have also been shown to regulate the expression of micro RNAs involved in plant development. The miR156 flowering pathway controls the vegetative juvenile-to-adult phase transition and is involved in floral initiation in *Arabidopsis* (Wang *et al.*, 2009; Wu *et al.*, 2009). A doubling in [CO<sub>2</sub>] reduced the expression of miR156/157, leading to a 7 d acceleration in *FLT* (May *et al.*, 2013). Despite these advancements, it is still unclear which upstream processes influence flowering gene expression and micro RNAs during growth at elevated [CO<sub>2</sub>].

One hypothesis linking elevated [CO<sub>2</sub>] to altered developmental timing proposes that sugars act as signaling molecules to influence flowering gene expression. Through this mechanism, plant carbohydrate status is sensed in leaves via the metabolite trehalose-6-phosphate (T6P) (Wahl *et al.*, 2013; Figueroa & Lunn, 2016), and *FLOWERING LOCUS T* (*FT*) is activated when carbohydrate levels become adequate to support reproduction. *FT* is then translocated to the meristem where floral induction occurs. Elevated [CO<sub>2</sub>] generally increases the sugar status of leaves, and altered sugar sensing via T6P may be one possible mechanism for how elevated [CO<sub>2</sub>] influences *FLT* (Springer & Ward, 2007; Coneva *et al.*, 2012). Studies in which elevated [CO<sub>2</sub>] or additions of exogenous sucrose were associated with delays in *FLT* support this potential mechanism (Posner, 1971). Despite the importance of understanding the mechanisms driving [CO<sub>2</sub>] effects on plant development and phenology, research that relates sugar-sensing mechanisms to elevated [CO<sub>2</sub>] is lacking. Further studies exploring molecular drivers of *FLT* in model and non-model systems will aid in accurately predicting how phenological responses to global change will manifest across spatiotemporal scales.

### III. CO<sub>2</sub> effects on plant–water dynamics

While we can predict general patterns of how [CO<sub>2</sub>] alters stomatal conductance, photosynthesis and transpiration in many systems (Ainsworth & Long, 2005), the strength of these effects (Haworth *et al.*, 2016) and major patterns in CO<sub>2</sub>-responsiveness across plant groups are still being debated (Brodribb & McAdam, 2011; Franks & Britton-Harper, 2016). Thus, despite decades of CO<sub>2</sub> research, surprises remain in how rising [CO<sub>2</sub>] affects plant–water dynamics (Franks *et al.*, 2013). Many of these questions may be better explained by improving our understanding of how plant hydraulics will respond to a range of [CO<sub>2</sub>], especially in conjunction with increased drought and warming.

When leaves are exposed to elevated [CO<sub>2</sub>], photosynthesis is stimulated, but stomatal conductance and transpiration decline, increasing leaf water use efficiency. It is commonly

assumed that plants grown at elevated  $[\text{CO}_2]$  will therefore use less water and better withstand drought stress; however, this is often not the case (Vaz *et al.*, 2012; Perry *et al.*, 2013; Duan *et al.*, 2014). Elevated  $[\text{CO}_2]$  can increase transpiration or reduce drought tolerance by increasing canopy leaf area (McCarthy *et al.*, 2006; Warren *et al.*, 2011), stimulating nocturnal stomatal conductance (Zeppel *et al.*, 2012), reducing rooting depth (Duursma *et al.*, 2011), and increasing leaf temperatures via lower latent heat loss (Warren *et al.*, 2011). Even when elevated  $[\text{CO}_2]$  promotes soil water savings at larger spatial scales, changes in plant community composition can increase total leaf area index and transpirational potential (Fay *et al.*, 2012), which may leave ecosystems equally vulnerable to drought under current and future  $[\text{CO}_2]$ . Without a greater understanding of how common these responses are under elevated  $[\text{CO}_2]$ , we cannot make robust predictions of how rising  $[\text{CO}_2]$  will alter drought tolerance, or properly capture  $\text{CO}_2$  effects on ecosystem water use efficiency (De Kauwe *et al.*, 2013).

Plant hydraulic responses to rising  $[\text{CO}_2]$  have received less attention than leaf-level gas exchange responses despite the importance of water transport traits in determining drought tolerance, carbon assimilation, and growth. Hydraulic conductance often decreases at elevated  $[\text{CO}_2]$  (Tognetti *et al.*, 2005; Domec *et al.*, 2009), but may also increase (Domec *et al.*, 2010) or show no response (Locke *et al.*, 2013), and can vary in an apparently species-specific manner that we cannot fully account for (Domec *et al.*, 2010). Likewise, cavitation resistance can increase at elevated  $[\text{CO}_2]$  in some species (Rico *et al.*, 2013), while vessel implosion strength, a correlate of cavitation resistance, decreases with rising  $[\text{CO}_2]$  in others (Medeiros & Ward, 2013). These findings highlight that we currently lack a generalized framework for predicting how  $[\text{CO}_2]$  rise will alter hydraulic traits. This issue becomes even more problematic when trying to predict integrated plant function at elevated  $[\text{CO}_2]$  in response to water stress. For example, plants grown at low  $[\text{CO}_2]$  maintained their hydraulic conductance and photosynthesis during drought, while these parameters declined in plants grown at elevated  $[\text{CO}_2]$  (Medeiros & Ward, 2013), indicating that rising  $[\text{CO}_2]$  could reduce plant growth and drought tolerance.

The uncertainties in how changes in  $[\text{CO}_2]$  affect plant water balance are exacerbated when we consider that warming, which will increase concurrently with  $[\text{CO}_2]$ , usually exacerbates water stress. While leaf-level physiological responses to these drivers suggest that rising  $[\text{CO}_2]$  can offset the negative effects of warming, evidence for this is mixed (Dieleman *et al.*, 2012; Way *et al.*, 2015). Instead, a growing number of studies find that elevated  $[\text{CO}_2]$  does not ameliorate the impact of high temperatures on drought stress, particularly when water is severely limited (Wertin *et al.*, 2012; Zeppel *et al.*, 2012; Duan *et al.*, 2014). Resolving the interactive effects of globally uniform increases in  $[\text{CO}_2]$  and regionally variable increases in temperature and drought will be necessary for scaling global change effects on plant–water dynamics.

#### IV. $\text{CO}_2$ effects on plant–microbe interactions

Changes in plant physiology, development, and growth with rising  $[\text{CO}_2]$  can influence global change responses at higher biological levels through cascading effects on interactions between plants and other organisms (Gilman *et al.*, 2010). Likewise, direct effects of plant

mutualists and antagonists on plant traits and fitness can mediate plant acclimation and evolutionary responses to global change (Lau & Lennon, 2012). [CO<sub>2</sub>] effects on species interactions and subsequent feedbacks between ecological and evolutionary processes may be especially important for plant–microbe symbioses where the functioning and fitness of the interacting organisms are tightly linked (terHorst & Zee, 2016). However, microbial communities, especially those belowground, remain one of the least understood components of terrestrial ecosystems (Bardgett & van der Putten, 2014), which presents a major challenge when modeling [CO<sub>2</sub>] effects across biological scales (Smithwick *et al.*, 2014).

Mycorrhizal associations, belowground plant–fungus symbioses in which host plants trade carbohydrates for soil nutrients, are one of the best-studied types of plant–microbe interactions. [CO<sub>2</sub>] effects on mycorrhizal associations have largely been explored in short-term studies from temperate ecosystems in the northern hemisphere, which limits our understanding of mycorrhizal processes at the global scale (Mohan *et al.*, 2014). Additionally, few studies have examined genetic and phenotypic variation in mycorrhizal traits (Johnson *et al.*, 2012), or the interactive effects of multiple global change drivers (Mohan *et al.*, 2014); thus, substantial questions remain regarding mechanisms driving mycorrhizal responses to rising [CO<sub>2</sub>] (Fig. 1).

Traditionally, mycorrhizal associations are viewed as nutritional mutualisms that are most beneficial to host plants when soil nutrients limit plant growth (Johnson & Graham, 2013). For this reason, rising [CO<sub>2</sub>] is predicted to promote stronger mycorrhizal mutualisms. However, observations of mycorrhizal responses to rising [CO<sub>2</sub>] do not always correspond to this theoretical prediction. In fact, competition between host plants and mycorrhizal fungi for increasingly limited nutrients can result in neutral associations, or even reduce plant growth under elevated [CO<sub>2</sub>] (Kivlin *et al.*, 2013; Becklin *et al.*, 2016).

Recent work calls for a broader view of mycorrhizal associations that incorporates the functional diversity exhibited by mycorrhizal fungi. For example, some fungi stimulate integrated signaling pathways that enhance their host’s ability to tolerate biotic (e.g. herbivore resistance, Pineda *et al.*, 2013) and abiotic stress (e.g. drought tolerance, Worchel *et al.*, 2013). In fact, most phytohormones examined thus far play a role in regulating mycorrhizal associations (Pozo *et al.*, 2015), and common mycorrhizal networks can serve as a conduit for these signals to pass between neighboring plants (Johnson & Gilbert, 2015). How these signals will integrate environmental cues and regulate multidimensional mycorrhizal responses to rising [CO<sub>2</sub>] in complex natural communities remains unclear.

There is growing evidence that within-population variation in mycorrhizal traits can affect mycorrhizal associations and ecosystem processes to the same degree as variation among mycorrhizal species (Johnson *et al.*, 2012). Furthermore, multiple experiments highlight the potential for genetically based variation in mycorrhizal traits to facilitate evolutionary responses to environmental conditions (Johnson *et al.*, 2012; van der Heijden *et al.*, 2015). Thus, it is likely that both inter- and intraspecific variation in mycorrhizal associations will generate feedbacks that can drive plant and community responses to global change (van der Putten *et al.*, 2013; terHorst & Zee, 2016). Characterizing adaptive potential in plant and fungal populations, the strength of selection exerted by rising [CO<sub>2</sub>] and other global change

drivers, and the magnitude of eco-evolutionary feedbacks between mycorrhizal partners will be especially important for scaling mycorrhizal responses to global change.

## V. The way forward

While we have had success in understanding some topics in CO<sub>2</sub> research, large gaps remain in our knowledge of plant responses to rising [CO<sub>2</sub>] (Fig. 1). Addressing these challenges will require collaboration across disciplines, experiments that span multiple scales of complexity, and the application of –omics technologies and new molecular tools, such as CRISPR-Cas9 that will allow for genome editing and precise mutations in order to study molecular pathways in ecologically relevant species. Additionally, plants host diverse communities of microorganisms that can affect molecular through ecosystem-level processes. Thus, we need to broaden our view of plants to consider how rising [CO<sub>2</sub>] impacts the physiology, ecology, and evolution of these symbiotic communities. Finally, understanding how rising [CO<sub>2</sub>] over recent geological history has affected plant traits will provide insights into the evolutionary pressures that have shaped modern plant species. Using a broader experimental [CO<sub>2</sub>] gradient will generate more informative response curves (rather than two-point comparisons) of plant and community traits, allowing us to better identify thresholds or nonlinear responses that could surprise us in the future. As we move into an even higher CO<sub>2</sub> world, it will be imperative that plant breeding programs account for potential developmental, physiological, and ecological shifts that may occur in response to rising [CO<sub>2</sub>] when developing future crop varieties and biofuels. Moreover, mechanistic studies of plant responses to rising [CO<sub>2</sub>] will become even more important for predicting global carbon, water, and nutrient cycling and for determining how ecosystem services may shift in the future.

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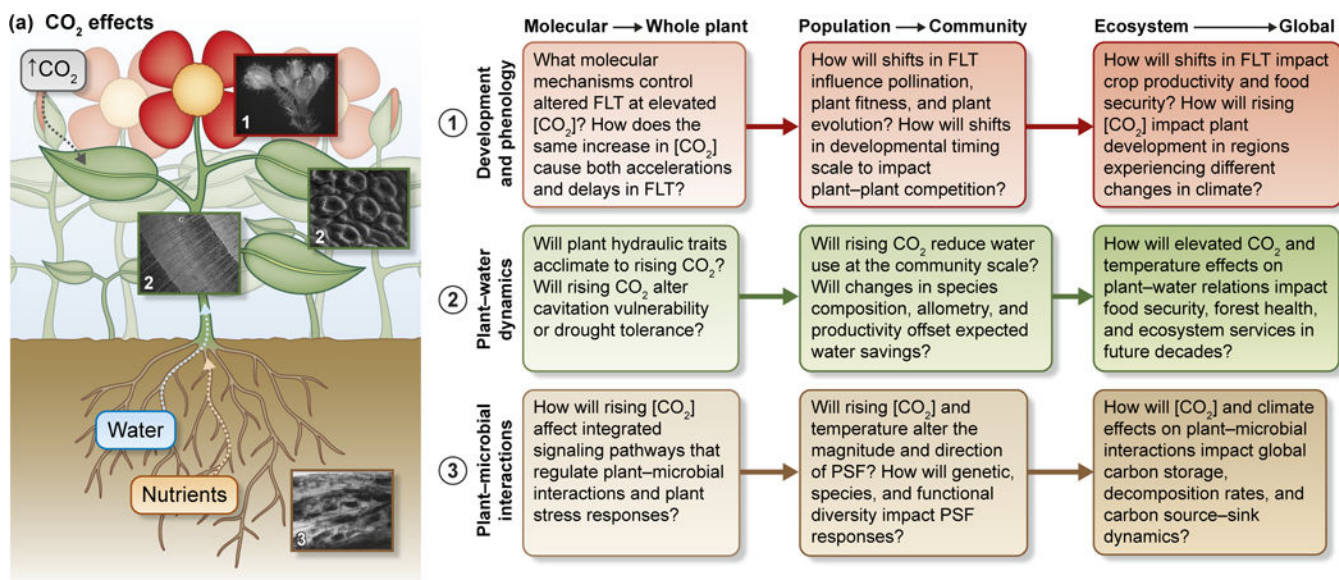
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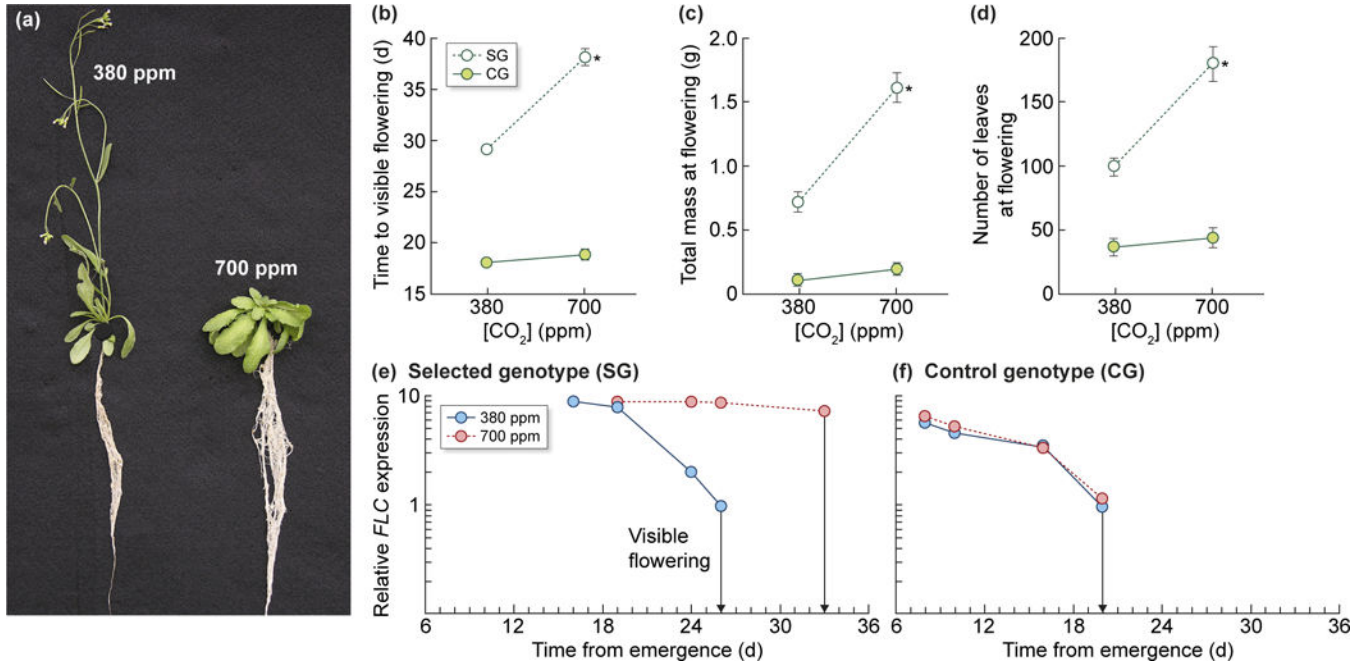


### (b) Grand Challenges

- How did interactions between rising [CO<sub>2</sub>] and climate regulate shifts in plant developmental timing, physiology, and species interactions from pre-industrial to modern times? How will adaptation to past conditions impact plant, community, and ecosystem responses to rising [CO<sub>2</sub>] in the future?
- How will rising [CO<sub>2</sub>] and climate change drive the evolution of plant traits (and traits of plant antagonists or mutualists)? How fast will adaptation to rising [CO<sub>2</sub>] occur, and what traits are potential targets for artificial selection in food crops?
- How is genetic and phenotypic variation distributed across the landscape? Can we identify populations or species that are more likely to acclimate or adapt to changing conditions?
- What are the mechanisms driving nonlinear responses to rising [CO<sub>2</sub>] across broad temporal scales and at different levels of biological organization? How can we incorporate nonlinear responses to rising [CO<sub>2</sub>] and changes in trait variability into global change models?
- How will complex biological interactions between plants and other organisms influence the trajectory of plant responses to rising [CO<sub>2</sub>]? How do these ecological effects scale up to impact food security and ecosystem services?
- How can recent advances in biotechnology aid in answering unsolved questions in CO<sub>2</sub> research? How can we scale molecular-level data up to ecosystem and global processes?

**Fig. 1.**

Direct and indirect effects of rising [CO<sub>2</sub>] on plant development and phenology, plant physiology and water dynamics, and plant interactions with other organisms can have cascading effects on ecological and evolutionary processes across spatial and temporal scales (a). Here we highlight examples of key questions in CO<sub>2</sub> research that will require an integrative approach that bridges gaps between molecular biology, plant physiology, ecological and evolutionary biology, and ecosystem science (b). This is by no means an exhaustive list, and not all questions are specifically discussed in the main text. Images show (a1) a developing *Arabidopsis* flower bud, (a2) stoma (upper image, *Juniperus*) and xylem (lower image, *Picea*) structures, and (a3) arbuscular mycorrhizal fungi colonizing herbaceous roots. Images were provided courtesy of R. Atkinson, K. M. Becklin, V. Bui, and S. M. Walker. FLT, flowering time; PSF, plant–soil feedback.



**Fig. 2.**

(a) A genotype of *Arabidopsis thaliana* (SG; see Springer *et al.*, 2008) that is sensitive to elevated [CO<sub>2</sub>] with respect to flowering time. Both representative plants are of the same genotype and age and were grown at 380 and 700 ppm [CO<sub>2</sub>]. Note that the elevated [CO<sub>2</sub>] plant has not yet flowered, although it has accumulated more total biomass and a greater number of leaves. (b) Time to visible flowering, (c) total biomass at flowering, (d) total number of leaves at flowering for the SG *Arabidopsis* genotype (white circles) and CG (green circles, control genotype) grown at 380 and 700 ppm [CO<sub>2</sub>]. (e) Real-time RT-PCR expression patterns for *FLC* in SG and (f) CG *Arabidopsis* genotypes grown at 380 ppm (solid blue line) and 700 ppm CO<sub>2</sub> (dashed red line). Sampling was performed at the same developmental stages for each genotype. Signals were normalized to the expression level at the time of flowering at 380 ppm within the same genotype. The photo in (a) is courtesy of B. Burgert, and (b–f) were reproduced from Springer *et al.* (2008).