



Evolution of Crassulacean acid metabolism in response to the environment: past, present, and future

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Abstract

Crassulacean acid metabolism (CAM) is a mode of photosynthesis that evolved in response to decreasing CO₂ levels in the atmosphere some 20 million years ago. An elevated ratio of O₂ relative to CO₂ caused many plants to face increasing stress from photorespiration, a process exacerbated for plants living under high temperatures or in water-limited environments. Today, our climate is again rapidly changing and plants' ability to cope with and adapt to these novel environments is critical for their success. This review focuses on CAM plant responses to abiotic stressors likely to dominate in our changing climate: increasing CO₂ levels, increasing temperatures, and greater variability in drought. Empirical studies that have assessed CAM responses are reviewed, though notably these are concentrated in relatively few CAM lineages. Other aspects of CAM biology, including the effects of abiotic stress on the light reactions and the role of leaf succulence, are also considered in the context of climate change. Finally, more recent studies using genomic techniques are discussed to link physiological changes in CAM plants with the underlying molecular mechanism. Together, the body of work reviewed suggests that CAM plants will continue to thrive in certain environments under elevated CO₂. However, how CO₂ interacts with other environmental factors, how those interactions affect CAM plants, and whether all CAM plants will be equally affected remain outstanding questions regarding the evolution of CAM on a changing planet.

Background

Whereas photosynthesis is arguably the most central component of a plant's metabolism, aspects of the photosynthetic machinery have evolved in response to environmental stressors. In particular, the carbon reactions (or "dark" reactions) are susceptible to conditions that promote photorespiration (**Box 1**) in plants, such as high temperatures or a lack of water. RuBisCO—the enzyme that catalyzes the fixation of atmospheric CO₂ in C₃ plants—can fix both CO₂ and O₂. When RuBisCO interacts with O₂, plants undergo photorespiration, a process that expends energy with no net

carbon gain. High temperatures favor O₂ fixation over CO₂ due to the enzymatic kinetics of RuBisCO and a lack of water promotes stomatal closure, limiting the amount of CO₂ available to RuBisCO. To combat this photorespiratory stress, some plants use carbon concentrating mechanisms (CCMs) to actively increase CO₂ concentrations around RuBisCO. C₄ photosynthesis and Crassulacean acid metabolism (CAM) are the two major CCMs in angiosperms, and each has evolved many times independently across diverse plant lineages (Keeley and Rundel, 2003; Edwards and Ogburn, 2012; Edwards, 2019).

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ADVANCES

- Climate change will impact plant growth and productivity not only through increasing CO₂ concentrations, but also via the interactive effects of CO₂, increasing temperatures, and increasing rainfall variability.
- The response of CAM plants to environmental perturbation that mirrors projected climate-change extremes—including elevated CO₂, higher temperatures, and drought stress—is highly variable across lineages.
- Physiological and genomic analyses of CAM plant responses to drought have shown alterations to photosynthesis, carbohydrate metabolism, stomatal regulation, light reactions, and the core CAM biochemical pathway.

CCMs function to increase the levels of CO₂ around RuBisCO but accomplish it in different ways (Sage et al., 2012; Edwards, 2019). C₄ plants spatially separate the initial atmospheric carbon capture from the subsequent RuBisCO-catalyzed conversion of carbon into sugars (Björkman and Gauhl, 1969; Berry et al., 1970; Edwards et al., 1970). C₄ plants use phosphoenolpyruvate carboxylase (PPC) expressed in the mesophyll cells to initially convert CO₂ (in the form of bicarbonate) into an organic acid, typically malic acid (Kortschak et al., 1965; Hatch and Slack, 1966, 1968). This acid is shuttled from the mesophyll to the adjacent bundle sheath cells, where it is decarboxylated, creating high concentrations of CO₂ around RuBisCO. CAM plants use a nearly identical biochemical pathway (Thomas, 1949), but the separation of atmospheric CO₂ capture and RuBisCO function happens temporally, rather than spatially. CAM plants open stomata for gas exchange predominantly during the night, convert incoming CO₂ into malic acid, and store that acid over the night period in the vacuole (Osmond, 1978). During the day, malic acid is moved out of the vacuole for decarboxylation, providing high CO₂ concentrations around RuBisCO. CAM plants, by limiting the bulk of gas exchange to the night period, reduce evapotranspiration and are considered to be considerably more water-use efficient than either C₃ or C₄ plants (Nobel, 1991; Borland et al., 2014).

CAM is found in over 33 plant families (Winter and Smith, 1996; Cushman, 2001), including desert species, tropical epiphytes, and even aquatic plants, resulting in an impressive amount of phenotypic variation (Dodd et al., 2002). CAM is typically divided into four distinct phases: phase I includes nocturnal CO₂ uptake and carboxylation into malic acid; phase II is distinguished by an early morning stomatal opening and carboxylation via RuBisCO, before malate is decarboxylated from the vacuole; phase III is a period of

stomatal closure during the majority of the day period, while malate is decarboxylated; and phase IV is characterized by stomatal opening and RuBisCO carboxylation driven by a draw down of malate concentrations (Figure 1A). The degree to which a plant obtains its carbon via these phases delineates different variants of CAM (Box 2). For example, constitutive CAM plants are those that take up the majority of their carbon via phase I; they are often treated as immune to environmental conditions; however, numerous studies have shown constitutive CAM species can modulate the proportion of CAM used depending on their environmental conditions (Nobel, 1988; Heyduk et al., 2016). C₃ + CAM species can obtain carbon through both C₃ and CAM pathways, including during phase III (Figure 1B), whereas facultative CAM species can upregulate phase I in response to abiotic stressors, such as drought and salt stress (Figure 1C).

Like C₄, CAM is thought to have evolved in response to decreasing CO₂ levels in the atmosphere some 20–30 million years ago (Edwards and Ogburn, 2012). The drop in atmospheric CO₂ relative to O₂ in the Miocene would have led to higher rates of photorespiration in C₃ plants, particularly in high-light and warm environments (Ehleringer et al., 1991). Phylogenetic studies show C₄ grasses diversified during the Miocene (Grass Phylogeny Working Group II, 2012), and similar ages are estimated for a number of eudicot C₄ lineages (Christin et al., 2011). CAM lineages likewise originated and diversified in the Miocene (Good-Avila et al., 2006; Arakaki et al., 2011; Hernández-Hernández et al., 2014). Whereas past environmental conditions may have promoted the evolution of C₄ and CAM, less is known about how future climate scenarios will affect plants using CCMs, particularly CAM. Increasing atmospheric CO₂ concentrations on their own should minimize the benefit of CCMs over C₃ photosynthesis, but climate change is not univariate: in addition to increasing CO₂, rising temperatures, and increasingly variable precipitation will interact to create novel environments. Herein described is what is known about how CAM species respond to environmental perturbations, including what has been learned from recent genomics studies in CAM stress tolerance, to hypothesize how CAM species will fare in a changing global climate.

CAM under stress

The intrinsic stress-adapted properties of CAM plants have garnered attention for the potential use of CAM species as climate-proofed food and fuel crops; a recent review of their leaf properties discussed both adaptations to potential future climate scenarios, as well as the implications of those adaptations (e.g. tissue succulence) to biofuel production (Pereira et al., 2021). Others have reviewed the predicted responses of CCMs more generally, particularly focusing on C₄ species, which have a larger body of prior research (Sage and Stata, 2021). Here, three specific environmental changes are considered—elevated CO₂, higher temperatures, and drought stress—and their effects on CAM.

Box 1 PHOTORESPIRATION AND THE EVOLUTION OF CCMS

RuBisCO is both a carboxylase and an oxygenase, and under certain conditions, RuBisCO's oxygenation function increases, leading to photorespiration (Bauwe et al., 2010; Eisenhut et al., 2019). Environmental conditions can cause an increase in oxygenation and therefore photorespiration: high temperatures alter RuBisCO's enzyme kinetics to increase its specificity for O₂, whereas drought conditions force the closure of stomata, leading to a draw down of available CO₂ for RuBisCO. Photorespiration refers specifically to the process by which plants remove O₂ from the substrate (ribulose biphosphate, or RuBP) so that it can be available again for CO₂. Photorespiration can be a costly process, accounting for greater than 20% yield losses in agricultural crops (Walker et al., 2016). Although photorespiration can have other benefits, including aiding in nitrogen and serine metabolism (Eisenhut et al., 2019), high rates of photorespiration can be detrimental to plant growth. Plants have evolved CCMS to cope with photorespiratory stress (Sage et al., 2012; Mallmann et al., 2014); CCMS concentrate CO₂ around the site of RuBisCO carboxylation, preventing RuBisCO from interacting with O₂ in any meaningful way (Keeley and Rundel, 2003; Raven et al., 2008).

Box 2 TYPES OF CAM PHOTOSYNTHESIS

- Constitutive CAM: when the majority of CO₂ is derived from nocturnal carboxylation via PPC. Constitutive CAM will still have phases II and IV stomatal opening and some carboxylation via RuBisCO.
- C₃ + CAM: the use of a mix of both pathways, though a substantial amount of CO₂ is obtained via the C₃ pathway. C₃ + CAM plants can also be facultative (see below). The typical threshold for defining this category of photosynthesis is that more than half of CO₂ is obtained via C₃.
- Facultative CAM: a CAM cycle that can be up-regulated in response to abiotic stress, including drought and salt stress. Typically found in plants that use C₃ + CAM.

CO₂

CCMs elevate CO₂ concentrations inside photosynthetic cells, theoretically saturating RuBisCO with CO₂ so that photorespiration is minimized. However, CAM plants still have direct carboxylation of atmospheric CO₂ by RuBisCO, particularly late in the day during phase IV. It is worth noting that photorespiratory genes are still present and active in CAM plants (Whitehouse et al., 1991; Lüttge, 2010; Heyduk et al., 2019), photorespiration provides benefits to plants through nitrogen and serine metabolism (Eisenhut et al., 2019), and generally the degree to which photorespiration is reduced in CAM species is unclear. The reliance on both PPC and RuBisCO in CAM plants has resulted in varying empirical responses to elevated CO₂ levels, though in general CAM plants respond to elevated CO₂ with increased biomass (Nobel and Hartsock, 1986; Drennan and Nobel, 2000; Ceusters and Borland, 2011). However, the mechanism of increased biomass varies; some species have increased nocturnal CO₂ uptake under elevated CO₂, whereas in others daytime CO₂ uptake increases at the expense of nocturnal carboxylation (Drennan and Nobel, 2000; Figure 2A). An increase in nocturnal PPC carboxylation is unexpected: PPC is CO₂ specific, unlike RuBisCO, and therefore CO₂ does not need to compete with O₂. In other words, PPC is saturated at current atmospheric CO₂ levels (Drennan and Nobel, 2000). However, PPC is thought to require carbonic anhydrase (CA), an enzyme that converts atmospheric CO₂ into bicarbonate (HCO₃⁻), which greatly speeds up the

process relative to passive CO₂ → HCO₃⁻. Recent functional genetics and genomic studies have shown limited activity of CA in both C₄ and CAM species (Studer et al., 2014; Brillhaus et al., 2016; Heyduk et al., 2019; Wai et al., 2019). If CA is indeed low-functioning or non-functional and plants are relying on passive conversion of CO₂ to bicarbonate, increases in atmospheric CO₂ could increase the rate of CO₂ to bicarbonate conversion in the absence of CA, and thus increase overall nocturnal carboxylation rates. Additionally, *Agave deserti* grown in elevated CO₂ levels had nocturnal CO₂ assimilation rates that peaked early in the night and dropped off (Nobel and Hartsock, 1979), perhaps indicating a limitation on storage of malate. In other words, the anatomy of the leaves and the function of the vacuole in CAM species are important when interpreting CAM responses to increased CO₂ (Töpfer et al., 2020) (see the “Anatomical considerations” and “Temperature”).

The effects of elevated CO₂ on facultative or C₃ + CAM species are even less studied than the responses of constitutive CAM species. Unlike constitutive CAM, C₃ + CAM plants are presumably not saturating RuBisCO via the CAM carboxylation pathway, and so could benefit from increased CO₂ levels in the atmosphere. Indeed, in a meta-analysis of C₃, C₄, and CAM plants, overall biomass increases in CAM species are intermediate to that of C₃ (large gains) and C₄ (small gains), likely because many of the species surveyed in the study have facultative CAM ability (Poorter and Navas, 2003). Even so, there were only nine CAM species available

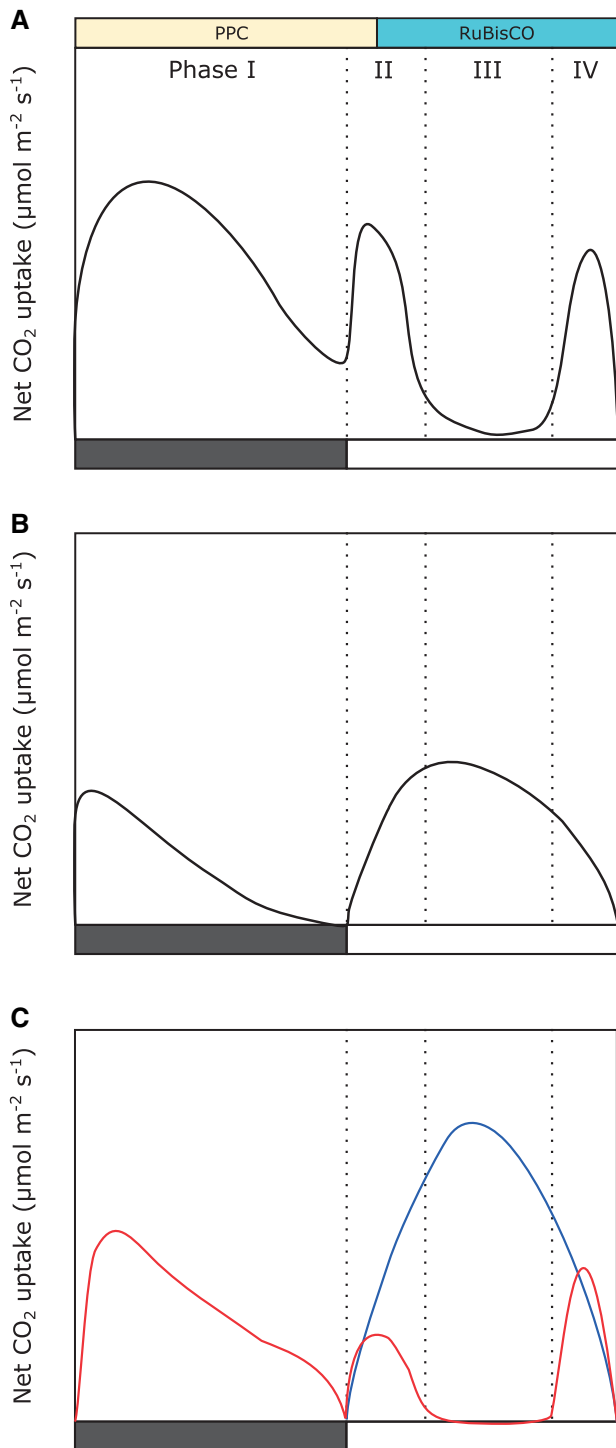


Figure 1 Daily gas exchange in the different types of CAM. General gas exchange plots for (A) constitutive CAM species, (B) C₃ + CAM species, and (C) facultative CAM species (well watered = blue, drought = red) across the four typical phases of CAM. Filled and unfilled boxes at the bottom of each graph represent night and day, respectively. After Winter and Smith (1996).

for the meta-analysis, precluding generalizations to all CAM species. This analysis also did not analyze carbon uptake patterns, so little is known about whether C₃ + CAM or facultative CAM plants will up- or downregulate across the four

phases. The large phenotypic diversity and flexibility of CAM—both within species and across lineages—likely means there is a high variability in CAM species' responses to elevated CO₂. Much of this variation will be driven by elevated CO₂ interactions with other environmental aspects, such as temperature and water availability.

Temperature

The effects of temperature on any physiological process are often integrated across biological levels; in the case of CAM, aspects of stomatal behavior, water relations, and enzymatic activity are all involved. CAM plants have the highest levels of nocturnal CO₂ fixation when nighttime temperatures are sufficiently lower than during the day—typically 5°C–10°C (Nobel, 1988). One potential explanation for this characteristic day–night difference in optimal temperatures lies in the properties of the tonoplast or the vacuolar membrane (Kluge and Schomburg, 1996). Tonoplast membrane properties change in response to temperatures: at high temperatures, they become more permeable, allowing greater rates of passive efflux of malate from the vacuole (Friemert et al., 1988; Kliemchen et al., 1993); cooler temperatures solidify the lipid membrane and prevent efflux. Despite these intrinsic properties, the tonoplast was found to play a role in high temperature acclimation in CAM species via increased rigidity of the membrane, but this acclimation came at the cost of daytime efflux of malate from the vacuole (Kliemchen et al., 1993). More recent work showed that although the tonoplast appears to be acclimating to warmer temperatures via an increase in the proportion of proteins:lipids in the membrane, the lipids were increasingly composed of unsaturated fatty acids, allowing the tonoplast to maintain fluidity even while overall rigidity increased (Lin et al., 2008). Lüttge (2000) postulated that the tonoplast serves as a central regulator of CAM, with more control over the entire pathway than even the expression of PPC; additional research in this area is warranted to understand the role of the tonoplast membrane in the regulation of CAM across a range of temperatures and species (Kluge and Schomburg, 1996).

Whereas movement of malic acid from the vacuole may occur partially through a passive mechanism, influx into the vacuole is thought to happen due to an electrochemical gradient generated by the active pumping of H⁺ into the vacuole (Lüttge and Ball, 1979; Cheffings et al., 1997; Holtum et al., 2005). There is also evidence for efflux from the vacuole via a malate transporter in the tonoplast (Emmerlich et al., 2003; Wai et al., 2017), but additional studies are needed to verify this transporter's role in CAM malate movement. The rate of efflux from the vacuole and subsequent decarboxylation of malate can impact the duration of daytime phases of CAM (Grams et al., 1997; Dever et al., 2015; Ceusters et al., 2021). Temperature changes affecting malate movement across the tonoplast are therefore important considerations for understanding CAM plants under climate change.

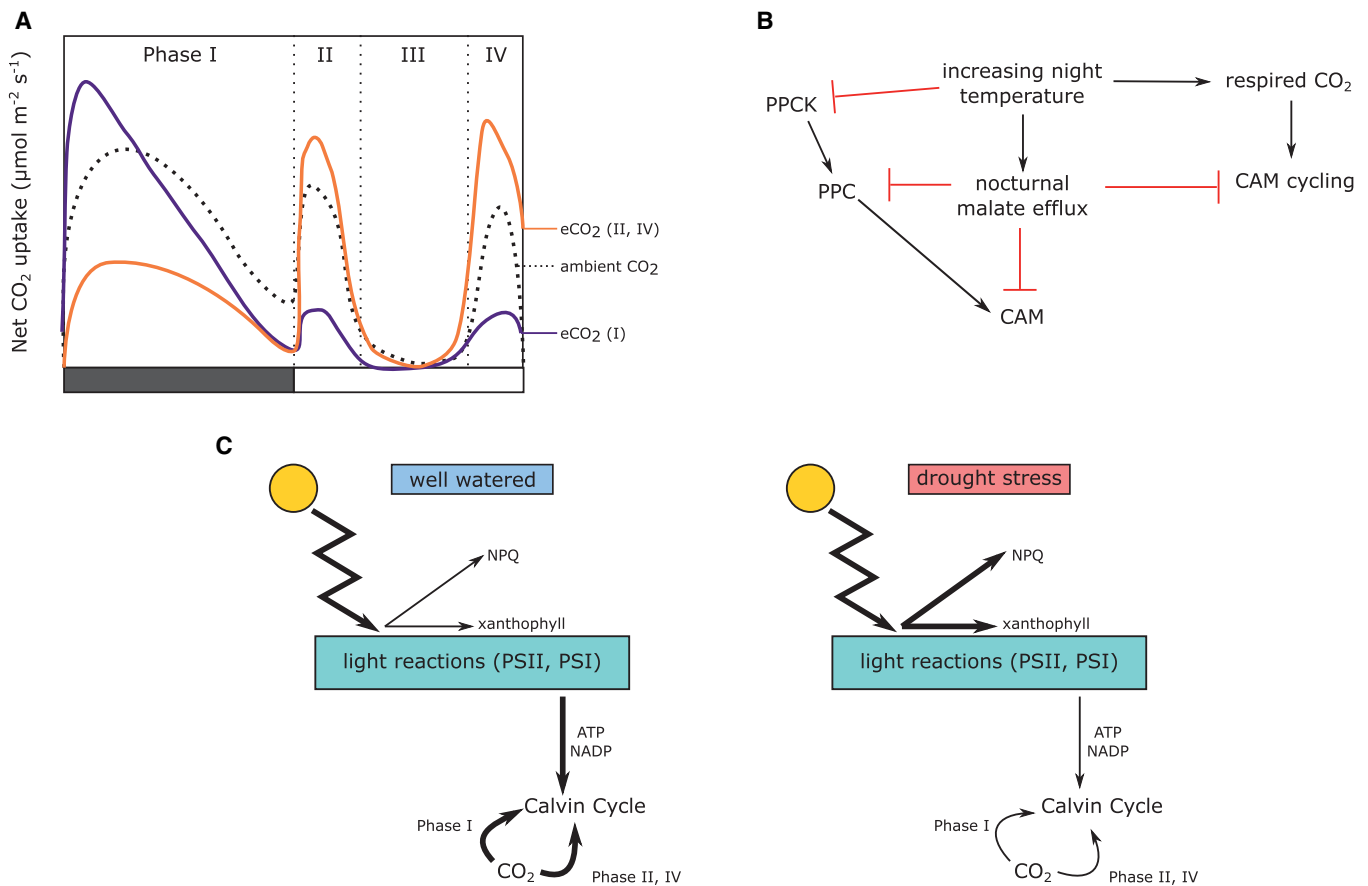


Figure 2 Possible effects of environmental perturbations on CAM. A, Effects of elevated CO₂ concentrations on the four phases of CAM (based on data summarized in Drennan and Nobel, 2000). Filled and unfilled boxes at the bottom represent night and day, respectively. Dashed line showed standard net CO₂ uptake curve at ambient CO₂ levels, whereas purple and orange lines indicate two possible CAM responses: increase in phase I CO₂ uptake (purple) or increase in phases II and IV uptake (orange). B, Schematic showing possible effects of increasing night temperatures on enzymes, malate efflux from the vacuole, and alterations to the carbon cycle in CAM plants. C, Changes to energy demands and dissipation under well-watered and drought-stress conditions for a CAM plant, where CO₂ can be supplied either by phase I or phases II and IV. Arrow thickness indicates the amount of flow through that part of the pathway. Drought stress can reduce CO₂ uptake and result in an imbalance of energy entering the system versus energy required; excess energy can be dissipated via NPQ and xanthophyll. ATP, adenosine triphosphate; NADP, nicotinamide adenine dinucleotide phosphate.

Enzymes involved in CAM biochemistry have temperature optima and can be adversely affected by higher or lower temperatures. PPC, the main carboxylating enzyme in the CAM pathway, is a critical component of nocturnal carbon fixation. Comparisons between CAM *Crassula argentea* and C₄ *Zea mays* showed that whereas the C₄ enzyme had a detrimental conformational change at temperatures above 27°C, the CAM enzyme continued to have increased activity at higher temperatures (Wu and Wedding, 1987). Similar experiments in *Kalanchoe* species show PPC enzyme activity is higher under warmer temperatures (Lee et al., 2007), but negative regulation via malate sensitivity also increases (Carter et al., 1995). This negative regulation by malate is avoided in CAM plants at night by the phosphorylation of PPC by a dedicated kinase, PPC kinase (PPCK). PPCK mRNA expression increases with increasing temperatures (Hartwell et al., 1996; Borland et al., 1999), though this has not been explored at temperatures higher than 30°C. Whereas enzyme activities appear to increase at warmer temperatures,

future research should experimentally test temperatures >30°C and effects on additional CAM enzymes. Moreover, broadening these studies to include transcription and translation in addition to enzyme activity will help to better characterize the complex response of CAM biochemistry to increasing temperatures (Figure 2B).

CAM plants will also be differentially affected by temperature extremes depending on the extent to which they rely on the CAM pathway for carbon gain. Whereas higher nocturnal temperatures decreased transpiration and overall photosynthesis in CAM species (Neales, 1973; Neales et al., 1980), higher daytime temperatures did not affect daytime conductance as greatly (Nobel and Hartsock, 1979; Nobel, 1988). In other words, CAM plants that have appreciable C₃ photosynthesis during the day may not be as affected by higher temperatures in terms of overall total carbon gain. High daytime temperatures will also affect other processes in CAM leaves, including light reactions and general cell stress, but those are unlikely to be unique to CAM plants.

Increased nocturnal temperatures might limit atmospheric CO₂ fixation, but will also increase respiration; many CAM plants can take respired CO₂ and convert it to malic acid for subsequent daytime fixation. Studies have shown that whereas some CAM species can have negligible CO₂ fixation at night under high temperatures, malic acid accumulation remains high, suggesting a recycling of respired CO₂ that can act as a buffer against photosynthetic loss under warmer night temperatures (Medina and Osmond, 1981; Nobel, 1988). However, negative feedback loops described above (e.g. malate efflux and decreased PPC activity) might hinder even CAM cycling under extreme temperatures (Figure 2B).

The diversity of CAM species both across the green plant phylogeny, as well as across habitat types, means any prediction of how CAM plants will fare under changing temperatures will need to be conditioned on their current temperature preferences. For example, there is no single temperature optimum for all CAM plants experimentally examined to date. In fact, work in *Agave* showed that optimal day/night temperature regimes for different species unsurprisingly mirrored temperatures found in their natural habitats; in another study, temperature had relatively little effect on biomass of *Agave angustifolia* (Holtum and Winter, 2014). In *Kalanchoe* species, plants acclimated to lower day/night temperature regimes did not have an endogenous CAM cycle at elevated temperatures, whereas plants acclimated to higher temperatures for 4 weeks were able to maintain CAM function at high temperatures (Grams et al., 1995; Yamori et al., 2014).

Drought

CAM is often discussed in the context of water limitation and whereas that is undoubtedly a major factor in the evolution and success of CAM plants, responses to drought in CAM plants are variable. Much of the drought-response research has understandably focused on C₃+CAM species, particularly those that can facultatively upregulate CAM in response to drought (Winter et al., 2008; Fleta-Soriano et al., 2015; Winter, 2019; Heyduk et al., 2020). Indeed, how facultative CAM plants sense drought stress and induce CAM photosynthesis remains unanswered. Less research has focused on how constitutive CAM plants respond to drought stress, even though abiotic factors can affect nocturnal CO₂ carboxylation rates (Winter, 2019). In seedlings of constitutive *Clusia* and *Kalanchoe* species, drought induced elevated nocturnal CO₂ fixation rates (Winter et al., 2008) and in CAM *Yucca aloifolia*, drought stress reduced nocturnal CO₂ fixation (Heyduk et al., 2016). The cause of the different drought responses may be differences across lineages or the ages of the plants studied (i.e. seedlings versus mature), but additional studies are required for generalizable conclusions.

Water limitation varies in strength and duration across the year for many plants and CAM plants are no different. Comparisons of CAM and C₃ tank bromeliads during the dry season in the Campo Rupestre of Brazil showed that whereas CAM plants could maintain metabolism under

drought via nocturnal photosynthesis, it came at a cost: CAM bromeliads lost larger amounts of leaf water whereas the C₃ species conserved water by severely limiting stomatal opening (Marques et al., 2021). The constitutive CAM saguaro cactus (*Carnegiea gigantea*) exhibits seasonal variation in the levels of CAM and C₃ photosynthesis employed (Bronson et al., 2011). Extreme drought over 6 months shifted constitutive CAM *Phalaenopsis* “Edessa” plants to CAM idling, whereby atmospheric CO₂ assimilation is abolished but respired CO₂ is refixed (Ceusters et al., 2019). Clearly, CAM species are also negatively affected by drought conditions, but can persist under those conditions for far longer than C₃ or C₄ species (Nobel, 1991). Additional studies on CAM species responses to drought stress are needed to better understand the diversity of mechanisms underlying their tolerance, as well as the variation in this trait across CAM phenotypes and lineages (though see “CAM under stress in the genomics era” for examples of drought studies on facultative CAM species).

In addition to empirical evidence, models of CAM photosynthesis have been developed to estimate productivity of CAM species on marginal lands (Hartzell et al., 2021), to assess limitations to and benefits of the CAM pathway (Töpfer et al., 2020), and to compare overall potential productivity of CAM species to those of C₃ and C₄ (Hartzell et al., 2018; Shameer et al., 2018). These models parameterize water relations and thus can make predictions of how plants fare under simulated drought conditions. Specifically, CAM had higher carbon gain compared with C₃ and C₄ under a prolonged drought and had overall lower transpiration rates, suggesting their ability to maintain a water supply near the roots for a longer period of time (Hartzell et al., 2018). A separate simulation study found that although CO₂ uptake was maintained under drought in CAM, CO₂ uptake rates decreased relative to control, particularly in phases II and IV, and eventually affecting even phase I (Bartlett et al., 2014). The existence of models that have parameterized the complexity of CAM systems—from circadian rhythms and water limitation to carbon assimilation and irradiance—holds great promise for simulations to understand how environmental perturbations like drought, temperature, and CO₂ levels will affect CAM species.

Beyond carbon: abiotic stress effects on photosystems

Environmental perturbations of water availability, temperature, and CO₂ concentration will impact carbon capture efficiency and overall growth of CAM plants. But like any organism, CAM plants are complex systems and focusing only on CO₂ uptake is narrow in scope. In particular, the carbon reactions of photosynthesis are directly tied to the light reactions; energy generated by photosystems II and I (PSII and PSI) is used in the Calvin cycle in *all* plants, regardless of any CCM. In CAM plants, both the light reactions and the Calvin cycle are temporally separated from the nocturnal CO₂ fixation via PPC. The amount of CO₂ decarboxylated

during the day during phase III, and the amount of CO₂ obtained from the atmosphere in phases II and IV, impacts the amount of energy from light reactions demanded by the Calvin cycle (Figure 2C). Therefore, environmental stressors like heat and drought can affect the photosystems of CAM plants directly and indirectly, the latter through their effects on carbon fixation.

In most plants, excess light will cause damage to the photosystems responsible for transferring energy through the light reactions; irreversible damage is known as photoinhibition and plants have multiple mechanisms to transfer extra energy either as heat (non-photochemical quenching, NPQ) or through other molecules (e.g. xanthophyll) (Demmig-Adams and Adams, 1992). Excess energy can also result if there is an imbalance between light availability and sink demand (i.e. the Calvin cycle). CAM plants employ the same NPQ strategies as C₃ species and appear to have evolved protection mechanisms that prolong the amount of time CAM plants can survive in environmental conditions that might lead to photoinhibition. For example, 6 weeks of drought in the CAM orchid *Phalaenopsis* “Edessa” induced CAM idling, whereby plants had constantly closed stomata and relied on recycled, respired CO₂ to maintain metabolism (Ceusters et al., 2019). Measures of photosystem performance showed reductions in photochemical activity in favor of energy dissipation, which matched the flux demands for carbon while CAM idling limited CO₂ availability (Ceusters et al., 2019). Four months of drought stress in *Agave salmiana* resulted in a decrease in chlorophyll *b* content, increased NPQ, and decreased PSII function; upon re-watering, all measures of photosystem function recovered to control levels, again suggesting that the integrity of photosystems was maintained over a long duration drought (Campos et al., 2014).

A number of other studies on desert and tropical CAM plants have suggested the same idea: CAM species, under drought, have enhanced abilities to reduce photoinhibition and can resume normal function rapidly post-drought (de Mattos et al., 1999; Cela et al., 2009; Masrahi et al., 2015). The ability of CAM plants to maintain low-level metabolic function (e.g. CAM idling) under drought allows maintenance of photosystems in a way that enables rapid recovery. In this respect, climate change-induced drought stress is likely to affect carbon reactions directly in CAM plants and indirectly affect light reaction efficiency and recovery. Light reactions are of course affected by other environmental cues, including higher temperatures. The effect of high temperatures on photoinhibition processes in CAM plants has not been extensively explored.

Anatomical considerations

Aside from a shared biochemical pathway, CAM plants often (though not always) have succulent leaves or stems that can store water for use during periods of drought (Borland et al., 2018; Grace, 2019). Succulent leaf morphology additionally benefits CAM by providing large cells for malate

storage, but can limit CO₂ diffusion by reducing air spaces between cells (Nelson and Sage, 2008; Zambrano et al., 2014; Males, 2018). The ability of CO₂ to move through the tissue is known as conductance; low conductance of CO₂ during the daytime when carboxylation occurs via RuBisCO increases the proportion of oxygenation to carboxylation, and likely leads to increased photorespiratory stress in plants. CAM species *Kalanchoe daigremontiana* has one of the lowest internal CO₂ conductance values measured in plants (Maxwell et al., 1997); such low conductance values could impede phases I, II, and IV carboxylation in CAM plants. Succulent leaves tend to also have a higher leaf mass per unit area (LMA); in general, high LMA leaves are often associated with resource-limited environments and tend to have lower nutrient concentrations than leaves with lower LMA (Reich et al., 1997; Poorter et al., 2009). A comparison of thick leaves (high LMA, both succulent and non-succulent) to thin leaves (low LMA) showed that thicker leaves will maintain lower nutrient status even under high-nutrient conditions. This leads to limitations in photosynthetic capacity, decreases the sink strength of RuBisCO, and therefore potentially limits conductance of CO₂ through the leaf (Nielsen et al., 1997).

Succulent tissues store large amounts of water, in addition to being reservoirs for malate. Both the water content and the overall thickness of succulent tissues can reduce heat stress in leaves by increasing the overall thermal mass—in other words, increasing how long it takes for temperatures within the tissue to rise. For example, thicker leaves (high LMA) were shown to reduce heat stress via models comparing arid plant species (Curtis et al., 2012; Leigh et al., 2012). This was especially true under environmental conditions that limited effective evaporative cooling, such as low wind speeds (Leigh et al., 2012). The water stored in succulent leaves is, of course, best used in times of drought; succulent plants can quickly mobilize water reserves to preserve metabolic function and growth (Ogburn and Edwards, 2010), even under prolonged drought (Goldstein et al., 1991; Pimienta-Barrios et al., 2002; Nobel, 2006). The succulent nature of many arid CAM plants means they are likely to have the additional benefit of stored water under future drought regimes over C₃ and C₄ species.

CAM under stress in the genomics era

In the last decade, a number of studies have begun to explore the genetic components of CAM photosynthesis, with reference genomes available (Cai et al., 2015; Ming et al., 2015; Yang et al., 2017; Wai et al., 2019; Wickell et al., 2021), additional transcriptomic studies (Abraham et al., 2016; Brillhaus et al., 2016; Heyduk et al., 2018a, 2018b, 2019; Gilman et al., 2022), and exciting functional genetics and genomics research ongoing (Boxall et al., 2017, 2020; Lim et al., 2019). Most studies that have examined abiotic stressors on CAM in a genomics context have focused on drought (though see Shakeel et al., 2013), limiting our understanding of how abiotic stress response is regulated in CAM plants

more generally, and providing an opportunity for further research. Nearly all studies that assess -omic changes in CAM species in response to abiotic stress focus on transcriptomic responses, with a few exceptions. [Brilhaus et al. \(2016\)](#) and [Heyduk et al. \(2019\)](#) also assessed metabolite changes and [Abraham et al. \(2016\)](#) assessed transcript, protein, and metabolite turnover across the diel cycle in *Agave americana*, but did not do so under abiotic-stress conditions. Additional studies exploring transcription to translation and the regulation of both are needed to understand the full response of CAM plants to abiotic factors.

Surprisingly, only a handful of the genomic studies in CAM have explored the effects of environmental factors on constitutive CAM species (the majority focus on facultative CAM). In the constitutive CAM *Agave sisalana*, over 3,000 genes had differential expression in response to drought stress, including genes involved in purine and thiamine metabolism and carbohydrate processing ([Sarwar et al., 2019](#)). This study only sampled at a single time point, however, and therefore did not capture the temporal dynamics of CAM and how they change under drought stress. In a comparison between constitutive CAM and C₃+CAM species in the Agavoideae (Asparagaceae), C₃+CAM species had an increase in the use of CAM photosynthesis under drought, whereas constitutive CAM species were only slightly negatively affected physiologically, evidenced by decreased leaf acid accumulation and a reduction in daytime CO₂ uptake ([Heyduk et al., 2018b, 2022](#)). The same study explored differences in expression of sugar metabolism genes and stomatal regulators, as CAM plants are thought to have dampened stomatal responses to daytime opening cues (e.g. blue light) and are likely affected by drought signals differently than C₃ stomata ([Males and Griffiths, 2017](#)). Sugar metabolism genes had differential expression under drought in CAM and C₃+CAM species, though analysis of stomatal regulatory genes was inconclusive. A drought experiment on constitutive CAM *Y. aloifolia* showed overall dampening of CAM physiology after 7 days drought and an almost total restriction of daytime CO₂ uptake ([Heyduk et al., 2016](#)). Gene expression of core CAM pathway genes also decreased under drought, corroborating measured physiological responses ([Heyduk et al., 2019](#)).

In facultative CAM plants, CAM is upregulated directly in response to an environmental stress. As a result, in-depth transcriptomic studies have been conducted on facultative CAM species with particular attention to drought response. Unsurprisingly, multiple studies find up-regulation of core CAM pathway transcripts in independent facultative lineages ([Brilhaus et al., 2016](#); [Heyduk et al., 2019](#); [Wai et al., 2019](#); [Gilman et al., 2022](#)). Whereas the photosynthetic response to drought in these species is predictable, several other pathways are affected by drought. Expression of ABA signaling transcripts was strongly induced during drought stress in facultative CAM *Talinum triangulare* ([Brilhaus et al., 2016](#)). Starch and sugar metabolic genes had differential

expression as well, though it's unclear if those are direct responses to drought or related to the induction of CAM. *Talinum triangulare* also showed induction of light stress-response genes, increase in gene expression of catabolic processes for fatty acids, and reduced expression of genes involved in the cell cycle and DNA replication. Together the transcriptomic responses in *T. triangulare* suggest that although CAM induction reduces water loss, multiple aspects of the plant are still dealing with stress incurred from drought. In general, describing the discrete pathways involved in CAM upregulation and a more general stress response in facultative CAM species is difficult ([Wai et al., 2019](#)), and would require the presence of a closely related outgroup without CAM induction. Whereas closely related C₃+CAM and C₃ species of the genus *Yucca* were drought stressed ([Heyduk et al., 2019](#)), analysis of the subsequent gene expression data focused on CAM induction but not drought responses directly.

Conclusions and future directions

CAM photosynthesis is a complex trait in that it requires the careful integration of multiple aspects of plant biology, including photosynthetic physiology and light reactions, metabolite movement, and anatomy. Whereas CAM plants are an evolutionary response to decreasing CO₂ concentrations, they are almost assuredly not doomed to nonexistence under our higher CO₂ future. CAM plants will likely continue to maintain an advantage of C₃ and C₄ in areas where water is limiting, though the extent to which that advantage continues under various CO₂ concentrations and drought extremes remains unknown. Importantly, the current understanding of CAM responses to climate-change factors is based on a very small number of species, concentrated in the Agavoideae, Cactaceae, and Crassulaceae. These represent a small fraction of the total diversity of CAM plants; future research should aim to increase the breadth of species considered, particularly tropical CAM plants. On the other hand, detailed functional genetics could be expanded in model CAM systems like *Kalanchoe* to further understand the mechanisms by which CAM plants respond to stressors associated with climate change. Distinctions in plant responses to climate should also be explored between constitutive CAM species and those that are C₃+CAM or facultatively CAM. Finally, the largest area lacking in our understanding of CAM plants under climate-change scenarios is how they will fare under the interactive effects of abiotic stress. Increasing CO₂ levels also will increase temperatures and can affect long-term weather patterns. A few notable studies have examined the effect of multiple variables on CAM: *Cylindropuntia imbricata* (CAM) had an advantage over *Bouteloua eriopoda* (C₃) under elevated CO₂ conditions and drought stress; that advantage disappeared in well-watered conditions ([Yu et al., 2019](#)). On the other hand, CAM and C₄ plants fared worse under elevated CO₂ when temperature was also elevated ([Wang et al., 2008](#)). Additional studies in diverse lineages are required to fully

OUTSTANDING QUESTIONS

- What are the interactive effects of drought, elevated CO₂ concentration, and high temperature on CAM photosynthesis?
- How does the response to these interactions vary across lineages? For example, will tropical and arid CAM plants have fundamentally different shifts in photosynthetic physiology and fitness in response to climate change?
- How will tropical species in the Orchidaceae and Bromeliaceae, which represent the largest diversity of CAM species but are relatively understudied in terms of physiological responses to environmental perturbation, respond to climate change?

understand how CAM plants respond to the interactions of abiotic stress.

CAM plants represent a sizable fraction of the flora on the planet and are important species in both tropical and arid environments. The current body of empirical evidence has barely scratched the surface of understanding how CAM plants respond to abiotic stress and the implications for climate change (see “Outstanding Questions”). The effects of climate change are already being seen. Our ability to predict threats to species and ecosystems requires further interrogation into how CAM species will fare under a changing climate.

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