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Evolution of Photosynthetic Metabolism in Terrestrial Plants.

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Introduction:

Photosynthetic metabolism has evolved in concert with the changing atmosphere of the earth. Central to this evolutionary interplay has been the dual catalytic nature of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The primary reaction of Rubisco is the carboxylation of RuBP to form PGA in photosynthetic carbon fixation. Rubisco also oxygenates RuBP, forming phosphoglycolate, a useless product that is toxic at elevated cellular concentrations. To avoid toxicity, plants must excrete p-glycolate, respire it, or metabolize it in the photorespiratory cycle. In the process, previously fixed CO₂ is lost, energy is expended and photosynthetic efficiency declines. For these reasons, RuBP oxygenation is considered a flaw in the function of Rubisco (Sage 1999).

The atmosphere of the early earth had very high CO₂ levels, and free O₂ was not present (Fig. 1). Under these conditions, RuBP oxygenation was extremely rare and likely had little evolutionary significance. By the time land plants appeared some 450 million years ago, O₂ had risen to 20%, and CO₂ levels had fallen substantially, thereby raising the potential for oxygenase activity. Further reductions in CO₂ occurred throughout the history of land plants, most notably in the past 50 million years, such that RuBP oxygenation became a significant limitation on photosynthesis. Because of this, evolutionary adjustments have occurred that compensated for photorespiratory limitations (Table 1). Here, I discuss two examples illustrating how the oxygenation flaw may have affected terrestrial plant evolution. The first is a possible influence of RuBP oxygenation on the origin of land plants. The second is C₄ plant evolution, a relatively recent development that is associated with the decline in atmospheric CO₂ that has occurred over the past 50 million years (Cerling 1999).

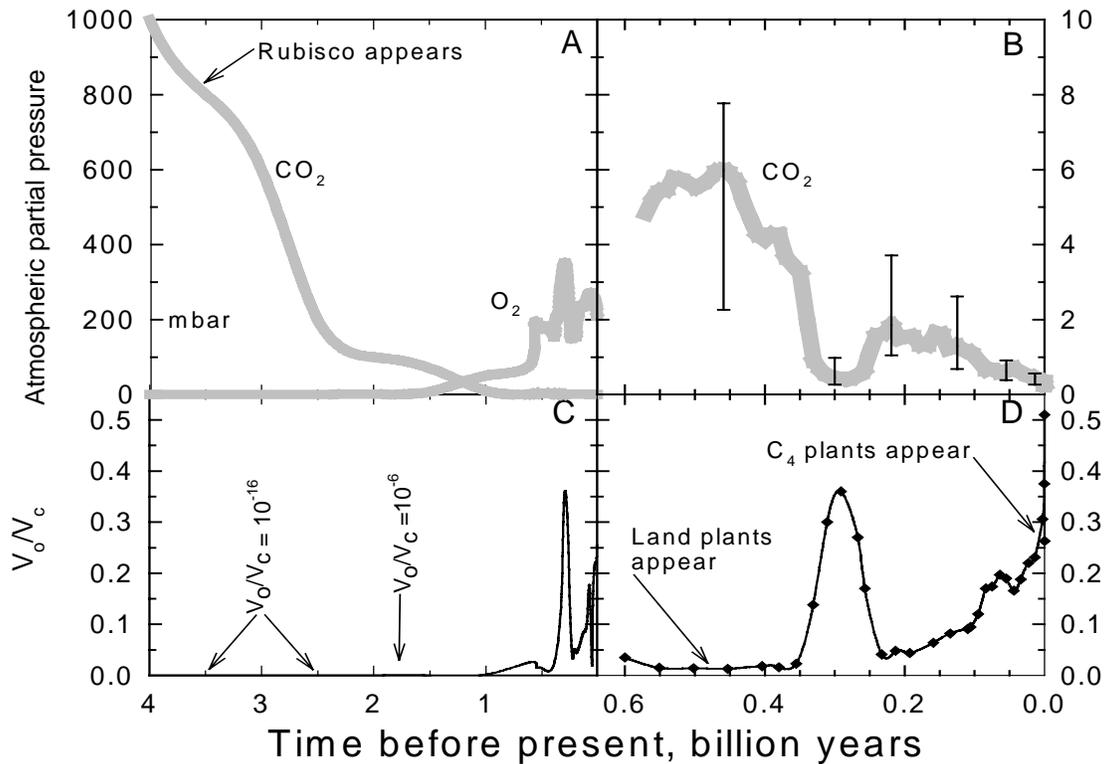


Figure 1: The atmospheric CO₂ and O₂ content since the origin of life (A) and the beginning of the Cambrian era (B). Panels C and D show the corresponding Rubisco oxygenase activity (v_o) relative to carboxylase activity (v_c) modeled at 30°C for the atmospheric conditions of the time (Sage 1999 using data from Berner 1994).

Table 1: Compensation mechanisms for high oxygenation potential in plants.

1) Compensation for rapid P-glycolate production (Tolbert 1992)	
a) Glycolate excretion	All forms of algae
b) Leaf-type peroxisomes and a complete C ₂ photorespiratory cycle	Charophycean green algae and terrestrial plants
2) Compensation by reducing oxygenation potential (Sage 1999)	
a) Lower the specificity of Rubisco for O ₂	C ₃ plants
b) Reduce leaf temperature	C ₃ plants
c) CO ₂ concentrating mechanisms	Most algae (HCO ₃ ⁻ pumping), C ₄ and CAM plants

RuBP Oxygenation and the Origin of Land Plants: In terrestrial plants, the photorespiratory cycle is well developed and is able to rapidly metabolize glycolate to PGA, even at proportionally high rates of oxygenase activity. Mutations in the cycle

are commonly lethal except under high CO₂ conditions where oxygenase activity is minimal. A key component of the photorespiratory cycle in terrestrial plants is the leaf-type peroxisome, which is unique to land plants and their immediate green algae ancestors, the Charophytes (Tolbert 1992). Leaf-type peroxisomes contain many key enzymes of the photorespiratory cycle, most notably glycolate oxidase and hydroxypyruvate reductase. With the exception of the Charophytes, most algae either lack advanced photorespiratory metabolism as expressed by the Charophytes and terrestrial plants, or they do not localize certain key steps within the peroxisome (Stabenau 1992; Tolbert 1992). As a consequence, they either cannot metabolize oxygenation products back to Calvin cycle intermediates such as PGA (as appears to be the case in most red, brown and xanthophyte algae, and the diatoms and dinoflagellates), or they have a low capacity to metabolize glycolate (Tolbert 1992). One advantage of incorporating the peroxisome into the photorespiratory pathway is that it allowed O₂ to be used for rapid oxidation of glycolate, with the resulting hydrogen peroxide being quickly detoxified by catalase activity within the peroxisome (Chapman and De Jesus, 1992). From these considerations, it appears that leaf-type peroxisomes are essential if plants are to have a high enough capacity to recover the carbon and energy diverted to glycolate when oxygenase activity is high.

In algae, the need for a high capacity to recycle photorespiratory metabolites is largely absent because RuBP oxygenation is rarely a problem. Algae have the ability to concentrate CO₂ around Rubisco and thereby suppress oxygenase activity (Graham and Wilcox 2000). When confronted with high rates of photorespiration, the response of most algae is to excrete glycolate or glyoxalate into the surrounding medium (Chapman and De Jesus 1992; Tolbert 1992). This is a temporary solution that lasts only until carbon concentrating mechanisms are activated.

It is now well established that land plants are monophyletic, having arising from green alga ancestors that are closely related to modern Charophytes (for example, *Chara*, *Nitella*, *Coleochaete*; Graham and Gray, 2001). Charophytes are common in fresh-water ponds, marshes and shallow lakes which are often ephemeral, and which are prone to substantial heating during the day (Smith 1933). While the ability of Charophytes to survive episodic drying is thought to be an important contribution to their ancestry of land plants (Graham and Gray 2000), the significance of advanced photorespiratory metabolism in the Charophytes has not been emphasized. This may be a result of the well-developed ability of the Charophytes to pump inorganic carbon, such that photorespiration is not viewed to be a major factor in their natural environment (Tolbert 1992). However, the freshwater habitats of the Charophytes commonly have a finite carbon supply owing to their shallow depth. This, coupled with substantial daytime heating that reduces CO₂ solubility and the CO₂ specificity of Rubisco, creates conditions that episodically promotes high oxygenase activity, even in species with a well-developed bicarbonate pump. (As an example of the chronic nature of CO₂ depletion in small ponds, submerged aquatic plants in the Isoetaceae use CAM-type photosynthesis to compensate for diurnal CO₂ deficiency). Thus, it can be argued that Charophytes periodically require a high capacity for photorespiratory metabolism, as it could be a critical carbon conservation mechanism during episodes when their CO₂ supply is depleted.

Was the evolution of high-capacity photorespiration a necessary precondition for plants to invade terrestrial landscapes? To address this, it is useful to compare photosynthetic possibilities in aquatic habitats versus that of terrestrial habitats. In most aquatic environments, the potential exists to concentrate inorganic carbon

dissolved in the surrounding media and excess photorespiratory metabolites can be dumped into the surroundings, if need be. In terrestrial plants, neither option exists. There isn't a large reservoir of surrounding water from which to concentrate carbonate ions, and the cuticle required for desiccation resistance in a terrestrial setting prevents the excretion of photorespiratory intermediates. The only way to survive RuBP oxygenase activity in terrestrial settings, therefore, is to fully metabolize the resulting products to CO₂, PGA or some other useful end. Under conditions where the oxygenation potential is high, the ability to respire glycolate to CO₂ is easily overwhelmed (Tolbert 1992; Chapman and De Jesus, 1992). High rates of glycolate oxidation require an abundance of electron acceptors, which become limiting in the absence of energy sinks. In addition, if glycolate is not converted back to PGA, the Calvin cycle intermediates are rapidly depleted, creating problems for photosynthesis. Finally, failure to rapidly metabolize or excrete glycolate would lead to its accumulation to toxic levels. Based on these considerations, the colonization of the land by complex photosynthetic life is hypothesized to have required a high capacity for photorespiratory metabolism. Until this happened, the terrestrial environment could only be exploited by simple algae that relied upon carbon concentration from surrounding water films, or alternatively were able to dump glycolate during intermittent rain events. The group of algae that first developed a high capacity for photorespiratory metabolism would then be able to invade and dominate the land without competition from other algae groups. This is consistent with the geological record, which shows land plants radiated from a common Charophycean ancestor 420 to 470 million years ago (Graham and Gray 2001).

CO₂ levels were high at the time land plants arose (Fig. 1), and therefore could have minimized the significance of RuBP oxygenation. Could this have eliminated a controlling role for photorespiration in the evolution of land plants? Two points relevant to this issue are worth noting. First, the climate 400 million years ago was torrid. Sea surface temperature was near 40°C (much greater than the current tropical average near 25°C; Algeo et al. 2001), indicating terrestrial environments were hot as well. CO₂ solubility is markedly depressed above 40°C, and Rubisco's affinity for CO₂ relative to O₂ is half of its value at 10°C (Jordan and Ogren, 1984). Hence, photorespiratory potential would be high under conditions where the CO₂ supply is depleted, as it can be in shallow lakes, or in the case of early terrestrial plants, when epidermal pores close to prevent desiccation. One important point about the hothouse climate of 450 million years ago is that the desiccation potential above 40°C is greatly increased, even when there is abundant soil water. For example, at 50% relative humidity, the transpiration driving force is over three times greater at 45°C than 25°C. As a result, the need to control water loss was great and likely led to substantial reduction in intercellular CO₂ levels, at least episodically. In turn, this could have caused enough RuBP oxygenation that a well-developed capacity for photorespiratory metabolism was required for survival.

In summary, it is hypothesized that a precondition for the rise of the land plant habit was the development of advanced photorespiratory metabolism as expressed by the Charophytes and terrestrial plants. If true, this proposal would explain why green algae, and not any other algae group, were able to give rise to terrestrial plant life. It would also mean that the green color that dominates our terrestrial world resulted in part from an ancient flaw in the function of Rubisco.

C₄ Plant Evolution: C₄ photosynthesis is a polyphyletic mode of metabolism that serves to concentrate CO₂ into a bundle sheath compartment where Rubisco has been localized. In the process, bundle sheath CO₂ levels are elevated to the point where RuBP oxygenase activity is reduced to below 5% of the carboxylation rate, and the overall rate and efficiency of photosynthesis is substantially enhanced. C₄ plants are successful in warm to hot environments where oxygenation rates in C₃ plants are high, and in drier environments where high water use efficiency has great value. They generally require moderate to high light intensities for ecological success, and hence are commonly found in open environments where dense shade from forest trees is not present. Most C₄ species are herbaceous, although there are dozens of C₄ shrubs, which are largely restricted to deserts. There are no robust C₄ trees. Currently, there are some 8000 C₄ species, all of which are angiosperms. Most (6500 species) are grasses or sedges, while approximately 1500 species are scattered amongst 16 diverse dicot families. Half the dicot families contain relatively few C₄ taxa; for example, five of the 16 have only one C₄ genus. (Sage et al. 1999a,b).

The earliest direct evidence for C₄ plants are fossils from 12 to 13 million years ago (Cerling 1999). Earlier fossils that may be from C₄ plants have been dated at about 14 million years ago, and there is some isotopic evidence for C₄ presence at 15 million years ago (Cerling 1999; Kellogg, 2000). Indirect taxonomic evidence indicates major C₄ grass taxa diverged by at least 25 to 30 million years ago (Kellogg 1999). Because the grasses examined share a common C₃ ancestor, the divergence time had to post-date C₄ plant evolution. From this, it has been suggested that the earliest C₄ plants date from at least the Oligocene epoch, between 23 and 35 million years ago (Kellogg 1999; Sage 2001). It is unlikely that C₄ photosynthesis is older than 50 to 60 million years, as the plant families that now contain C₄ species are generally younger than this time, or as is the case with the grasses, were relatively uncommon in the fossil record until after 50 million years ago (Fig. 2). The earliest C₄ species were grasses, as the diversity of C₄ plants in the grass family is greater than any other family, indicating an older origin (Fig. 2). The relatively low diversity of C₄ species in most dicot families indicates a recent origin, possibly as late as the Pleistocene epoch between 12,000 and 2.5 million years ago (Ehleringer et al. 1997).

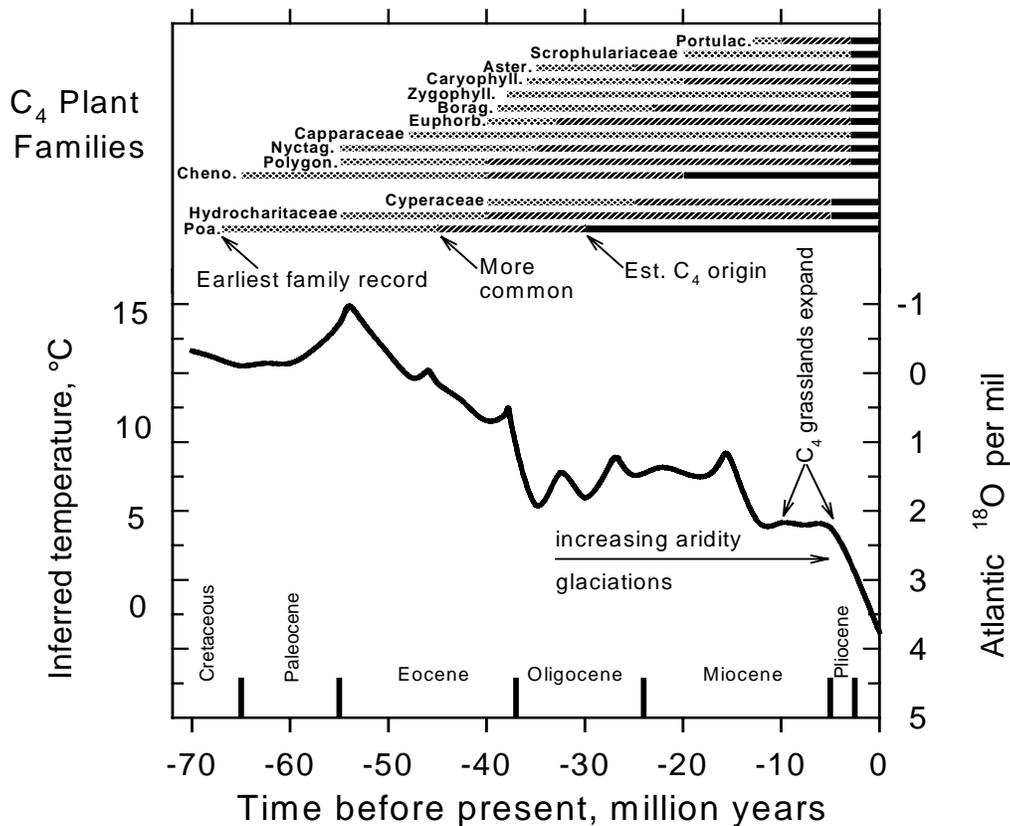


Figure 3: The temporal occurrence of plant families containing C_4 photosynthesis (from Sage 2001). The inferred temperature was derived from oxygen isotope ratios in deep ocean cores from the mid-Atlantic ocean, Prothero 1994). Abbreviations: Poa. – Poaceae; Cheno. – Chenopodiaceae; Nyctag. – Nyctaginaceae; Euphorb. – Euphorbiaceae; Borag. – Boraginaceae. All other abbreviations end in “aceae”.

At 8 million years ago, shifts in carbon isotope ratios from C_3 to C_4 values occur in soil deposits and herbivore remains from low latitudes around the world, indicating a widespread expansion of C_4 vegetation to ecological dominance (Cerling et al. 1997). Prior to this time, there is little evidence that C_4 plants were more than modest members of plant communities, and it is possible that for millions of years they were largely restricted to extreme locations where the advantages of the C_4 syndrome were most pronounced (Kellogg 1999; Sage 2001).

Because C_4 plants are most successful in hot, arid and high light habitats, it had been suggested that these conditions promoted the evolutionary diversification of the syndrome. The realization that C_4 photosynthesis appeared relatively recently in geological time raised questions about the role of temperature and precipitation as evolutionary forcing agents, because these conditions had always existed, and the earth’s climate was cooling, rather than warming, during the period of C_4 diversification (Fig. 2; Ehleringer et al. 1991). In addition, the multiple number of origins of C_4 photosynthesis (over 30 in a recent count; Sage 2001) indicate that it is not an extremely difficult syndrome to evolve. Hence, it seems C_4 photosynthesis should have appeared earlier in the history of land plants. The key development that

clarified this uncertainty was the characterization of atmospheric change through geological time (Berner 1994 and references there-in). Over much of the time since the rise of terrestrial plants, the atmospheric CO₂ level was elevated with respect to today, by 5 times or more (Fig. 1). Under these high CO₂ conditions, C₄ photosynthesis is at a disadvantage because it has a higher energy requirement than C₃ photosynthesis in high CO₂, and is less effective exploiting cool, shaded situations than C₃ species (Ehleringer et al. 1997). In the past 50 million years, atmospheric CO₂ levels have declined from high values to low levels well below current values (Fig. 1). At the height of the ice age, for example, CO₂ levels fell below 200 μbar CO₂ for tens of thousands of years at a time (Sage 1999). Conditions below 200 μbar clearly favor C₄ plants in warm environments, as the absolute photosynthesis rate, and the efficiency of light, nitrogen and water use is superior in C₄ relative to C₃ species (Ehleringer et al. 1991; 1997; Sage 2001). Once the CO₂ record was clarified, it became possible to hypothesize that atmospheric CO₂ reduction was the selection agent for the initial rise of C₄ photosynthesis and for the later ecological expansion of C₄-dominated vegetation 8 million years ago (Ehleringer et al. 1991; 1997; Cerling et al. 1997). Initially, the focus was on late-Miocene reductions in CO₂ (Ehleringer et al. 1991); however, recent evaluations indicate CO₂ levels favoring C₄ photosynthesis, at least in hot climates, may have first appeared between 30 and 40 million years ago (Pearson and Palmer, 2000). Subsequent reductions in CO₂ are postulated to have occurred in the late-Miocene, and this may have favored the expansion of C₄ plants to the ecological dominance observed for this time (Cerling et al. 1997). The recent origin of C₄ dicots may have followed CO₂ reduction below 200 μbar that occurred in the Pleistocene epoch (Ehleringer et al. 1997).

While the need to reduce photorespiration provides a rational for C₄ plant evolution, an equally important consideration is that high rates of photorespiration in C₃ plants may have provided a metabolic framework upon which evolutionary selection pressures could act. In doing so, photorespiration may have provided the evolutionary bridge from C₃ to C₄ photosynthesis (H. Bauwe, personal communication; Monson 1999). Under conditions of high photorespiration, C₃ plants generate substantial CO₂ through the decarboxylation of glycine. Some C₃ species localize glycine decarboxylase in bundle sheath tissues, creating an internal compartment where CO₂ can be elevated, which then enhances the efficiency of nearby Rubisco. This localization of glycine decarboxylase in the bundle sheath may have been the initial step in the evolution of C₄ photosynthesis (Monson 1999). Subsequent steps would then involve the elaboration of mechanisms to scavenge CO₂ exiting the bundle sheath, and increased exploitation of the high CO₂ produced in the bundle sheath by increasing Rubisco expression in this compartment (Monson 1999). Eventually, PEP carboxylase is incorporated into the CO₂ pumping mechanism, allowing Rubisco to be completely shifted to the bundle sheath compartment. At this point, plants have essentially made the transition from C₃ to C₄ forms.

Reduction in atmospheric CO₂ alone does not ensure the advent of C₄ plants. Indeed, the vast majority of land plants are C₃, and most ecosystems have been dominated by C₃ species, even at the very low CO₂ levels of the last ice age (Sage et al. 1999a,b). It is probably better to view CO₂ reduction and the subsequent rise in photorespiratory potential as a necessary precondition for the evolution of C₄ plants. In the absence of low CO₂, C₄ plant evolution would be unlikely, while in low CO₂, it become a possibility. Whether C₄ photosynthesis evolves following CO₂ reduction would likely depend upon additional, accessory conditions. An accessory condition

does not need to be an absolute requirement for C₄ evolution, but its presence could promote C₄ evolution once the preconditions have been met. Combinations of numerous accessory conditions would increase the likelihood of C₄ evolution, particularly if essential preconditions were barely met. Accessory conditions promoting C₄ evolution include warm growing season temperature, high light, drought, high salinity, and ecological disturbance from fire and megaherbivores (Sage 2001). The plant taxa involved are also important. No primitive angiosperm, gymnosperm or fern is C₄ (Sage et al, 1999a), indicating only advanced angiosperms are able to evolve the C₄ habit. The appearance of advanced angiosperms in the past 70 million years may therefore have been an additional precondition for C₄ evolution.

Despite being widely successful in open habitats of warm-temperate to tropical landscapes, few (~4%) of the higher plant families have evolved C₄ photosynthesis. However, within some of these families, C₄ photosynthesis has evolved numerous times. In the grasses (Poaceae), two to four distinct origins are proposed, four are suspected in the sedges (Cyperaceae), and the chenopods may have five (Kellogg 1999). The multiple origins of C₄ photosynthesis in these families indicates they are predisposed to evolve C₄ photosynthesis once other preconditions such as atmospheric CO₂ reduction have been met. Why they may be predisposed for C₄ evolution is unclear, but recent suggestions are that in the course of evolving drought or salinity tolerance, the C₃ progenitors in these families may have developed traits that facilitated subsequent C₄ evolution (Sage 2001). Suggested drought adaptations include enlargement of the bundle sheath for improved hydraulic storage or safety. Numerous C₃ species from arid environments exhibit enlarged bundle sheath tissues and some were even classified as Kranz anatomy before C₄ photosynthesis was discovered (Sage 2001). Enlargement of the bundle sheath tissue as a drought adaptation could have established compartments that could later be exploited for biochemical specialization in photorespiratory CO₂ pumping. This possibility demonstrates how drought may have been an accessory to C₄ evolution in a manner distinct from the more traditional view, which is that drought favors C₄ species because of higher water use efficiency.

The period between 35 and 25 million years ago was a time of global cooling (Fig. 2), which should not have favored C₄ plants. However, a cooler climate is typically drier because of reduced evaporation from the oceans (Prothero 1994). In the tropics where cooling was less pronounced, drying of the atmosphere promoted ecosystem aridification. These changes in climate, along with concurrent CO₂ reduction, caused a host of changes in plants and ecosystems. For example, the herbaceous habits became pronounced, and many arid zone taxa evolved (Sage 2001). Drought put a premium on high water use efficiency, stomatal closure, and possibly anatomical traits that facilitated biochemical specialization. Aridification enhanced fire frequency and promoted diversification of mammalian taxa, most notably, the rise of large herbivores capable of tearing down forest canopies (Sage et al. 1999b). The reduction of woody canopies by drought, fire and herbivore action enhanced light intensity at ground level, intensifying surface heating. Taken together, these developments indicate the Oligocene was a time when changes in the atmosphere, climate, and biotic composition of the earth established for the first time a combination of conditions that led to the evolution of C₄ photosynthesis.

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