

Review

The function of the aerenchyma in arborescent lycopsids: evidence of an unfamiliar metabolic strategy

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Most species of the modern family Isoëtaceae (Quillworts) some other modern hydrophytes, use a metabolic pathway for carbon fixation that involves uptake of sedimentary carbon and enrichment of CO₂ in internal gas spaces as a carbon-concentrating mechanism. This metabolism, which is related to ‘aquatic CAM’, is characterized by morphological, physiological and biochemical adaptations for decreasing photorespirative loss, aerating roots and maintaining high growth rates in anoxic, oligotrophic, stressed environments. Some of the closest relatives of the Isoëtaceae were the ‘arborescent lycopsids’, which were among the dominant taxa in the coal swamps found in lowland ecosystems during the Carboniferous and Permian periods (approx. 300 Ma). Morphological, ecological and geochemical evidence supports the hypothesis that the arborescent lycopsids had an unusual metabolism similar to that of modern Isoëtaceae and processed a biogeochemically significant proportion of organically fixed carbon over a period of about 100 million years in the late Palaeozoic. The temporal coincidence between the dominance of plants with this metabolism and an anomalous global atmosphere (high O₂; low CO₂) supports the idea that biosphere feedbacks are important in regulating global climatic homeostasis. The potential influence of this metabolism on the global carbon cycle and its specific adaptive function suggest that it should perhaps be considered a fourth major photosynthetic pathway.

Keywords: aerenchyma; aquatic CAM; arborescent lycopsids; metabolic pathways; parichnos

1. INTRODUCTION

Three routes through which plants fix carbon from atmospheric CO₂ to carbohydrates are generally accepted as major photosynthetic pathways: C₃, C₄ and CAM (Crassulacean acid metabolism). Since the 1980s, however, evidence from physiological experiments has been accumulating that there are both intermediates between these categories and variations upon them (Holaday & Bowes 1980; Koch & Kennedy 1980; Keeley 1981; Cockburn 1985; Brown & Hattersley 1989; Ehleringer & Monson 1993; Li & Jones 1995; Lüttge 1996; Mazon 1996; Smirnoff 1996; Winter & Smith 1996; Ehleringer *et al.* 1997; Keeley 1998; Lambers *et al.* 1998; Voznesenskaya *et al.* 2001; Hibberd & Quick 2002). Three major pathways are nevertheless distinguished from many intermediates; they dominate habitats or higher taxonomic groups and have been evolutionarily important in the development of terrestrial ecosystems or in the production and regulation of the Earth’s atmosphere. The evolutionary role and importance of variant pathways is less clear.

One particular variant metabolism has been called ‘diurnal/diel acid metabolism’ (Keeley 1982; Aulio 1985), ‘aquatic acid metabolism’ (Cockburn 1985), ‘CAM-like metabolism’ (Raven *et al.* 1988), ‘specialized

mechanisms associated with photosynthetic carbon acquisition in aquatic plants’ (Lambers *et al.* 1998, p. 80), and, most frequently, ‘aquatic CAM photosynthesis’ (Keeley 1998, etc.). It is considered a CAM variant because plants that display it sometimes have diurnal cycles of acidification due to the nocturnal accumulation of 4-carbon acids, one of the defining characteristics of ordinary CAM. More importantly, however, many aquatic plants that have been described as demonstrating aquatic CAM photosynthesis have organized aerenchyma (internal gas spaces) connecting buried and photosynthetic organs, and transport of O₂ downward and CO₂ upward in these spaces. Stomates may be present (frequently deeply sunken, occluded by cuticular wax, or in low numbers) or entirely lacking. The habitat of these species is predominantly aquatic, hypoxic or anoxic and oligotrophic. Fixed carbon may be obtained from the atmosphere, from the sediment, or from respiration, usually with a substantial component from the latter two sources.

In this paper, I offer morphological, ecological and biogeochemical evidence that a variant metabolism similar to aquatic CAM was much more prevalent in the late Palaeozoic than it is now. This metabolism is accompanied by recognizable morphological adaptations; it shows ecological and phylogenetic specificity; it has evolved more than once in response to the same ecological conditions; its ability to recycle sedimentary carbon could have potential long-term effects on the global

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carbon cycle. I argue that these characteristics constitute an evolutionarily important adaptive strategy (in which diurnal acidity cycles are incidental). Therefore it should perhaps be considered an independent metabolic pathway rather than a variant of CAM. More importantly, the realization that the late Palaeozoic ecosystems were populated by plants with a radically different metabolism from those dominating modern floras suggests that uniformitarian assumptions about flora–atmosphere feedbacks should be re-examined.

2. THE ROLE OF AERENCHYMA IN GAS-PHASE CARBON CONCENTRATION: DETAILS OF THE UNUSUAL PATHWAY

Until the 1980s, CAM was considered primarily an adaptation to physiologic drought: it allows plants to open their stomata during the night, when water loss is minimized, and accumulate atmospheric carbon in 4-carbon acids (malate or aspartate) that are then converted to carbohydrates during the day with less water loss (Kluge & Ting 1978; Winter & Smith 1996). The water stress that makes this strategy advantageous does not affect plants living submerged or in waterlogged soils. As Keeley (1981) and many subsequent authors have noted, some aquatic plants do store up carbon fixed from dissolved CO₂ as 4-carbon acids during the night; they do so not in order to prevent transpirative water loss during the day but because dissolved CO₂ levels in water usually rise at night above their daytime values. Expanding the term CAM to describe aquatic plants blurs its ecological specificity and obscures its function as an adaptation to specific environmental conditions.

The term ‘aquatic CAM’ is used because the diurnal acidity cycles of terrestrial and aquatic CAM plants are biochemically identical. Not all aquatic plants, however, show diurnal acidity cycles, and tying the definition of CAM to a single measurement (the presence of diurnal acidity cycles) seems arbitrary when individuals of the same species of aquatic plants and even organs of the same plant can show acidity cycles when submerged but not when emergent (Keeley 1998).

Aquatic CAM does not satisfactorily describe a plant that has absent or weak diurnal acidity cycles but does have an aerenchyma system in which CO₂ is either accumulated during the night for daytime fixation or merely concentrated above ambient atmospheric levels to reduce photorespiration without significant nocturnal storage. Since there seems to be no convenient term to describe this metabolic pathway, for the purposes of this paper I refer to it as the ‘lycopsid photosynthetic pathway’ (LPP). The term ‘photosynthetic pathway’ rather than ‘metabolism’ underlines the route taken by carbon. The enzymes necessary for any metabolic pathway have been found in virtually all plant groups (albeit in different concentrations), so it is the pathway and timing of carbon movement between atmosphere or sediment and carbohydrate, rather than the biochemistry, that distinguishes a photosynthetic pathway.

LPP metabolism is defined by the presence of internal gas spaces showing CO₂ enrichment above ambient levels in subaerial portions and O₂ in buried portions, with a substantial proportion of net carbon fixation coming from respired or sedimentary carbon. The term ‘aquatic

CAM’ as currently used corresponds to a CAM-LPP intermediate: an aquatic or wetland plant that shows CAM-like biochemistry (diurnal acidity cycles and fixation of a large proportion of its acquired carbon via phosphoenolpyruvate carboxylase and the Hatch/Slack/Korshak cycle) as well as an aerenchyma system used for gas-phase CO₂ concentration and transport (figure 1; see the electronic supplementary material, table S1).

The largest group of plants that show aquatic CAM and LPP (extensively documented by Keeley 1981, 1982; Keeley & Busch 1984; Keeley *et al.* 1984; Keeley 1998) are species of the genus *Isoetes* (Isoëtaceae), which includes the nearest living relatives of the arborescent lycopsids of the Palaeozoic. LPP is not restricted to the isoëtaleans. In his 1998 review of CAM-like photosynthesis in aquatic plants, Keeley listed 180 submerged aquatics whose metabolisms have been described since the 1980s, of which 69 have CAM-like diurnal malate cycles. Other examples include *Cyperus papyrus* (Cyperaceae), studied by Li & Jones (1995), who found that the O₂ in submerged (hypoxic) rhizomes rose from 10.3 per cent at night to 15.1 per cent during the day, while the CO₂ remained stable at 4.5 per cent. The maximum concentration of CO₂ in the culm rose to about 74 times its ambient atmospheric level (2.6%), averaging about 0.07 per cent during the day and 0.16 per cent at night. About half (35–57%) of the respired CO₂ was refixed. Measurements of the internal atmosphere of *Lobelia dortmanna* (Campanulaceae) by Pedersen & Sand-Jensen (1992) show that the concentration of CO₂ in its lacunae rose to 23 times its ambient atmospheric level, ranging from about 0.3 per cent during the day to about 0.7 per cent at night while the sediment was heavily enriched in O₂ in the vicinity of the roots. Similar results were obtained from *Typha latifolia* (Typhaceae) by Constable *et al.* (1992) in which the gas in the leaf aerenchyma ranged from ambient CO₂ levels around noon to about 0.6 per cent CO₂ (18 times ambient) in the early morning, and a dye tracer showed interconnection of the aerenchyma system from rhizomes to leaves. This pathway also appears to be inducible: Nielsen *et al.* (1991) measured lacunar CO₂ from 1.1 to 1.3 per cent (about 40 times ambient) in *Littorella uniflora* (Plantaginaceae) which has a diurnal malate cycle if grown submerged but not when emergent (Aulio 1985). Experiments on *Phaseolus vulgaris* (Fabaceae) using a ¹⁴C tracer to measure root uptake of carbon showed carbon uptake rates in aerated conditions that did not exceed passive transpiration rates. When the nutrient solution was not aerated, however, facultative aerenchyma formed in the stem and a higher rate of root uptake of carbon was measured, due at least in part to mass flow as the observed rate was too high for passive uptake or diffusion alone (Amiro & Ewing 1992). Root aeration via mass flow has been documented in *Nuphar* as a result of heat pressurization (Dacey 1980), and in *Phragmites* and *Equisetum* caused by a combination of humidity-driven convection and the Venturi effect (Armstrong & Armstrong 1988, 2009; Armstrong *et al.* 1992).

These examples are selected from a much larger body of existing literature to illustrate the variety of aquatic plants that show diurnal cycles of gas phase accumulation and transport (CO₂ up, O₂ down), and fixation of

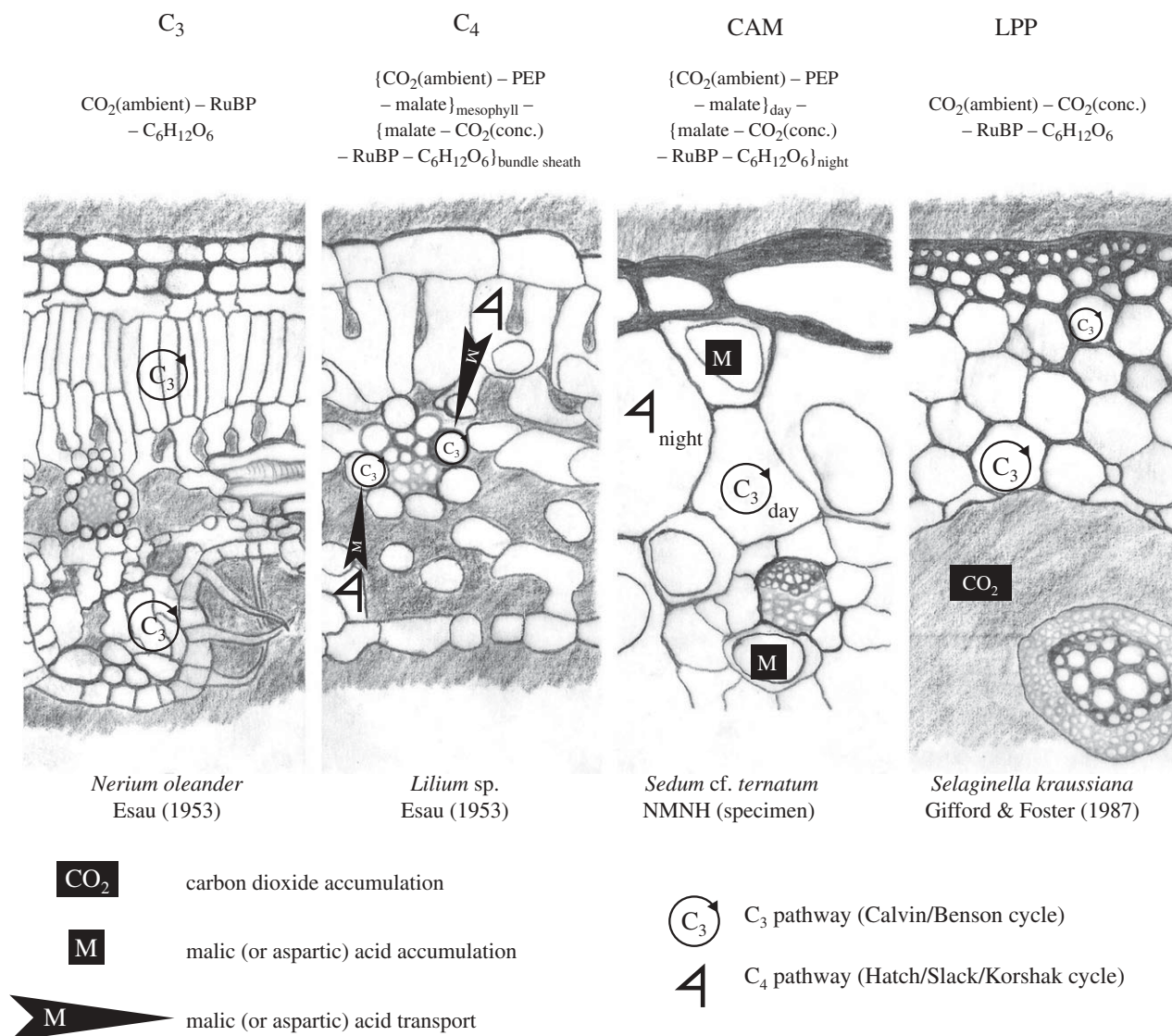


Figure 1. Comparison of photosynthetic pathways. Transverse sections showing the different anatomies characteristic of the pathways and schematic descriptions of the route taken by carbon during photosynthesis. The path taken by a carbon molecule during fixation is given at the top: C₆H₁₂O₆ (glucose) is used as a proxy for more complex carbohydrates; RuBP, ribulose-1,5-bisphosphate; PEP, phosphoenolpyruvate; conc., concentrated. Sections are modified from illustrations in Esau (1953); Gifford & Foster (1987), and an unaccessioned anatomical slide from the botany collections of the National Museum of Natural History. Light grey shading shows gas spaces and the icons show the different locations of carbon storage, fixation, and transport via the C₃ and C₄ cycles in each metabolic pathway.

sedimentary and respired carbon. If this metabolic pathway only appeared in a few modern plants, it might be reasonable to continue treating it as a relatively rare and evolutionarily insignificant oddity. Instead, however, evidence seems to suggest that it was much more prevalent during the late Palaeozoic.

3. INTERPRETATION OF THE PARICHNOS SYSTEM: MORPHOLOGICAL EVIDENCE

The arborescent lycopsids¹ constitute a group of extinct plants most of which are thought to be phylogenetically bracketed (Witmer 1995) by the extant genera *Selaginella* and *Isoetes* (Bateman *et al.* 1992; Pigg 1992; Kenrick & Crane 1997; Korall & Kenrick 2002; figure 2). They were among the taxa that dominated biomass production in the coal-swamp ecosystems during the Late Carboniferous (Pennsylvanian) in Euramerica (Phillips *et al.*

1985; Phillips & DiMichele 1992) and the Early Permian in China (Hilton & Cleal 2007). Arborescent lycopsids are preserved as fossil casts and moulds as well as in carbonate concretions known as coal balls, which preserve internal anatomical details at a cellular level; their physiology, of course, cannot be directly observed.

According to Hill (1906), the term ‘parichnos’ (from Greek *παρά*, beside + *ἕλκνος*, trace) was coined by Bertrand (1891) to describe what he took to be mucilage ducts surrounding the leaf trace in the axis of the arborescent lycopsid *Lepidodendron harcourtii*. The term has since come to be used to describe the pits initially described as ‘glanduoli’ (little glands) by Sternberg (1820–1838) that are visible in the fossilized ‘bark’ of arborescent lycopsids, where the parichnos system leaves the axis. The anatomical studies by DiMichele (e.g. DiMichele 1979, 1981, 1985) show how a single parichnos strand splits either once or twice under the surface of the leaf cushion to

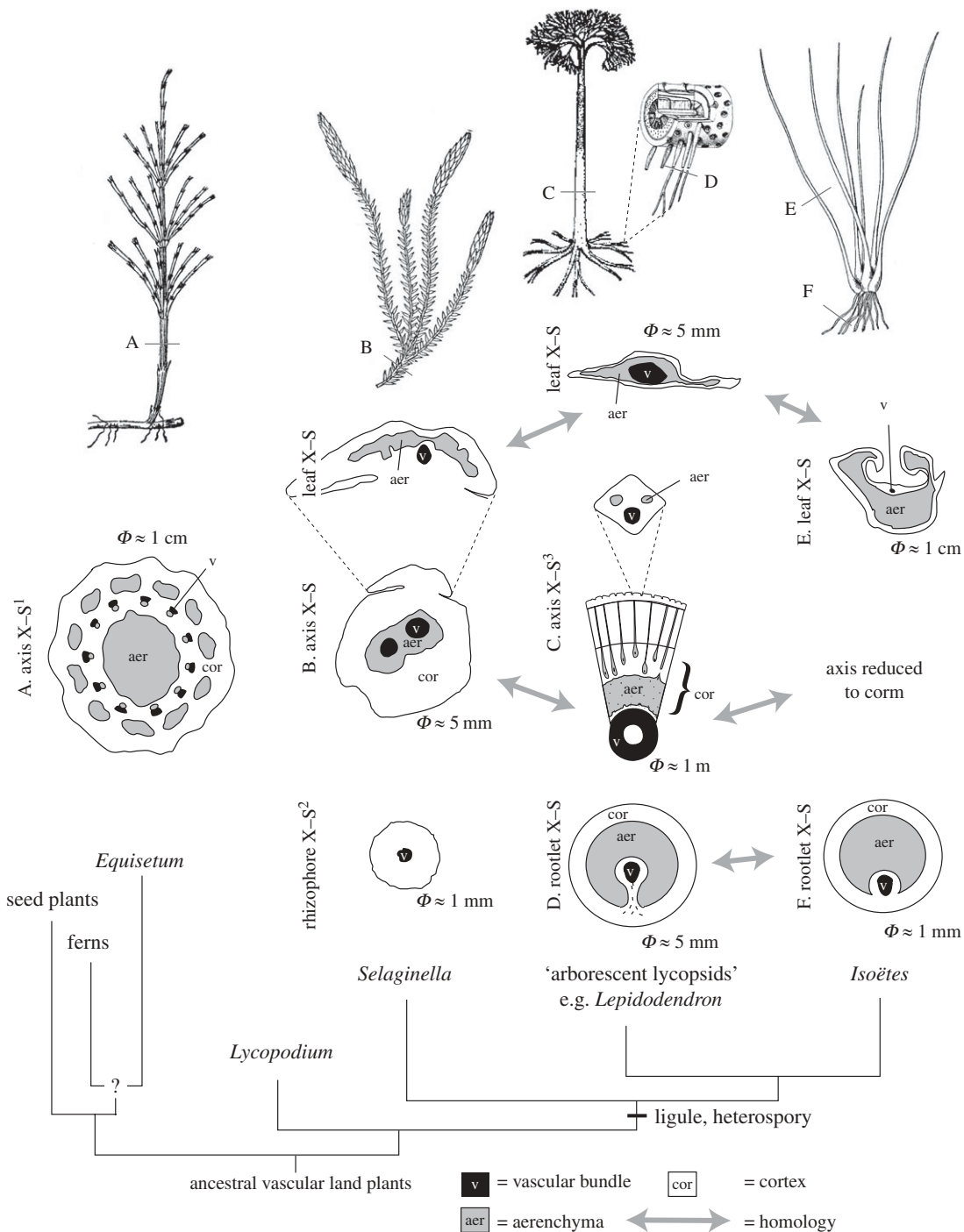


Figure 2. The parichnos system. Schematics showing aerenchyma system connecting buried and subaerial organs in *Equisetum*, *Selaginella*, the arborescent lycopsid *Lepidodendron* and *Isoetes*. Note positional homology of the aerenchyma across the lycopsids. The phylogenetic relationships are shown by a schematic tree in which the branch lengths are not significant and many taxa are omitted for clarity. Φ indicates approximate diameters of organs. Parts of the illustration are adapted from Britton & Brown (1913), Stewart (1947), Gifford & Foster (1987), Pigg (1992) and Stewart & Rothwell (1993). Note 1. At internode; rhizome is similar. 2. Rhizophore is shown, though the species of *Selaginella* pictured at the top is *S. selaginoides*, which lacks a rhizophore. Some species of *Selaginella* show aerenchyma development in the rhizophore, but it is probably not homologous with the aerenchyma in the rooting organ of the arborescent lycopsids, *Stigmaria* (Karrfalt 1981). 3. With expanded paradermal section of the leaf base.

form two pits in the leaf scar (representing canals feeding the leaf before abscission). In some cases, two additional pits below the leaf scar are present, representing connections to the atmosphere. From the leaf cushion, the parichnos system can be traced back into the 'middle cortex'—the aerenchymatous middle of the primary cortex—which is composed of thin-walled parenchyma

cells separated by large intercellular spaces. The middle cortex is continuous down into the rooting organ associated with the arborescent lycopsids (the form-genus *Stigmaria*, sometimes called a 'rhizomorph' or 'rhizophore') and connects with an air canal in the stigmarian rootlets (DiMichele 1979, 1981, 1985; Gifford & Foster 1987; Stewart & Rothwell 1993; figure 2). To

avoid ambiguity, I use the terms '(stigmarian) root' to describe the radially symmetric, branched, rooting axis with secondary growth and '(stigmarian) rootlet' to describe the bilaterally symmetric appendages radiating from it. I also extend the term 'parichnos' to describe any continuous aerenchymatous tissue system connecting buried or submerged and subaerial organs of a plant.

As figure 2 illustrates, transverse sections of *Selaginella*, *Lepidodendron*, and *Isoetes* all show an organized parichnos system. The middle cortex in the axis of arborescent lycopsids occupies the same relative position as the trabecular aerenchyma in the axis of *Selaginella*, and the air space in the stigmarian rootlet is homologous with that in the 'rootlet' of modern *Isoetes* (Stewart 1947; Rothwell & Erwin 1985). Therefore, making allowances for the reduction of the stem in *Isoetes* and the absence of a clearly homologous rooting organ in *Selaginella*,² the parichnos system is positionally homologous across the three groups.

The identification of the parichnos system with aerenchyma in modern plants is not a new idea: as early as 1893, Bower suggested that 'the trabecular [aerenchyma] development in *Selaginella* is a specialized and more definite example of that lacunar development which appears in such various forms and positions in cortical tissues of various other Lycopodinous plants' (Bower 1893, p. 349). A few years later, it was suggested that the leaf traces in the woody base of the genus *Isoetes* are surrounded by 'strands of degenerating [lysigenous] tissue . . . representing the parichnos occurring in *Lepidodendron*, *Sigillaria*, *Lepidocarpon*, &c.' (Hill 1904, p. 654).

Figure 2 shows other strong similarities between the rootlets of the arborescent lycopsids and the rootlets of modern *Isoetes*. As has been recognized for some time, both stigmarian appendages and *Isoetes* rootlets are spirally arranged on an axis and bear a leaf-trace-like single monarch xylem strand suspended in a gas-filled lacuna on parenchymatous trabeculae or bars. Stewart (1947) concluded that stigmarian and *Isoetes* rootlets are indeed homologous. More recent work (Raven & Edwards 2001) supports this homology and convincingly identifies *Isoetes* rootlets as leaf homologues, which lack root hairs and have a documented role in CO₂ uptake.

The functional significance of the parichnos system has also been suggested: Hill (1906) argued that the parichnos found in extant *Isoetes* had a secretory function, but did not rule out its use in aeration or respiration, as suggested by Scott (1900) and Weiss (1903). The interpretation of the parichnos system as having a poorly defined role in gas transport seems to be the most current view (Stewart & Rothwell 1993). Both CO₂ uptake and root aeration have been specifically mentioned: 'the early colonists (of the land) were . . . able to take up CO₂ through their roots' (Moore 1984, p. 633); 'the parichnos would appear to be an internal system of gas exchange associated with photosynthesis, corresponding more to recycling of CO₂ and O₂ than to external diffusion balances' (Phillips & DiMichele 1992, p. 568).

Barclay (1931) and Jagels (1970_{a,b}) have also observed that chloroplast densities in some modern species of *Selaginella* are higher in the cells bounding internal gas spaces than in subdermal parenchyma cells. Keeley (1998) reports the same distribution in species of *Isoetes* as well as in *Littorella*, another aquatic of 'isoëtoid'

life form. This supports the conclusion that carbon fixation in these plants is primarily from internal gases rather than from direct communication with the atmosphere via stomata. Cell contents are not preserved in permineralized arborescent lycopsids, so chloroplast densities cannot be directly measured, but the structural and positional homologies between the preserved parichnos system and aerenchyma in modern LPP plants suggest that the arborescent lycopsids also probably relied more heavily on internal than on external atmosphere to supply carbon for photosynthesis.

The results reviewed in this section are intended to document the link between the parichnos system in the arborescent lycopsids and aerenchyma found in their modern relatives. The previous section covered the functional role of aerenchyma in modern plants. It seems reasonable, therefore, to suggest on grounds of functional morphology alone that the arborescent lycopsids showed the upward CO₂ transport and gas-phase carbon concentration that is characteristic of modern LPP plants. Carbon concentration to reduce photorespirative loss is only one of the adaptive advantages provided by LPP metabolism. As discussed below, this would have been particularly important in the high O₂ to CO₂ ratio atmosphere of the late Palaeozoic. Another major function performed by LPP metabolism, which will be discussed in the following section, is to supply O₂ to roots via the parichnos system as an adaptation to growth submerged or in waterlogged soils.

4. ECOLOGICAL EVIDENCE

In addition to documenting an enrichment of CO₂ in internal gas spaces, recent research on aerenchyma has firmly associated it with a need for root aeration. Aerenchyma (*sensu lato*) is simply parenchymatous tissue with a large volume of intercellular space. The amount of organization implied by the term varies, as it can be applied both to the slightly porous parenchyma found in the root cortices of many wetland plants and to the well-defined longitudinal canals found in the stems and rhizomes of *Equisetum*. The spongy mesophyll in dicot leaves is also referred to as aerenchyma. In some species (e.g. *Zea mays*, *Spartina patens*), aerenchyma forms when the plant is grown in oxygen-depleted soils (this is called 'induced' or 'facultative' aerenchyma); in other species (e.g. *Tripsicum dactyloides*, *Zea luxurians*, *Sagittaria lancifolia*), it is formed regardless of the environmental conditions in which the plant is grown ('constitutive' aerenchyma; Drew *et al.* 2000).

In developmental terms, aerenchyma can be formed by the physical separation of cells at the middle lamella during ontogeny (schizogeny), by the death and lysis of cells (lysigeny), by the physical rupture of cells (rexigeny) or by some combination of these modes. Justin & Armstrong (1987), who examined aerenchyma formation in 91 angiosperms, and Drew (1997) document some patterns: the propensity of wetland species to form constitutive aerenchyma in any environmental conditions and to form induced aerenchyma when subjected to a given level of oxygen depletion. They also point out a connection between the cubic packing of parenchyma cells (as opposed to hexagonal close packing) and the formation of aerenchyma. Despite recent studies that have examined

the details of aerenchyma formation in certain species (Drew *et al.* 2000; Longstreth & Borkhsenius 2000), there seems to be no simple link between the mode of formation or degree of organization of the aerenchyma and whether it is constitutive or induced. On one hand, Drew *et al.* (2000) conclude that hypoxia-induced ethylene promotes lysigenous productions of induced aerenchyma in maize roots; but 'gas space formation does not require lysis and cell death in at least three wetland species' (Longstreth & Borkhsenius 2000, p. 642). So, although there is considerable variation in the conditions and cellular processes that give rise to aerenchyma in different plants, several studies of angiosperm roots have clearly established that induced aerenchyma forms as an adaptation to low O₂ in the rhizosphere.

General morphological studies of the genus *Selaginella* (Harvey Gibson 1894; Browne 1908; Cusick 1953; Rosello 1966; Buck & Lucansky 1976) deal with the air spaces in the cortex merely in passing; Jagels (1970*a,b*) concentrates exclusively on photosynthetic apparatus; Uphof (1920) briefly discusses aerenchyma in xerophytic species; and recent developmental studies (Webster & Steeves 1964, 1967; Webster & Jagels 1977; Karrfalt 1981; Imaichi & Kato 1989; Webster 1992) are largely focused on identifying the the origin of the rhizophore, or have been concerned with other anatomical features such as the ligule. No recent study seems to have concentrated on aerenchyma formation or function, with the result that the best obtainable description of lycopsid aerenchyma formation comes from 1931: 'The rate of growth of the endodermis mother cells does not keep up with the differential increase in diameter and length of the cortical cylinder and central bundle. As a result, the endodermis mother cells become stretched radially between the pericycle and inner cortex.... An air cavity is thus formed... bridged by the endodermis mother cells.... This is the 'trabecular cortex' (Barclay 1931, p. 458). In modern terminology, this would be considered schizogenous and therefore developmentally unrelated to the lysigenous aerenchyma appearing in *Arabidopsis* (Mühlenbock *et al.* 2007). The relationship between rhizosphere anoxia and aerenchyma formation in extant lycopsids has not been studied in detail, but in many of the environments in which they grow, root respiration would not be possible without some oxygen transport downward through an aerenchyma system. Therefore, like the other three major photosynthetic pathways, LPP metabolism has an element of habitat specificity; it is at least partially an adaptation to low oxygen levels in the rhizosphere.

From the sedimentology of the carbonaceous shales and coals in which they are typically found, it is clear that the arborescent lycopsids grew predominantly in backswamp floodplain communities with permanently inundated soils (DiMichele & Phillips 1994). The plant fossil record is generally considered to be biased towards such lowland, anoxic settings, so upland settings dominated by ferns or pteridosperms may be under-represented. Even allowing for this bias, however, it seems reasonable to assume that (as is the case today) the greatest accumulation of biomass was in lowland communities, which contained a large proportion of arborescent lycopsids.

Other important components of these communities were horsetail relatives (Sphenophyta), tree-ferns (Marattiales) and presumed conifer relatives (Cordaitales). Most Palaeozoic sphenophytes, tree-ferns like *Psaronius*, and *Cordaites* roots (the form-genus *Amyelon*) show aerenchyma development, so it is important to distinguish between LPP metabolism (connected aerenchyma, CO₂ concentration in internal gas spaces, use of sediment-derived carbon, root aeration) and the aerenchyma formation simply to provide root aeration found, for instance, in modern bald cypress (*Taxodium*), which relies on pneumatophores for root respiration when growing in standing water. Like succulence in CAM plants; a parichnos system is a necessary but insufficient condition for diagnosing LPP metabolism. With that important proviso, LPP metabolism may indeed have been more common across taxonomic groups throughout lowland, backswamp communities in the late Palaeozoic. In some plants, there is also evidence for axial carbon transport in the xylem (Zelawski *et al.* 1970; Martin *et al.* 1994; Hibberd & Quick 2002), so there may be intermediate metabolic pathways between LPP and C₃, represented by taxa with weak aerenchyma formation and some recycling of respired CO₂ via the transpiration stream instead of as gas-phase CO₂. As discussed above, LPP-CAM intermediates have been well characterized as aquatic CAM plants. Further investigation will help to characterize variants and details of LPP; this paper is primarily concerned with defining it as a metabolic pathway and documenting its importance in the late Palaeozoic.

In addition to CO₂ concentration and root oxygenation, LPP would have enabled Palaeozoic arborescent lycopsids to sustain a higher rate of growth than would otherwise have been possible. Phillips & DiMichele (1992) suggested that the arborescent phase of the arborescent lycopsid life history was much more transient than previously assumed. Instead of spending most of their lives as 20–30 m trees, they may have shot up from low rhizomatous structures in the last few years before reproduction. It seems probable that rapid accumulation of biomass would have been limited by the amount of carbon that could be obtained from a low CO₂ atmosphere via stomata. If carbon was obtained from the sediment, however, growth rate would not be limited by carbon availability.

According to the species diversity curves of Nikas *et al.* (1985), the arborescent lycopsids increased in diversity beginning in the Upper Devonian, reaching a maximum that may have been as high as 100 described species at the end of the Carboniferous (figure 3*c*). Most of the taxa with large adult stature went extinct by the Middle Triassic, leaving only diminutive or herbaceous taxa extant (Pigg 1992). Note that modern *Isoetes* is considered non-arborescent in the compilation by Nikas *et al.* (1985).

From the mid-Carboniferous to the mid-Permian period, the arborescent lycopsids probably constituted a substantial proportion of terrestrial vegetation, but they have slowly declined in ecological importance since then and now are represented only by a few genera of widely distributed herbaceous and shrubby plants. Many species of modern isoëtaleans are found in oligotrophic lakes and bogs, which, like backswamps, are considered stressed environments because of their high acidity and low

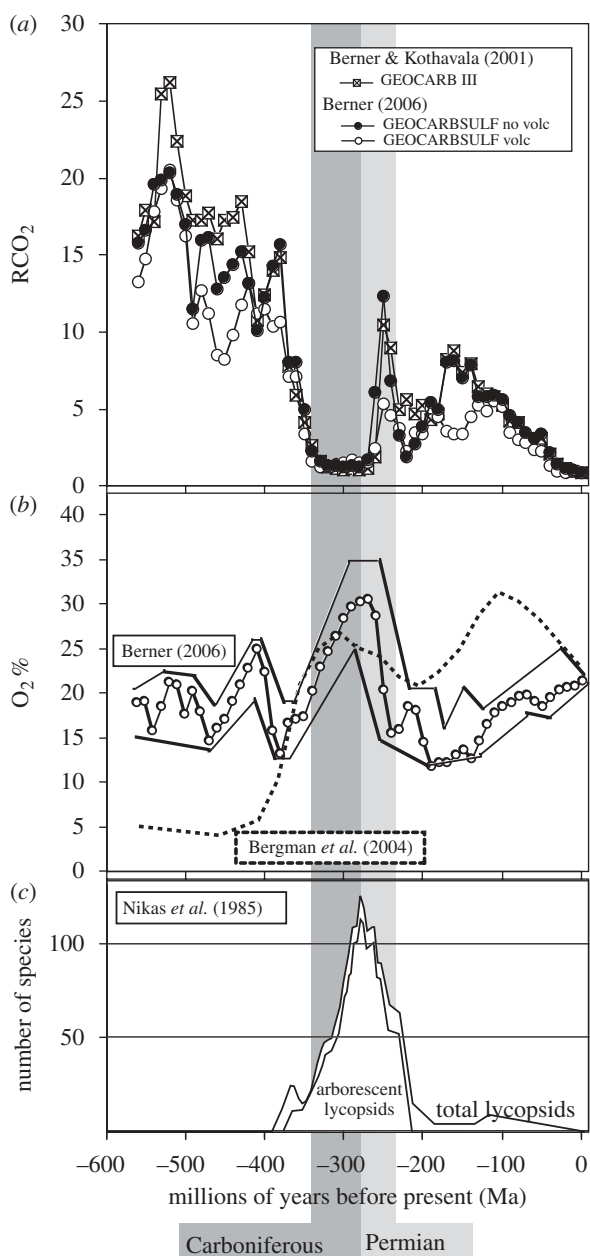


Figure 3. (a) CO₂, (b) O₂ and (c) numbers of described species of lycopsids through time. Note that arborescent lycopsids were diverse only in the high O₂, low CO₂ atmosphere of the Carboniferous and Permian. In (a), CO₂ values are given as RCO₂; relative enrichment over modern (preindustrial) level. Redrawn from figures in Berner (2006), Bergman *et al.* (2004), Berner & Kothavala (2001) and Nikas *et al.* (1985). The curves in (c) are traced compilations of several figures and therefore represent approximate rather than exact values.

nutrient availability. This implies that the isoëtalian lycopsid lineage has maintained a degree of habitat specificity since the Palaeozoic (DiMichele *et al.* 2001). Nowhere do modern lycopsids provide more than a small fraction of the biomass of an ecosystem. Nevertheless, the remaining isoëtaleans remain of particular interest because we can infer that their LPP metabolism was inherited from their extinct relatives, the arborescent lycopsids. These flourished in the late Palaeozoic, when LPP would have been far more evolutionarily advantageous than subsequently.

5. BIOGEOCHEMICAL EVIDENCE

Computer modelling based in part on the amount of organic carbon and pyrite in sedimentary rocks suggests that the mid-Carboniferous through mid-Permian was a period of extremely low CO₂ (figure 3a) and high O₂ (figure 3b) levels in the atmosphere (Berner & Canfield 1989; Berner 2001, 2006; Berner & Kothavala 2001; Bergman *et al.* 2004). These conditions would have been particularly favourable for a metabolic pathway such as LPP, which allowed utilization of sedimentary carbon and reduced the apparent atmospheric ratio of partial pressures of O₂ to CO₂ by concentrating CO₂ in internal spaces. This is metabolically important because the oxygenase versus carboxylase activity of rubisco is dependent on the O₂ to CO₂ ratio in the gas available for carbon fixation. So photorespiration is greatly increased (leading to a loss of net photosynthetic efficiency) in a high O₂, low CO₂ atmosphere.

The high O₂, low CO₂ excursion coincides both with the greatest diversity of arborescent lycopsids (figure 3c) and with the stratigraphic distribution of coal balls in the fossil record. Coal balls were formed by carbonate precipitation in coal swamp environments between about 290 and 320 Ma (latest Mississippian through earliest Permian; Phillips *et al.* 1985; Hilton *et al.* 2001). The large-scale local removal of carbon dioxide from groundwater would drive to the left the equilibrium $\text{CO}_2 + \text{CaCO}_3 + \text{H}_2\text{O} \rightleftharpoons 2\text{HCO}_3^- + \text{Ca}^{2+}$, and may have helped to precipitate coal balls where they are found, in an anoxic rhizosphere.

In modern floras, measurement of carbon isotope ratios allows the statistical separation of C₃ from C₄ plants, although intermediate carbon fractionation shown by CAM plants can blur the picture. It is possible that isotopic data on fossil plants would allow identification of LPP metabolism in fossil material. Since the arborescent lycopsids may have extracted a substantial proportion of their carbon from the sediments, they are likely to reflect the isotopic ratios of their substrates, a hypothesis supported by the measurements made of *Stylites* (a segregate of *Isoëtes* lacking stomata; -25.7‰), the peat in which it was growing (-26.6‰) and groundwater carbonates (-25.0‰) by Keeley *et al.* (1984).

The lack of fractionation in *Stylites* indicates that essentially all of the carbon taken up by the plant from the sediments was eventually absorbed. In the case of plants with functional stomata, fractionation may show the use of some atmospheric oxygen or loss of sedimentary carbon, but there is clearly no dramatic signal like that shown by C₄ plants (Lloyd & Farquhar 1994). Because of the overlap between the expected carbon isotope ratios in C₃ and LPP plants, available data on the isotope ratios of fossil lycopsids (Raven & Spicer 1996; Beerling *et al.* 2002; Osborne & Beerling 2006) is not sufficient to identify LPP metabolism. Measurements of sedimentary organic carbon in coal swamp environments seldom differ greatly from -25‰, so isotopic discrimination of LPP would require many paired measurements of plant tissues along with the organic carbon in the sediments surrounding them. Finding these paired measurements significantly correlated would suggest sedimentary carbon utilization and LPP metabolism. Unlike modern C₄ grassland ecosystems, which can show substantially higher δ¹³C fractionations,

the majority of buried carbon in Carboniferous coal swamps must ultimately have been obtained from the atmosphere via the rubisco-mediated Calvin/Benson (C_3) cycle and then reprocessed by LPP, providing a selective advantage for the arborescent lycopsids, but not dramatically altering the ecosystem-scale C_3 isotopic signal.

6. BROADER SIGNIFICANCE

Study of forest ecosystems—ecosystems with complex and dense photosynthetic canopies, generally found in regions where precipitation exceeds evaporation—has a long, productive history in palaeontology because trees provide a comparatively good fossil record compared with that of smaller plants (e.g. Behrensmeyer *et al.* 1992). Forests have also been a particular concern of the environmental movement because loss of diversity in tropical rainforests, massive deforestation for agricultural purposes with the consequent loss of biomass to greenhouse CO_2 , and the growing problem of forest fires in residential areas are well-known social and economic problems.

In the past (Green & Hickey 2005), I have argued that if leaf architecture is used as a proxy for ecosystem structure, the available data show structural continuity in forest ecology since before the Cretaceous/Tertiary boundary. Many species of plants have been replaced, but the basic architecture of a late Cretaceous forest would not seem out of place in the modern world. Palaeozoic ecosystems with complex, differentiated photosynthetic canopies, on the other hand, were dominated by plants such as the arborescent lycopsids, which were only distantly related to the angiosperms and may have functioned very differently. These ecosystems may have been so architecturally and physiologically different from modern forests that they should probably not be referred to as ‘forests’ at all. In order to appreciate the global environmental significance of plant ecosystems in the Palaeozoic, it is therefore necessary to examine carefully any uniformitarian assumptions about plant metabolism.

Although details of the metabolism of the arborescent lycopsids remain to be determined, available evidence seems sufficient to demonstrate that (i) the arborescent lycopsids relied heavily on sedimentary/respired carbon concentrated in internal gas spaces for photosynthesis; (ii) the internal gas spaces also had a function in oxygen transport downward for root respiration; (iii) the metabolism associated with these two functions is usually identifiable from the presence of an organized aerenchyma/parichnos system connecting buried and photosynthetic organs; (iv) the temporal co-occurrence of this metabolic pathway with the high O_2 , low CO_2 late Palaeozoic atmosphere is strong if not conclusive evidence for its adaptive significance in a major clade (lycopsids) and an important biome (lowland coal-forming swamps). I would argue that the importance of the LPP in the late Palaeozoic and its ecological specificity warrants consideration of it as a fourth major photosynthetic pathway.

There remain many details to be ascertained: (i) whether the arborescent lycopsids relied primarily on dissolved CO_2 or also on HCO_3^- ; (ii) how prevalent LPP or intermediate metabolic pathways were among other Palaeozoic taxa with organized aerenchyma, such

as the sphenophytes and marattialean tree ferns; (iii) what percentage of fixed carbon in a Palaeozoic ecosystem was atmospheric as opposed to sedimentary; and (iv) how often LPP evolved. In addition to the lycopsids, it has clearly appeared in both dicots and monocots and possibly in the sphenophytes and ferns; physiological study of more extinct taxa and phylogenetic analysis will be necessary to ascertain the number of independent origins.

Even without these details, the evidence offered here seems to suggest that the arborescent lycopsids demonstrated an aberrant metabolism that may not be well described by, for instance, isotopic fractionation models intended for C_3 or C_4 plants (Lloyd & Farquhar 1994). Similarly, stomatal atmospheric proxies are sensitive even to species-level differences (Royer 2003) and therefore application of them to arborescent lycopsids in extinct families (such as Lepidodendraceae) requires phylogenetic bracketing (Witmer 1995) and conservative error calculations. Both analytic models of photosynthesis and statistical proxies based on extant C_3 plants may need modifications to describe Palaeozoic plants with LPP metabolism accurately; discussing these in detail is beyond the scope of this review, but may form the basis of future work.

In the last half century, both scientific research programs and political and social movements have become deeply concerned with the rate and significance of change in the global biosphere and atmosphere. It has frequently been pointed out, perhaps most influentially by Lovelock (1979), that the biosphere—in particular the dominant primary producers—has played an active role in the maintenance of conditions suitable for life. Other things being equal, organic carbon burial is dependent on the balance between net photosynthesis and respiration, so relatively small changes in the residence times or annual fluxes of carbon can cumulatively affect the balance between buried organic carbon and atmospheric CO_2 . The success of the arborescent lycopsids is temporally associated with an anomalously low CO_2 global atmosphere and the deposition and burial of large coal beds. A metabolism such as LPP may have created a positive feedback, increasing net photosynthesis, sequestering more atmospheric carbon in coal swamps, and thus promoting conditions favourable for the arborescent lycopsids. Currently available evidence only allows identification and recognition of the aberrant metabolism; additional investigation may allow further description of the LPP and elucidation of its links to change in the global atmosphere and long-term climate change.

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ENDNOTES

¹This term is used throughout in its loose traditional sense to describe extinct tree-like lycopsids, excluding modern *Isoetes*. Bateman *et al.* (1992) has pointed out that 'arborescent' (having tree-like adult stature) would be a more accurate term for this group than 'arborescent' (having extensive secondary growth) because *Isoetes* has secondary growth in its corm and therefore is also technically an arborescent lycopsid. For the purposes of this paper, however, I need to refer to the group of fossil taxa that are phylogenetically bracketed between extant *Selaginella* and *Isoetes*. This paraphyletic group is probably roughly coextensive with the group of taxa that have traditionally been called arborescent lycopsids, so I employ the term loosely to avoid the precise phylogenetic hypothesis that would be implied by the use of 'Lepidodendrales'. See Bateman *et al.* (1992), DiMichele & Bateman (1996) and Kenrick & Crane (1997) for further discussion of the taxonomy and systematics of the lycopsids.

²Note that many derived species of *Selaginella* have an organ called a rhizophore that serves the same functional role as aerial roots in angiosperms but is not homologous either to true angiosperm roots or to the isoëtalian/stigmarian root or rootlet.

REFERENCES

- Amiro, B. D. & Ewing, L. L. 1992 Physiological conditions and uptake of inorganic C₁₄ by plant roots. *Environm. Exp. Botany* **32**, 203–211. (doi:10.1016/0098-8472(92)90003-K)
- Armstrong, W. & Armstrong, J. 1988 *Phragmites australis*—a preliminary study of soil-oxidizing sites and internal gas transport pathways. *New Phytol.* **108**, 373–382. (doi:10.1111/j.1469-8137.1988.tb04177.x)
- Armstrong, W. & Armstrong, J. 2009 Record rates of pressurized gas-flow in the great horsetail, *Equisetum telmateia*. Were Carboniferous *Calamites* similarly aerated? *New Phytol.* **184**, 202–215. (doi:10.1111/j.1469-8137.2009.02907.x)
- Armstrong, J., Armstrong, W. & Beckett, P. M. 1992 *Phragmites australis*—venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. *New Phytol.* **120**, 197–207. (doi:10.1111/j.1469-8137.1992.tb05655.x)
- Aulio, K. 1985 Differential expression of diel acid metabolism in two life forms of *Littorella uniflora* (L.) Aschers. *New Phytol.* **100**, 533–536. (doi:10.1111/j.1469-8137.1985.tb02799.x)
- Barclay, B. D. 1931 Origin and development of tissues in stem of *Selaginella willdenovii*. *Botanical Gazette* **91**, 452–461. (doi:10.1086/334168)
- Bateman, R. M., DiMichele, W. A. & Willard, D. A. 1992 Experimental cladistic analysis of anatomically preserved arborescent lycopsids from the Carboniferous of Euramerica: an essay on paleobotanical phylogenetics. *Ann. Mo Botanical Garden* **79**, 500–559. (doi:10.2307/2399752)
- Beerling, D. J., Lake, J. A., Berner, R. A., Hickey, L. J., Taylor, D. W. & Royer, D. L. 2002 Carbon isotope evidence implying high O₂/CO₂ ratios in the Permo-Carboniferous atmosphere. *Geochem. Cosmochem. Acta* **66**, 3757–3767. (doi:10.1016/S0016-7037(02)00901-8)
- Behrensmeyer, A. K., Damuth, J. D., DiMichele, W. A., Potts, R., Sues, H. & Wing, S. L. (eds) 1992 *Terrestrial ecosystems through time*. Chicago, IL: University of Chicago Press.
- Bergman, N. M., Lenton, T. M. & Watson, A. J. 2004 COPSE: a new model of biogeochemical cycling over Phanerozoic time. *Am. J. Sci.* **304**, 397–437. (doi:10.2475/ajs.304.5.397)
- Berner, R. A. 2001 Modeling atmospheric O₂ over Phanerozoic time. *Geochem. Cosmochem. Acta* **65**, 685–694. (doi:10.1016/S0016-7037(00)00572-X)
- Berner, R. A. 2006 GEOCARBSULF: a combined model for Phanerozoic atmospheric O₂ and CO₂. *Geochim. Cosmochim. Acta* **70**, 5653–5664. (doi:10.1016/j.gca.2005.11.032)
- Berner, R. A. & Canfield, D. E. 1989 A new model for atmospheric oxygen over Phanerozoic time. *Am. J. Sci.* **289**, 59–91.
- Berner, R. A. & Kothavala, Z. 2001 GEOCARB III: a revised model of atmospheric CO₂ over Phanerozoic time. *Am. J. Sci.* **301**, 182–204. (doi:10.2475/ajs.301.2.182)
- Bertrand, C. E. 1891 Remarques sur le *Lepidodendron harcourtii* de Witham. *Travaux et Mémoires des Facultés de Lille*, vol. 2.
- Bower, F. O. 1893 On the structure of the axis of *Lepidostrobus brownii* Schpr. *Ann. Bot.* **7**, 329–354.
- Britton, N. L. & Brown, A. 1913 *An illustrated flora of the northern United States, Canada, and the British possessions*, 2nd edn. New York, NY: Charles Scribner's Sons.
- Brown, R. H. & Hattersley, P. W. 1989 Leaf anatomy of C₃–C₄ species as related to evolution of C₄ Photosynthesis. *Plant Physiol.* **91**, 1543–1550. (doi:10.1104/pp.91.4.1543)
- Browne, I. 1908 The phylogeny and inter-relationships of the Pteridophyta. IV. The Lycopodiales (continued). *New Phytol.* **7**, 181–197. (doi:10.1111/j.1469-8137.1908.tb06086.x)
- Buck, W. R. & Lucansky, T. W. 1976 An anatomical and morphological comparison of *Selaginella apoda* and *Selaginella ludoviciana*. *Bull. Torrey Bot. Club* **103**, 9–16. (doi:10.2307/2484743)
- Cockburn, W. 1985 Variation in photosynthetic acid metabolism in vascular plants: CAM and related phenomena. *New Phytol.* **101**, 3–24. (doi:10.1111/j.1469-8137.1985.tb02815.x)
- Constable, J. V. H., Grace, J. B. & Longstreth, D. J. 1992 High carbon dioxide concentrations in aerenchyma of *Typha latifolia*. *Am. J. Bot.* **79**, 415–418. (doi:10.2307/2445153)
- Cusick, F. 1953 Experimental and analytical studies of Pteridophytes. XXII. Morphogenesis in *Selaginella willdenovii* Baker. *Ann. Bot.* **17**, 369–383.
- Dacey, J. W. H. 1980 Internal winds in water lilies: an adaptation for life in anaerobic sediments. *Science* **210**, 1017–1019. (doi:10.1126/science.210.4473.1017)
- DiMichele, W. A. 1979 Arborescent lycopods of Pennsylvanian age coals; *Lepidophloios*. *Palaeontographica B* **171**, 57–77.
- DiMichele, W. A. 1981 Arborescent lycopods of Pennsylvanian age coals; *Lepidodendron*, with description of a new species. *Palaeontographica B* **175**, 85–125.
- DiMichele, W. A. 1985 *Diaphorodendron*, gen. nov., a segregate from *Lepidodendron* (Pennsylvanian age). *Syst. Bot.* **10**, 453–458. (doi:10.2307/2419138)
- DiMichele, W. A. & Bateman, R. M. 1996 Plant paleoecology and evolutionary inference: two examples from the Paleozoic. *Rev. Palaeobot. Palynol.* **90**, 223–247.
- DiMichele, W. A. & Phillips, T. L. 1994 Paleobotanical and paleoecological constraints on models of peat formation in the Late Carboniferous of Euramerica. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **106**, 39–90. (doi:10.1016/0031-0182(94)90004-3)
- DiMichele, W. A., Stein, W. E. & Bateman, R. M. 2001 In *Evolutionary paleoecology* (eds W. D. Allmon & D. J. Bottjer), pp. 285–335. New York, NY: Columbia University Press.
- Drew, M. C. 1997 Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 223–250. (doi:10.1146/annurev.arplant.48.1.223)

- Drew, M. C., He, C.-J. & Morgan, P. W. 2000 Programmed cell death and aerenchyma formation in roots. *Trends Plant Sci.* **5**, 123–127. (doi:10.1016/S1360-1385(00)01570-3)
- Ehleringer, J. R. & Monson, R. K. 1993 Evolutionary and ecological aspects of photosynthetic pathway variation. *Annu. Rev. Ecol. Syst.* **24**, 411–439. (doi:10.1146/annurev.es.24.110193.002211)
- Ehleringer, J. R., Cerling, T. E. & Helliker, B. R. 1997 C₄ photosynthesis, atmospheric CO₂, and climate. *Oecologia* **12**, 285–299. (doi:10.1007/s004420050311)
- Esau, K. 1953 *Plant anatomy*. New York, NY: Wiley.
- Gifford, E. M. & Foster, A. S. 1987 *Morphology and evolution of vascular plants*, 3rd edn. New York, NY: W. H. Freeman and Company.
- Green, W. A. & Hickey, L. J. 2005 Leaf architectural profiles of angiosperm floras across the Cretaceous/Tertiary boundary. *Am. J. Sci.* **305**, 983–1013. (doi:10.2475/ajs.305.10.983)
- Harvey Gibson, R. J. 1894 Contributions towards a knowledge of the anatomy of the genus *Selaginella* Spr. *Ann. Bot.* **8**, 133–206.
- Hibberd, J. M. & Quick, W. P. 2002 Characteristics of C₄ photosynthesis in stems and petioles of C₃ flowering plants. *Nature* **415**, 451–454. (doi:10.1038/415451a)
- Hill, T. G. 1904 On the presence of a parichnos in recent plants. *Ann. Bot.* **8**, 654.
- Hill, T. G. 1906 On the presence of a parichnos in recent plants. *Ann. Bot.* **20**, 267–273.
- Hilton, J. & Cleal, C. J. 2007 The relationship between Euramerican and Cathaysian tropical floras in the Late Palaeozoic: palaeobiogeographical and palaeogeographical implications. *Earth Sci. Rev.* **85**, 85–116.
- Hilton, J., Wang, S.-J., Galtier, J. & Li, C.-S. 2001 An Early Permian plant assemblage from the Taiyuan Formation of northern China with compression/impression and permineralized preservation. *Rev. Palaeobot. Palynol.* **114**, 175–189. (doi:10.1016/S0034-6667(01)00045-8)
- Holaday, A. S. & Bowes, G. 1980 C₄ acid metabolism and dark CO₂ fixation in a submersed aquatic macrophyte (*Hydrilla verticillata*). *Plant Physiol.* **65**, 331–335. (doi:10.1104/pp.65.2.331)
- Imaichi, R. & Kato, M. 1989 Developmental anatomy of the shoot, apical cell, rhizophore and root of *Selaginella uncinata*. *Bot. Mag. Tokyo* **102**, 369–380. (doi:10.1007/BF02488120)
- Jagels, R. 1970a Photosynthetic apparatus in *Selaginella*. I. Morphology and photosynthesis under different light and temperature regimes. *Can. J. Bot.* **48**, 1843–1852. (doi:10.1139/b70-270)
- Jagels, R. 1970b Photosynthetic apparatus in *Selaginella*. II. Changes in plastid ultrastructure and pigment content under different light and temperature regimes. *Can. J. Bot.* **48**, 1853–1860. (doi:10.1139/b70-271)
- Justin, S. H. F. W. & Armstrong, W. 1987 The anatomical characteristics of roots and plant response to soil flooding. *New Phytol.* **106**, 465–495. (doi:10.1111/j.1469-8137.1987.tb00153.x)
- Karrfalt, E. E. 1981 The comparative and developmental morphology of the root system of *Selaginella selaginoides* (L.) Link. *Am. J. Bot.* **68**, 244–253. (doi:10.2307/2442856)
- Keeley, J. E. 1981 *Isoetes howellii*: a submerged CAM plant. *Am. J. Bot.* **68**, 420–424. (doi:10.2307/2442779)
- Keeley, J. E. 1982 Distribution of diurnal acid metabolism in the genus *Isoetes*. *Am. J. Bot.* **69**, 254–257. (doi:10.2307/2443012)
- Keeley, J. E. 1998 CAM photosynthesis in submerged aquatic plants. *Botanical Rev.* **64**, 121–175. (doi:10.1007/BF02856581)
- Keeley, J. E. & Busch, G. 1984 Carbon assimilation characteristics of the aquatic CAM plant *Isoetes howellii*. *Plant Physiol.* **76**, 525–530. (doi:10.1104/pp.76.2.525)
- Keeley, J. E., Osmond, C. B. & Raven, J. A. 1984 *Stylites*, a vascular land plant without stomata absorbs CO₂ via its roots. *Nature* **310**, 694–695. (doi:10.1038/310694a0)
- Kenrick, P. & Crane, P. R. 1997 *The origin and early diversification of land plants: a cladistic study*. Washington, DC: Smithsonian Institution.
- Kluge, M. & Ting, I. P. 1978 *Crassulacean acid metabolism: analysis of an ecological adaptation*. Berlin, Germany: Springer.
- Koch, K. & Kennedy, R. A. 1980 Characteristics of Crassulacean acid metabolism in the succulent C₄ dicot, *Portulaca oleracea* L. *Plant Physiol.* **65**, 193–197. (doi:10.1104/pp.65.2.193)
- Korall, P. & Kenrick, P. 2002 Phylogenetic relationships in Selaginellaceae based on *rbcL* sequences. *Am. J. Bot.* **89**, 506–517. (doi:10.3732/ajb.89.3.506)
- Lambers, H., Chapin III, F. S. & Pons, T. L. 1998 *Plant physiological ecology*. Berlin, Germany: Springer.
- Li, M. & Jones, M. B. 1995 CO₂ and O₂ transport in the aerenchyma of *Cyperus papyrus* L. *Aquat. Bot.* **52**, 93–106. (doi:10.1016/0304-3770(95)00484-H)
- Lloyd, J. & Farquhar, G. D. 1994 ¹³C discrimination during CO₂ assimilation by the terrestrial biosphere. *Oecologia* **99**, 201–215. (doi:10.1007/BF00627732)
- Longstreth, D. J. & Borkhsenius, O. N. 2000 Root cell ultrastructure in developing aerenchyma tissue of three wetland species. *Ann. Bot.* **86**, 641–646. (doi:10.1006/anbo.2000.1151)
- Lovelock, J. 1979 *Gaia: a new look at life on Earth*. Oxford, UK: Oxford University Press.
- Lüttge, U. 1996 Clusia: plasticity and diversity in a genus of C₃/CAM intermediate tropical trees. In *Crassulacean acid metabolism* (eds K. Winter & J. A. C. Smith), pp. 296–311. Berlin, Germany: Springer.
- Martin, T. A., Teskey, R. O. & Dougherty, P. M. 1994 Movement of respiratory CO₂ in stems of loblolly pine (*Pinus taeda* L.) seedlings. *Tree Physiol.* **14**, 481–495.
- Mazen, A. M. A. 1996 Changes in levels of phosphoenolpyruvate carboxylase with induction of Crassulacean acid metabolism (CAM)-like behavior in the C₄ plant *Portulaca oleracea*. *Plant Physiol.* **98**, 111–116. (doi:10.1111/j.1399-3054.1996.tb00681.x)
- Moore, P. D. 1984 Novel carbon supply on land. *Nature* **310**, 633. (doi:10.1038/310633a0)
- Mühlenbock, P., Plaszczyca, M., Plaszczyca, M., Mellerowicz, E. & Karpinski, S. 2007 Lysigenous aerenchyma formation in *Arabidopsis* is controlled by Lesion Stimulating Disease 1. *The Plant Cell* **19**, 3819–3830.
- Nielsen, S. L., Gacia, E. & Sand-Jensen, K. 1991 Land plants of amphibious *Littorella uniflora* (L.) Aschers. maintain utilization of CO₂ from the sediment. *Oecologia* **88**, 258–262. (doi:10.1007/BF00320820)
- Nikas, K. J., Tiffney, B. H. & Knoll, A. H. 1985 Patterns in vascular land plant diversification: an analysis at the species level. In *Phanerozoic diversity patterns* (ed. J. Valentine), pp. 97–127. Princeton, NJ: Princeton University Press.
- Osborne, C. P. & Beerling, D. J. 2006 Nature's green revolution: the remarkable evolutionary rise of C₄ plants. *Phil. Trans. R. Soc. B* **361**, 173–194. (doi:10.1098/rstb.2005.1737)
- Pedersen, O. & Sand-Jensen, K. 1992 Adaptations of submerged *Lobelia dortmanna* to aerial life form: morphology, carbon sources, and oxygen dynamics. *Oikos* **65**, 89–96. (doi:10.2307/3544890)
- Phillips, T. L. & DiMichele, W. A. 1992 Comparative ecology and life-history biology of arborescent lycopsids in

- Late Carboniferous swamps of Euramerica. *Ann. Mo Botanical Garden* **79**, 560–588. (doi:10.2307/2399753)
- Phillips, T. L., Peppers, R. A. & DiMichele, W. A. 1985 Stratigraphic and interregional changes in Pennsylvanian coal-swamp vegetation: environmental inferences. *Int. J. Coal Geol.* **5**, 43–109. (doi:10.1016/0166-5162(85)90010-2)
- Pigg, K. B. 1992 Evolution of isoetalean lycopsids. *Ann. Mo Botanical Garden* **79**, 589–612. (doi:10.2307/2399754)
- Raven, J. A. & Edwards, D. 2001 Roots: evolutionary origins and biogeochemical significance. *J. Exp. Bot.* **52**, 381–401.
- Raven, J. A. & Spicer, R. A. 1996 The evolution of Crassulacean acid metabolism. In *Crassulacean acid metabolism* (eds K. Winter & J. A. C. Smith), pp. 360–385. Berlin, Germany: Springer.
- Raven, J. A., Handley, L. L., MacFarlane, J. J., McInroy, S., McKenzie, L., Richards, J. H. & Samuelsson, G. 1988 The role of CO₂ uptake by roots and CAM in acquisition of inorganic C by plants of the isoetid life-form: a review, with new data on *Eriocaulon decangulare* L. *New Phytol.* **108**, 125–148. (doi:10.1111/j.1469-8137.1988.tb03690.x)
- Rosello, S. 1966 L'anatomie de *Selaginella willdenowii* Baker et la notion de polystélie. *Naturalia Monspeliensia, série Botanique* **17**, 189–207.
- Rothwell, G. W. & Erwin, D. M. 1985 The rhizomorph apex of *Paurodendron*; implications for homologies among the rooting organs of Lycopsida. *Am. J. Bot.* **72**, 86–98. (doi:10.2307/2443571)
- Royer, D. L. 2003 Estimating latest Cretaceous and Tertiary atmospheric CO₂ from stomatal indices. *Geol. Soc. Am. Special Papers* **369**, 79–93.
- Scott, H. D. 1900 *Studies in fossil botany*. London, UK: Adam and Charles Black.
- Smirnoff, N. 1996 Regulation of Crassulacean acid metabolism by water status in the C3/CAM intermediate *Sedum telphium*. In *Crassulacean acid metabolism* (eds K. Winter & J. A. C. Smith), pp. 176–191. Berlin, Germany: Springer.
- Sternberg, K. 1820–1838 *Versuch einer geognostisch-botanischen Darstellung der Flora der Vorwelt*. Leipzig and Prague: Fleischer.
- Stewart, W. N. 1947 A comparative study of stigmarian appendages and *Isoetes* roots. *Am. J. Bot.* **34**, 315–324. (doi:10.2307/2437143)
- Stewart, W. N. & Rothwell, G. W. 1993 *Paleobotany and the evolution of plants*, 2nd edn. Cambridge, MA: Cambridge University Press.
- Uphof, J. C. Th. 1920 Physiological anatomy of xerophytic Selaginellas. *New Phytol.* **19**, 101–131. (doi:10.1111/j.1469-8137.1920.tb07321.x)
- Voznesenskaya, E. V., Franceschi, V. R., Kiirats, O., Freitag, H. & Edwards, G. E. 2001 Kranz anatomy is not essential for terrestrial C₄ plant photosynthesis. *Nature* **414**, 543–546. (doi:10.1038/35107073)
- Webster, T. R. 1992 Developmental problems in *Selaginella* (Selaginellaceae) in an evolutionary context. *Ann. Mo Botanical Garden* **79**, 632–647. (doi:10.2307/2399757)
- Webster, T. R. & Jagels, R. 1977 Morphology and development of aerial roots of *Selaginella martensii* grown in moist containers. *Can. J. Bot.* **55**, 2149–2158. (doi:10.1139/b77-243)
- Webster, T. R. & Steeves, T. A. 1964 Developmental morphology of the root of *Selaginella kraussiana* A. Br. and *Selaginella wallacei* Hieron. *Can. J. Bot.* **42**, 1665–1676. (doi:10.1139/b64-165)
- Webster, T. R. & Steeves, T. A. 1967 Developmental morphology of the root of *Selaginella martensii* Spr. *Can. J. Bot.* **45**, 395–404. (doi:10.1139/b67-039)
- Weiss, F. E. 1903 A biseriata halonial branch of *Lepidophloios fuliginosus*. *Trans. Linn. Soc. Lond. Ser. 2, Bot.* **6**, 217–235.
- Winter, K. & Smith, J. A. C. (eds) 1996 *Crassulacean acid metabolism*. Berlin, Germany: Springer.
- Witmer, L. M. 1995 The extant phylogenetic bracket and the importance of reconstructing soft tissues in fossils. In *Functional morphology in vertebrate paleontology* (ed. J. J. Thompson), pp. 19–33. New York, NY: Cambridge University Press.
- Zelawski, W., Riech, E. P. & Stanley, R. G. 1970 Assimilation and release of internal carbon dioxide by woody plant shoots. *Can. J. Bot.* **48**, 1351–1354. (doi:10.1139/b70-204)