Trends in Plant Science

CellPress
REVIEWS

Review

Photosynthesis Optimized across Land Plant Phylogeny

Jorge Gago,[1,*] Marc Carriquí,[1] Miquel Nadal,[1] María José Clemente-Moreno,[1] Rafael Eduardo Coopman,[2] Alisdair Robert Fernie,[3] and Jaume Flexas[1,*,@]

Until recently, few data were available on photosynthesis and its underlying mechanistically limiting factors in plants, other than crops and model species. Currently, a new large pool of data from extant representatives of basal terrestrial plant groups is emerging, allowing exploration of how photosynthetic capacity (A_{max}) increases from minimum values in bryophytes to maximum in tracheophytes, which is associated to an optimization of the balance between its limiting factors. From predominant mesophyll conductance limitation (l_m) in bryophytes and lycophytes (fern allies) to stomatal conductance (l_s) and l_m colimitation in pteridophytes (ferns) and gymnosperms, a balanced colimitation by the three limitations is finally reached in angiosperms. We discuss the implications of this new knowledge for future biotechnological attempts to improve crop photosynthesis.

Highlights

A new emerging pool of gas-exchange data from mosses to angiosperms is widening our knowledge about photosynthesis across the land plant groups.

More basal groups achieve lower photosynthetic benefits per leaf mass area (LMA) and/or N content compared with angiosperms, likely due to a differential resource investment that could indicate some trade-offs between maximizing photosynthesis and stress tolerance strategies.

Photosynthetic capacity increases along the phylogenetic continuum, reaching its maximum in angiosperms, based on a balanced colimitation among its diffusional and biochemical determinants, which questions the possibility of improving crop maximum photosynthetic capacity beyond its current limits.

Terrestrial Photosynthesis: An Ongoing Ancient Story

Photosynthesis is the basis of life on Earth. As such, together with respiration, it is the primary process leading to plant growth and crop yields. Improving photosynthesis to achieve higher and/or more resource-use-efficient crop yields has been the subject of several large scale projects, including the C_4-rice project (https://c4rice.com/) and realizing increased photosynthetic efficiency (RIPE; http://ripe.illinois.edu/).

In C_3 plants, which represent the vast majority of plants, photosynthesis has long been considered to be limited either by CO_2 diffusion (regulated by stomatal aperture/closure) or by CO_2 fixation, which depends on the functioning of leaf photochemistry and/or photosynthetic enzymes [1]. However, it is now well established that a third major factor plays a key role: mesophyll conductance to CO_2 diffusion (g_m), that is, the facility of diffusion from the substomatal cavities to the sites of carboxylation inside chloroplast stroma (see [2] for a description and critical appraisal of methods to estimate g_m and [3] for a recent review of the variability of g_m and the factors influencing it). Therefore, it is nowadays considered that photosynthesis can be limited by three major factors, which can be assessed in a quantitative manner [4]: stomatal, mesophyll conductance, and biochemical/photochemical limitations (Box 1). However, until recently, few data were available on both the photosynthetic capacity (i.e., the light-saturated net photosynthesis rate, A_{max}) and its underlying mechanistically limiting factors in plants other than angiosperms, including crops and model species. Nowadays, a large pool of data from extant species of the major clades of terrestrial plants is emerging, allowing the exploration of how A_{max} and its limitations vary across the land plant phylogeny, from mosses and liverworts to tracheophytes (i.e., fern allies, ferns, gymnosperms, and angiosperms). This information, and the knowledge of the mechanisms behind each limitation, may help in understanding the extent to which photosynthetic capacity improvement in crops can be expected and which are the key factors to achieve it.

[1] Research Group on Plant Biology under Mediterranean conditions, Departament de Biologia, Universitat de les Illes Balears / Institute of Agro-Environmental Research and Water Economy – INAGEA, Carretera de Valldemossa, 07122, Palma, Spain
[2] Ecophysiology Laboratory for Forest Conservation, Instituto de Conservación, Biodiversidad y Territorio, Facultad de Ciencias Forestales y Recursos Naturales, Universidad Austral de Chile, Campus Isla Teja, Casilla 567, Valdivia, Chile
[3] Central Metabolism Group, Molecular Physiology Department, Max-Planck-Institut für Molekulare Pflanzenphysiologie, 14476 Golm, Germany

*Correspondence:
xurxogago@gmail.com (J. Gago) and
jaume.flexas@uib.es (J. Flexas).
@Twitter: @JaumeFlexas73 (J. Flexas).

Filling the Gap: What Limits Photosynthesis across the Land Plant Phylogeny?

To answer this question, we compiled gas-exchange and chlorophyll fluorescence data from several papers (detailed information in Table S1 in the supplemental information online) in which $A_{\max}$ and the other gas-exchange parameters were measured in different plant species belonging to diverse phylogenetic groups, in healthy and nonstressed individuals, at saturating light, ca. 400 mmol CO_2 mol^{-1} air and temperatures ranging from 23 to 25°C. As was previously anticipated [15], after filling the gaps for mosses [16], ferns allies [16–18], ferns [17,19], and gymnosperms [18,20–22], a phylogenetic trend for $A_{\max}$ emerged among land plants. The lowest $A_{\max}$ occurred in mosses and liverworts and largest $A_{\max}$ in angiosperms, with intermediate values in fern allies, ferns, and gymnosperms (Figure 1A). This phylogenetic continuum has implications for the leaf economics spectrum (LES) [23] (i.e., the relationship between photosynthetic capacity and other leaf traits) (Box 2).

Besides the phylogenetic trends in $A_{\max}$ and LES, another phylogenetic trend is observed for photosynthetic limitations, from predominant mesophyll conductance limitation (l_m) in mosses, liverworts, and fern allies to stomatal conductance (l_s) and l_m colimitation in ferns and gymnosperms, finally reaching a balanced colimitation by l_s, l_m, and biochemical limitations (l_b)

Box 1. Photosynthesis Limitations: The Power of Three

In gas-exchange studies and since the introduction of the leaf photosynthesis model of Farquhar-von Caemmerer-Berry (FvCB) [5], photosynthesis was commonly viewed as limited merely by two factors: stomata-driven CO_2 diffusion versus photochemistry and/or photosynthetic biochemistry. This essentially means that, although stomatal conductance (g_s) and the rates of photochemistry and/or photosynthetic biochemistry [i.e., the electron transport rate (ETR) in the thylakoids, the maximum carboxylation rate by Rubisco ($V_{c,\max}$), and RuBP regeneration in the Calvin cycle] are all finite, only three limiting conditions are possible: only one or the other factor are the most limiting at any time, or the two factors equally colimit. This is illustrated by the 'Greek temple column' diagram analogy (Figure IA), where the roof indicates the height of the total limitation and the two columns indicate the partial limitations, with the tallest column always being the one which sets the upper height of the roof (i.e., total limitation). However, many reports suggested discrepancies between the two (separately analyzed) limitations and the observed rate of photosynthesis, which is illustrated by the unbalanced inclined roof and the adjacent question mark in Figure IA. For instance, when stomatal limitations were assessed by gas exchange and carboxylation limitations by the biochemically determined Rubisco activity, the resulting total limitation under water stress was lower than the observed decline of $A_{\max}$ [6,7]. These discrepancies suggested that an additional limiting factor could be involved.

Early works, prior to the FvCB model, already proposed the nature of the third limitation (i.e., the CO_2 diffusion through the mesophyll, from the substomatal cavities to the carboxylation sites in chloroplasts stroma) [8]. In the 1970s, the so-called mesophyll conductance for CO_2 (g_m) was often estimated, yet the methods used had some fundamental misconceptions [9–12]. Since the broad adoption of the FvCB model [5], it was often assumed that g_m was large and constant, and thus the chloroplast CO_2 concentration (C_c) was considered to be equal to the concentration in substomatal cavities (C_i). However, thanks to new methods based on combined measurements of ^{13}C-discrimination and gas exchange during photosynthesis [13] or chlorophyll fluorescence and gas exchange [14], which were introduced in the late 1980s, this assumption was challenged. Now there is ample evidence demonstrating that C_c is significantly lower than C_i and that g_m is finite and variable, imposing a significant limitation to photosynthesis of similar magnitude to those imposed by g_s and the rates of photo/biochemistry. Thus, the current view is that photosynthesis can be limited by three factors, which implies scaling regulatory complexity from the mere three limiting combinations described for the two-factor model to up to seven combinations for the three-factor one: a colimitation by the three factors, three different combinations of colimitation by just two of the factors; and another three combinations for single-factor limitation, as one factor alone can lead to unincreased $A_{\max}$ even if the other two limitations are reduced. This is illustrated as the stability of the roof, even with a single limitation, in Figure IB.

Thus, the regulation of $A_{\max}$ is complex, as it involves diffusion of CO_2 from the stomata pores, through the substomatal cavities and intercellular air spaces, cell walls, plasma membrane, cytosol, chloroplast membrane, and stroma, and finally fixation by Rubisco in coordination with all the photochemical and biochemical processes (Figure IC).

Using the photosynthesis limitation model established by Grassi and Magnani [4], from measurements of $A_{\max}$, g_s, g_m, and either ETR or $V_{c,\max}$, it is possible to estimate the percentage of each relative limitation (stomatal, l_s; mesophyll, l_m; and biochemical, l_b limitations) to photosynthesis [4].

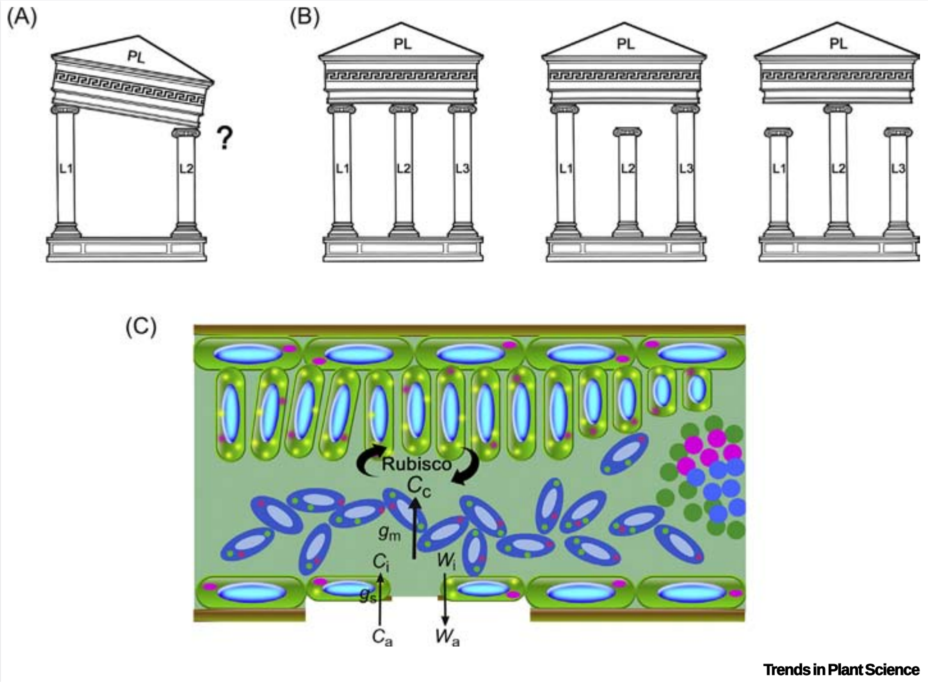


Figure I. The Power of Three: Photosynthetical Capacity (A_{max}) Regulation. Photosynthesis was classically considered as limited, either by the limitation imposed by stomata closure [i.e., the stomatal limitation (l_s)] or by that imposed by insufficient function of leaf biochemistry (Rubisco) and/or photochemistry (thylakoid electron transport) [i.e., the globally called biochemical limitation (l_b)] (A). This is illustrated as a ‘Greek temple’, with the stomata (L1) and the combined Rubisco–PSII complex proteins (L2) as the ‘columns’ supporting the ‘roof’ (total photosynthesis limitation) (PL). The regulation of total photosynthesis limitation is simple in this model: either one factor (i.e., one partial limitation) or the other limits A_{max} the most, or the two factors colimit in a balanced manner. However, it is currently known that a third ‘column’, the mesophyll conductance limitation (l_m)(L3), is an essential limitation to photosynthesis (B). This changes the regulation of total photosynthesis limitation to a much more complex scenario: each one of the three factors can limit photosynthesis the most, or three different combinations of two factors can colimit, or the three factors can colimit photosynthesis in a balanced manner. The complex nature of A_{max} regulation is shown in (C) as a leaf cross-section integrating a diagram of the CO_2 and H_2O diffusion pathways and the final CO_2 assimilation by photobiochemistry: Abbreviations: C_a, ambient CO_2 concentration; C_c, CO_2 concentration at the site of Rubisco carboxylation within the chloroplast stroma; C_i, CO_2 concentration at the substomatal cavities; g_s, stomatal conductance; g_m, mesophyll conductance; Rubisco, employed here to illustrate all photobiochemical reactions needed for carboxylation; and W_i and W_a, the concentrations of water vapor in the substomatal cavity and the atmosphere, respectively.

in angiosperms (Figure 1B). Recalling the ‘Greek temple analogy’ of Box 1, one can now imagine the limitation bars in Figure 1B as the ‘columns’ and imagine the position of the ‘roof’; it becomes apparent that only the l_m column supports the roof in all plant groups, while the other limitations cosupport it in some of the groups. Thus, optimization of A_{max} along the land plant’s phylogeny is tightly driven by changes in nonstomatal diffusional limitations, particularly in the mesophyll, that appear as a major factor in all of the groups evaluated.

Prospects for Understanding the Nature of Nonstomatal Diffusional Limitations

Mesophyll conductance importantly limits photosynthesis in the extant representatives of bryophytes and fern allies and colimits with other factors in ferns, gymnosperms, and angiosperms. Indeed, it is a complex trait that is likely determined by both biochemical and anatomical features. It has been suggested that it is dynamically regulated by aquaporins [29–33] and carbonic anhydrases [34–38], it responds to external (e.g., water stress, temperature, or CO_2) and internal

Box 2. Leaf Economics Spectrum across Phylogeny: More for Less?

Wright and coworkers [23] established several ‘universal’ trade-offs among leaf traits, which were collectively defined as the ‘leaf economics spectrum’ (LES). However, their dataset was largely dominated by angiosperms, including only a few fern and gymnosperm species and no mosses, liverworts, or fern allies. From the current available data for different phylogenetic groups, both a negative correlation between mass-based photosynthesis and leaf mass area (LMA) and a positive correlation between mass-based photosynthesis and nitrogen content were observed [16] (Figure I). While slopes are common for all groups for both relationships, the intercepts differ for both relationships along the phylogenetic continuum, so that the more basal a group is along the phylogeny, the less photosynthetic benefit it has per unit LMA or N [16].

Whatever the possible adaptive significance, the different intercepts may have a physiological meaning. On one hand, the fact that extant representatives of most basal phylogenetic groups have less photosynthesis per unit LMA suggests that a larger fraction of their leaf mass is devoted to nonphotosynthetic structures. On the other hand, they have less photosynthesis per unit N, suggesting that they devote a larger fraction of their nitrogen to nonphotosynthetic elements such as, for example, cell walls. Likewise, the fact that ferns are positioned with angiosperms in this relationship is intriguing and could perhaps be due to the fact they showed a notable different cell wall composition [27] (Box 3), so maybe the elemental chemistry of their structural components is different and requires less nitrogen. Both observations suggest an increasing role of mesophyll conductance limitations from the modern to the most basal groups of the land plant phylogeny [28], which can be assessed by the limitation analysis mentioned in Box 1 [4].

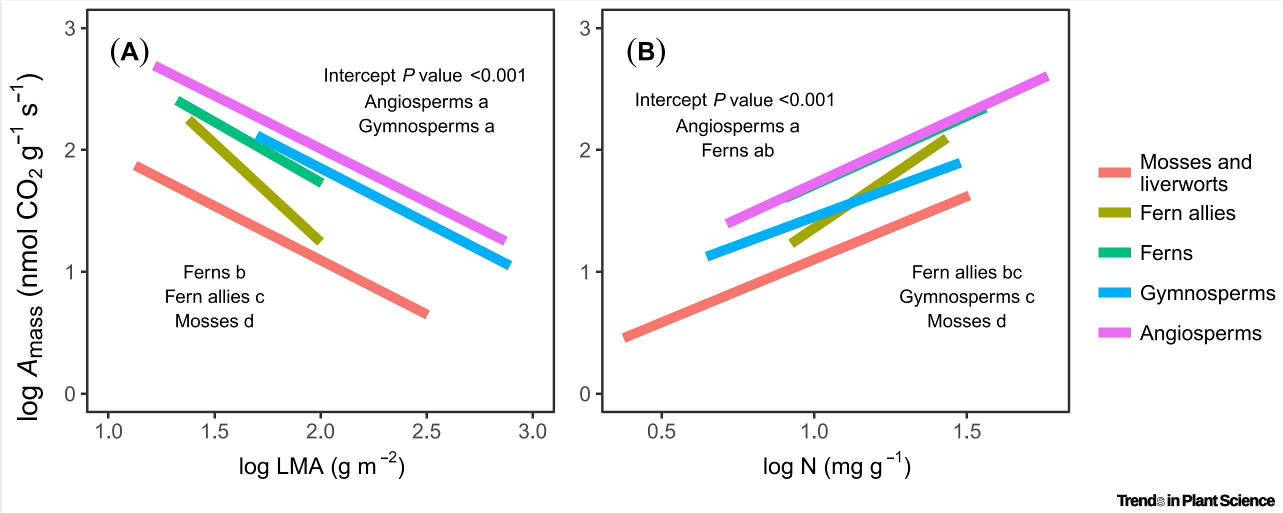
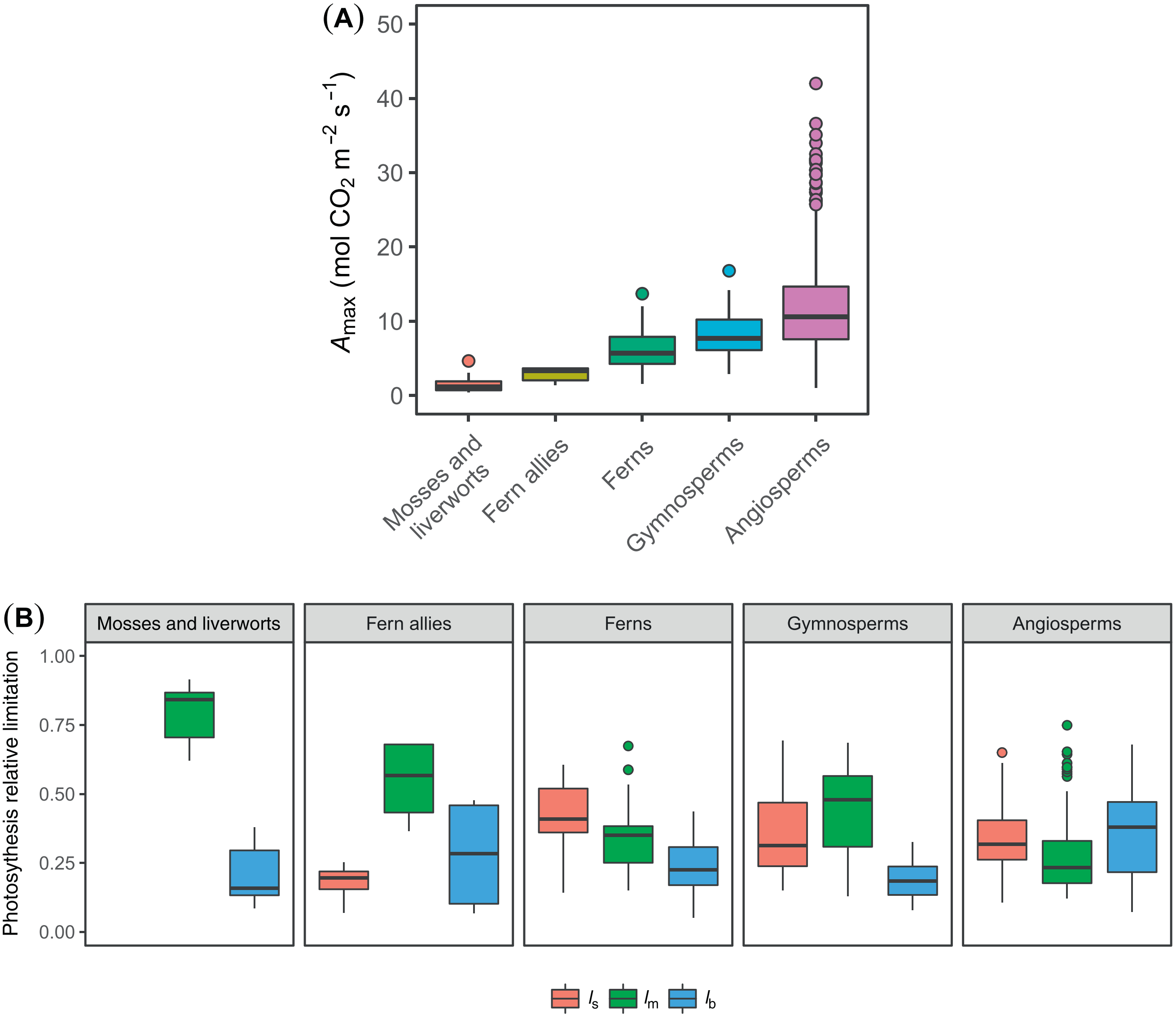


Figure I. Photosynthesis and Leaf Economics Spectrum: More for Less? Trait relationships between log A_{mass} and log leaf mass area (LMA) (A), and log A_{mass} versus log N (B) across plant groups: mosses and liverworts (n = 31 for A, 32 for B), fern allies (n = 6 for both subplots), ferns (n = 37 for both subplots), gymnosperms (n = 90 for A, 95 for B), and angiosperms (n = 727 for A, 665 for B). Data (dots) are not shown for clarity. Lines indicate the linear function for each group. Data are compiled from several articles [14–16,19,21]. In the case of mosses, canopy mass area is considered instead of LMA for the leaf economics spectrum relationships, since moss canopy has been hypothesized [24] and later confirmed [16,25,26] to be analogous to the tracheophyte leaf. Common slopes were tested using a likelihood ratio test and intercepts by calculating the Wald statistic across groups [standardized major axis (SMA)]; different letters mean statistical differences per group at $P < 0.05$. SMA statistics can be found in Table S3 in the supplemental information online.

(e.g., abscisic acid) factors [39–44], and shows a complex dependency on metabolic pathways driving some of the previous mentioned traits in leaves [45]. Also, the maximum g_m for a given species strongly depends on several leaf anatomical characteristics in vascular plants, especially on mesophyll cell wall thickness (CWT) and the total chloroplast surface area exposed to mesophyll intercellular air spaces (S_c/S) per leaf area (or exposed to ambient air in mosses because they do not present intercellular airspaces), which in turn depends on the number, size, and distribution of chloroplasts. These traits largely explain the variations of g_m among plant species and growth forms [46–51]. Although mesophyll conductance can be now better represented with recent 3D leaf models [52], current available anatomical data only allow for the construction of a 1D analytical model for g_m, using CWT, S_c/S, and other measured anatomical traits as the main inputs [49,51]. The values of g_m modeled using this anatomical model are fully independent of estimates made by combined gas exchange and chlorophyll fluorescence or isotope discrimination, but still the two values strongly correlate when comparing different species under

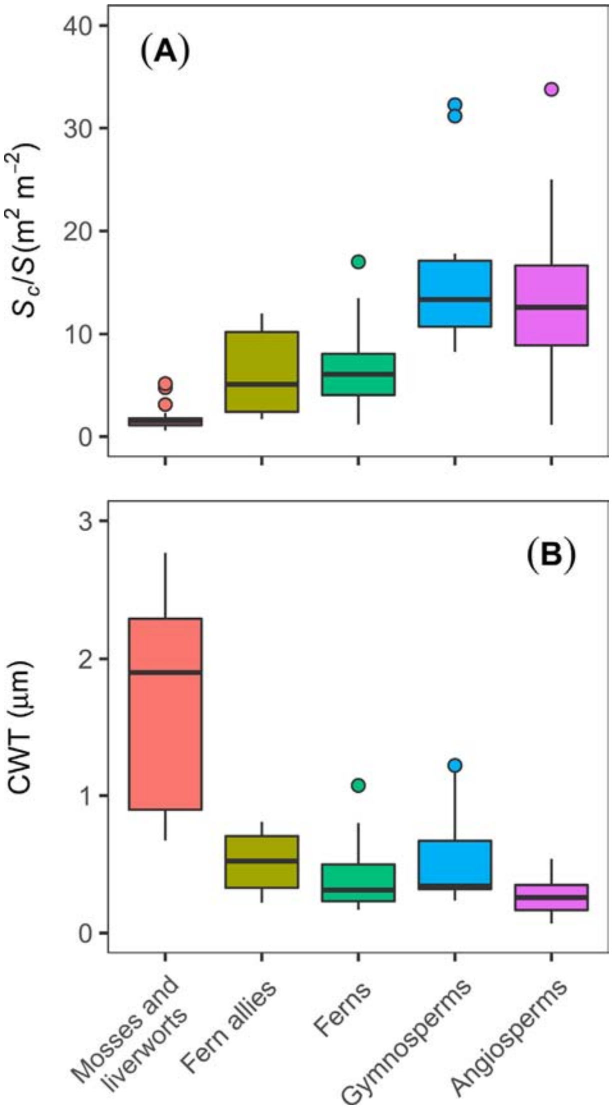


Trends in Plant Science

Figure 1. Net Photosynthesis (A_{max}) and its Physiological and Biochemical Limitations along the Land Plant Groups. (A) Boxplots of net assimilation (A_{max}) across plant groups: mosses and liverworts (n = 32), fern allies (n = 6), ferns (n = 37), gymnosperms (n = 90), and angiosperms (n = 783), data were compiled from several papers [14–16,19,21]. (B) Boxplots of relative stomatal (l_s), mesophyll (l_m), and biochemical (l_b) photosynthesis limitations in mosses and liverworts (n = 11), fern allies (n = 5), ferns (n = 41), gymnosperms (n = 15), and angiosperms (n = 70). Notice the absence of stomatal limitations in mosses and liverworts, this is because they are most often found in their gametophyte stage, which lacks stomata. The employed dataset and references are shown in Table S1 in the supplemental information online and in open repository figshare (https://figshare.com/search?q=10.6084%2Fm9.figshare.8378297&searchMode=1). The two extreme lines of the boxplot (whiskers) show the 5% and 95% percentiles, the two bounds of the box the 25% and 75% percentiles, and the center thick line the median. Dots represent data out of the shown percentiles.

nonstress conditions [50]. This confirms the importance of anatomy in setting g_m. Recent studies suggest that the anatomy of photosynthetic tissues also explains most of the differences in g_m among phylogenetic groups [16–19,28,53]. Thus, using the data available for quantitative anatomical traits of photosynthetic tissues in extant species, the emerging picture displays a phylogenetic trend towards decreasing CWT and increasing S_c/S (Figure 2).

The two anatomical characteristics that constrain g_m the most, CWT and S_c/S, are negatively related to one another along the phylogeny (Figure 2), but also when comparing different species within a genus or genotypes within a species [53,54]. This observation is intriguing as the cytological and molecular drivers of cell wall construction and chloroplast differentiation and positioning during cell and tissue development are quite independent. Chloroplasts arise from plastids and they are positioned by actin filaments of the cytoskeleton. By contrast, Golgi bodies, endoplasmic reticulum, and microtubules are concerned with the organization and synthesis of materials that are incorporated into the cell walls [55]. Yet, despite their apparently independent origins, cell walls and chloroplasts are developed and positioned in a manner such that CWT and S_c/S negatively correlate, which suggests a possible coordination during leaf development that deserves future studies. Regardless of the causes for covariation, this is an example of how the photosynthetic organelles have been able to optimize their design to maximize the use of CO_2



Trends in Plant Science

Figure 2. Anatomical Transect of the Chloroplast Surface Area Exposed to the Intercellular Air Spaces per Leaf Area (S_c/S) and Cell Wall Thickness (CWT) across Land Plant Groups. Chloroplast surface area exposed to intercellular air spaces per leaf area (S_c/S (A) and mesophyll cell wall thickness (CWT (B) for mosses and liverworts (*n* = 20 for S_c/S, 20 for CWT), fern allies (*n* = 5, 6), ferns (*n* = 35, 37), gymnosperms (*n* = 16, 17), and angiosperms (*n* = 159, 132). The two extreme lines of the boxplot (whiskers) show the 5% and 95% percentiles, the two bounds of the box the 25% and 75% percentiles, and the center thick line the median. Dots represent data out of the shown percentiles. Data and references can be found in Table S2 in the supplemental information online and in open repository figshare (https://figshare.com/search?q=10.6084%2Fm9.figshare.8378297&searchMode=1).

for photosynthesis. This resembles other anatomical phylogenetic patterns suggested to optimize both carbon and water supply in the photosynthetic organs, like the tight correlation between hydraulic and stomatal conductances (K_{leaf} and g_s, respectively). Higher K_{leaf} and g_s, which allow the optimization of transpiration, have been possible by concomitantly increasing vein and stomatal density and miniaturizing both veins and stomata, which was mostly or partially driven by a decrease in cell size and genome size [56–62]. Thus, although little is known about how CWT and S_c/S concomitant changes are mediated through phylogeny, it is possible that they could also be associated with cell size changes [48,63].

In addition to the developmental events required to establish chloroplast number, size, and position, it is important to consider that chloroplasts can move dynamically. However, it is mostly unknown how these movements may affect S_c/S in coordination with g_m. Only one study showed that the 'light avoidance' chloroplast response decreased g_m in *Arabidopsis thaliana* and this effect was not observed in *phot2* mutants lacking chloroplast movements [64]. However, it has been recently demonstrated that rapid changes of g_m in response to light, CO_2, or temperature are not associated with changes in S_c/S and therefore it is likely that they do not involve chloroplast movements [65,66].

In addition, it is widely accepted that the plant cell wall cannot be considered a static structure, but rather a dynamic element that plays a critical role in the growth and phenological stages of the plants, as well as rapidly interacting with the environment in response to biological attacks or abiotic stresses [67–71]. Concerning the diffusive properties of the cell walls, it is assumed that the pore sizes are large enough to freely facilitate the diffusion of CO_2 through them [72–74], however cell wall composition and its biochemical properties could change rapidly in response to stress conditions and could potentially affect their interactions with CO_2 diffusion [70,75] (Box 3).

Reduced Mesophyll Conductance Limitations Lead to Maximized Photosynthetic Capacity in Angiosperms

Besides the mechanisms setting g_m and its regulation, what appears is that its dominant role as a limiting factor for photosynthetic capacity has decreased along the land plant's phylogeny and that, in angiosperms in general and in herbaceous crops in particular [84], photosynthesis is already maximized and limited in a very well balanced manner by the three limitations (Figure 1B). This is illustrated graphically in Figure 3, which shows the ratio between the diffusive (l_d, the sum of stomatal and mesophyll limitations) and the biochemical limitations along the land plant phylogeny. Mosses, liverworts, fern allies, ferns, and gymnosperms showed low A_{max} and g_m values related to higher l_d/l_b ratio, however angiosperms showed higher A_{max} and g_m associated with a more even balance between diffusive and biochemical limitations (l_d/l_b). Interestingly, elevated values for A_{max} (>20 μmol CO_2 m^{-2} s^{-1}) and g_m (>0.2 mol CO_2 m^{-2} s^{-1}) are found only within the angiosperms and most of them do not exceed a balanced colimitation between the three limitations (unbroken line, $l_s = 0.3$, $l_m = 0.3$, and $l_b = 0.3$). Indeed, many species show a balanced behavior between diffusive and biochemical limitations ($l_s + l_m = 0.5$ and $l_b = 0.5$, broken line) (Figure 3). A more balanced photosynthesis limitation requires higher g_s as g_m increases. Angiosperms have indeed increased g_s through changes in stomatal size and arrangement [59] and vein density [58,61,85]. These changes are necessarily linked to leaf hydraulics in order to cope with increased transpiration [60,86]. Furthermore, leaf hydraulic conductance (K_{leaf}), specifically its extra-xylem component (K_{ox}), and g_m may also be directly related by a common diffusion pathway for H_2O and CO_2 through the mesophyll apoplast [54,87].

Altogether, coregulation of leaf hydraulics and gas-exchange traits as photosynthesis and stomatal and mesophyll conductances has been described in vascular plants [22,86,87]. Coordination

Box 3. Cell Wall Biochemistry: A Previously Neglected Key Factor?

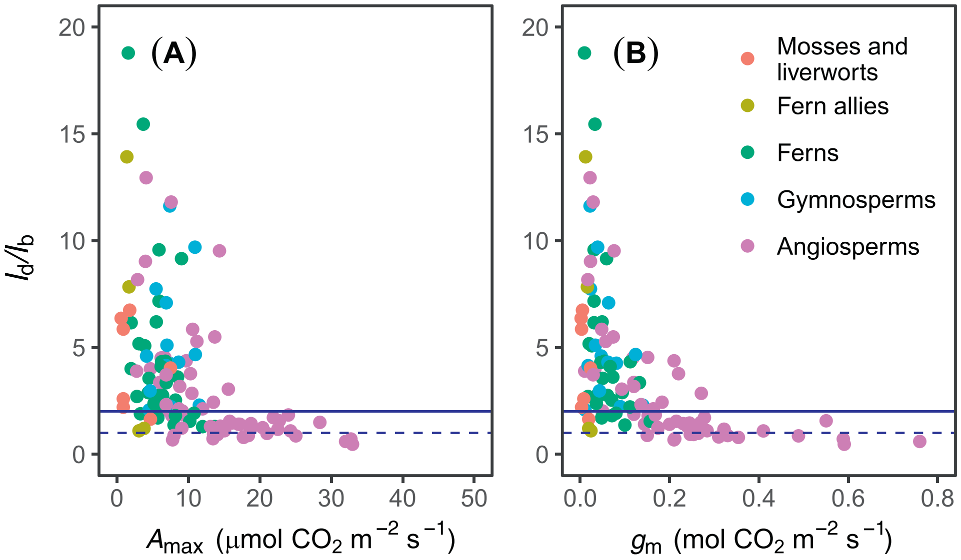
There are many ways by which cell wall composition can potentially affect CO_2 diffusion, although they have been scarcely investigated. For instance, the protonation or ionization of chemical groups in polysaccharides and proteins present in the cell wall would alter the pH, which may, in turn, affect porosity and tortuosity [48]. Moreover, the apoplast acts as a signaling bridge between the environment and the symplast [76], where several enzymes (expansins, peroxidases, hydrolases, transferases, methylesterases, etc.), that can be activated in response to stress, are responsible for the rapid modification of noncellulosic sugar contents (rhamnose, arabinose, xylose, galactose, and galacturonic acid), changing the conformation and complexity of the cell walls [61,63,64]. Interestingly, changes in some cell wall-related metabolites were specifically linked to g_m in a recent multispecies modeling approach, indicating that cell wall structure and composition could significantly affect photosynthesis [45].

Cell wall structure and composition have evolved from algae to higher plants, with important differences in the main cell wall components from mosses to ferns, gymnosperms, and angiosperms [27,77]. All groups possess cellulose microfibrils, but in non-grass angiosperms high quantities of hemicelluloses [as fucoside xyloglucans (XG)], pectins [homogalacturonans (HG), rhamnogalacturonan (RG)], and lignins (guaiacyl and syringil units) are observed, while grasses instead display higher amounts of mixed-linkage glucans. Cell wall composition in gymnosperms is similar to non-grass angiosperms but with higher quantities of glucomannans and lignins (although just guaiacyl units). In leptosporangiate ferns, cell walls display higher quantities of xylans, mannans, uronic acids, and lignins but lower amounts of XGs, HGs, and RGs, while in eusporangiate ferns and mosses xylans and lignins are absent but other phenolic compounds are present, and another important aspect is that primary and secondary cell walls cannot be differentiated in these groups ([78] and references therein).

Current knowledge does not permit us to establish precise mechanistic links between cell wall thickness, structure, composition, and CO_2 diffusion through mesophyll cells to the chloroplast carboxylation sites. However, it has been shown recently that rice mutants with disruptions in cell wall mixed-linkage glucan production have a significantly reduced g_m [79]. Other cell wall components facing the internal side of the pores could potentially affect the physicochemical interactions with the CO_2. For instance, thanks to the activity of apoplastic peroxidases, some cell wall polymers can behave as hydrocolloids that could link phenolic compounds through oxidative crossreactions within cell walls, improving water holding capacities under drought stress [80–82]. Whether reduced water movement can result in reduced diffusion of water-diluted CO_2 has, as yet, not been proven. However, a consistent negative relationship between photosynthesis and the concentration of leaf phenolic compounds was recently reported across a range of different species [83]. That said, additional research efforts are clearly needed for a better understanding of the mechanistic basis of coregulation of cell wall thickness and chloroplast distribution and positioning and how the cell wall composition affects CO_2 diffusion.

between K_{leaf} and A_{max} in nonvascular plants has only been shown in [86] with a *Polytrichum* that has functional water conducting tissues, but there are few moss species that possess it, so it is difficult to generalize for all bryophytes. Further studies should demonstrate if this coordination is present across the entire terrestrial plant phylogeny.

The tight colimitation by the three factors in angiosperms implies that it might be difficult to improve leaf-based maximum light-saturated photosynthesis rates in future crops [88], as improving any of the three limiting factors would immediately render the other two as the most limiting, thus achieving little improvement in the overall photosynthesis rate (see the three-column analogy in Box 1). Certainly, in nature, most of the realized photosynthesis is not performed under conditions favoring the maximum rate (i.e., A_{max}). However, improving A_{max} has been and continues to be a long-term goal for improving crop productivity [89] because of its nonnegligible role in crops, which typically have canopies designed to optimize light capture, for example, in minimizing auto-shading. For instance, in grapevines the small fraction of leaves exposed to saturated light along the whole day has been shown to contribute to more than half of the total daily canopy assimilation [90]. In this sense, although improving stomatal conductance is feasible in many ways, it results in limited improvements of photosynthesis at the expense of reduced water use efficiency [91,92]. In turn, increasing photosynthesis by improving Rubisco has not been achieved yet, but is a goal envisaged as feasible for the next decade or so [93, 94]. Interestingly, alternative photorespiratory pathways for glycolate metabolism (a toxic by-product of RuBP Rubisco oxygenation through photorespiration) showed that it is possible to reduce the costs of this process, improving growth and productivity in tobacco plants at field



Trends in Plant Science

Figure 3. Diffusive and Biochemical Limitation Balance in Relationship to Photosynthesis and Mesophyll Conductance through Land Plant Phylogeny. Relationship between gas exchange parameters and photosynthesis relative limitations across plant groups. The ratio of diffusive (l_d; the sum of stomatal and mesophyll limitations) and biochemical limitations (l_d/l_b) against net assimilation (A_{max}) (A) and mesophyll conductance to CO$_2$ (g_m) (B). Broken lines show that the diffusive (the sum of stomatal and mesophyll) and biochemical limitations are equal, the continuous lines show that the three limitations are equal. Data and references can be found in Table S1 in the supplemental information online and in the open repository figshare (https://figshare.com/search?q=10.6084%2Fm9.figshare.8378297&searchMode=1).

conditions [95]. Instead, improving g_m seems more complicated, despite success following the overexpression of some aquaporins [32,33], because of its very complex nature, which implies that it is not only a highly ‘multigenic trait’, but also a trait for which most of the potentially implicated genes are unknown. Therefore, improving g_s together with Rubisco seems achievable in the short term, yet both theoretical modeling and empirical evidence suggests that (without simultaneously improving g_m) this would somehow resemble going back through the phylogeny, (i.e., recovering a dominant mesophyll conductance limitation that may severely limit further improvements of photosynthesis rate). While significantly improving g_m seems a more long-term goal, the analysis of *Arabidopsis* mutants with different anatomical characteristics suggests that it might be possible [96] and stomatal mutants prove that this could be achieved without simultaneously increasing g_s, thus resulting in improved water-use efficiency [97].

Additionally, g_m and photosynthesis could be improved in nonleaf green organs of angiosperms to increase whole plant photosynthesis, as it has been recently found in cotton, while photosynthesis in leaves is mostly colimited by the three limitations; in bracts it is largely limited by g_m (i.e., like in the most basal phylogenetic groups) [98]. To advance in this direction, a renewed effort is needed to study the mechanisms underlying g_m, from the gene to metabolite scales.

Concluding Remarks and Future Perspectives

Just recently, an important pool of data on photosynthetic limitations has become available for different species across the entire land plant phylogeny. From these, a pattern emerges in extant plant groups from a predominant mesophyll conductance limitation in bryophytes to a well-balanced colimitation by stomatal conductance, mesophyll conductance, and leaf biochemistry/photochemistry in angiosperms. This transition is due, at least in part, to a concomitant transition in some anatomical traits, notably decreasing CWT and increasing the chloroplast

Outstanding Questions

A strong coordination among stomatal, mesophyll conductance, and biochemical limitations drive maximal photosynthetic capacities in angiosperms; to what extent does this require optimization of hydraulic (xylem and phloem) properties?

Can light-saturated maximum photosynthesis be improved in a simple manner? Photosynthesis is a complex process driven by several physiological factors, hence, is it possible to increase it by improving one of these factors alone?

Is there a trade-off between photosynthesis and stress tolerance? Along the land plant phylogeny, a differential resource investment into photosynthetical traits can be observed from mosses to angiosperms, yet the reason for this remains obscure; thus, does maximizing photosynthesis impose penalties to the stress tolerance trait investment?

exposure area. Some intriguing questions emerge that may deserve future studies, for example, how is the coordination between chloroplasts and cell walls achieved during leaf development; what is the role of the cell wall composition in the CO_2 diffusion; and if the thicker cell walls translate into increased stress tolerance in the plants with lower A_{max} [99,100]. In addition, the balanced coregulation of the three photosynthetic limiting factors in angiosperms suggests that it might be very difficult to improve maximum leaf photosynthesis in these plants, including crops; improvements in just one or two of them will directly force the nonimproved factor to limit maximal photosynthesis. Thus, future breeding strategies to significantly increase A_{max} should coordinate improvements in all the limiting factors, from the CO_2 diffusive limitations, the stomatal and mesophyll conductances, to the photobiochemistry. Clearly, other strategies like optimizing canopy light saturation, increasing the velocity of photosynthesis recovery after photoprotective stages, speeding up stomatal kinetics to environment, selection for increased daily carbon gain, or improving photosynthesis of nongreen leaf organs [98,101–104] are likely to be more feasible in improving crop photosynthesis in the near future (see Outstanding Questions).

Acknowledgments

The research of J.G., M.C., M.N., M.J.C-M., and J.F. is supported by the projects CTM2014-53902-C2-1-P from the Ministerio de Economía y Competitividad (MINECO, Spain), PGC2018-093824-B-C41 from the Ministerio de Ciencia, Innovación y Universidades, and the ERDF (FEDER). J.G. want also to thank the postdoctoral contract from Universitat de les Illes Balears, M.C. acknowledges his predoctoral fellowship FPI/1700/2014 from the Conselleria d'Educació, Cultura i Universitats (Govern de les Illes Balears), in addition, M.N. thanks his predoctoral fellowship BES-2015–072578 from the Ministerio de Economía y Competitividad (MINECO, Spain); both fellowships are co-financed by the European Social Fund. R.E.C. is supported by the project FONDECYT-1171640 from the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT, Chile). Authors also want to thank Dr Danilo Daloso for his comments after a critical reading of the manuscript.

Supplemental Information

Supplemental information associated with this article can be found online at https://doi.org/10.1016/j.tplants.2019.07.002.

References

1. Jones, H.G. (1985) Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant Cell Environ.* 8, 95–104
2. Pons, T.L. *et al.* (2009) Estimating mesophyll conductance to CO_2: methodology, potential errors, and recommendations. *J. Exp. Bot.* 60, 2217–2234
3. Flexas, J. *et al.* (2018) CO_2 diffusion inside photosynthetic organs. In *The Leaf: A Platform for Performing Photosynthesis, Advances in Photosynthesis and Respiration*In (44) (Adams, W.W. and Terashima, I., eds), pp. 163–208, Springer
4. Grassi, G. and Magnani, F. (2005) Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ.* 28, 834–849
5. Farquhar, G.D. *et al.* (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta* 149, 78–90
6. Bota, J. *et al.* (2004) Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytol.* 162, 671–681
7. Flexas, J. *et al.* (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. *Plant Biol.* 6, 269–279
8. Gaastra, P. (1959) Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. *Meded. Landbouwhogeschool, Wageningen* 59, 1–68
9. Jones, H.G. and Slatyer, R.O. (1972) Effects of intercellular resistances on estimates of the intracellular resistance to CO_2 uptake by plant leaves. *Aust. J. Biol. Sci.* 25, 443–453
10. Jones, H.G. and Slatyer, R.O. (1972) Estimation of the transport and carboxylation components of the intracellular limitation to leaf photosynthesis. *Plant Physiol.* 50, 283–288
11. Jones, H.G. (1973) Moderate-term water stresses and associated changes in some photosynthetic parameters in cotton. *New Phytol.* 72, 1095–1105
12. Samsuddin, Z. and Impens, I. (1979) Photosynthesis and diffusion resistance to carbon dioxide in *Hevea brasiliensis* muell. agr. clones. *Oecologia* 37, 361–363
13. Evans, J.R. *et al.* (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. *Aust. J. Plant Physiol.* 13, 281–292
14. Harley, P.C. *et al.* (1992) Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. *Plant Physiol.* 98, 1429–1436
15. Flexas, J. *et al.* (2012) Mesophyll conductance to CO_2: an unappreciated central player in photosynthesis. *Plant Sci.* 193–194, 70–84
16. Carriquí, M. *et al.* (2019) Anatomical constraints to non-stomatal diffusion conductance and photosynthesis in lycophytes and bryophytes. *New Phytol.* 222, 1256–1270
17. Tosens, T. *et al.* (2016) The photosynthetic capacity in 35 ferns and fern allies: mesophyll CO_2 diffusion as a key trait. *New Phytol.* 209, 1576–1590
18. Veromann-Jürgenson, L.L. *et al.* (2017) Extremely thick cell walls and low mesophyll conductance: welcome to the world of ancient living! *J. Exp. Bot.* 68, 1639–1653
19. Carriquí, M. *et al.* (2015) Diffusional limitations explain the lower photosynthetic capacity of ferns as compared with angiosperms in a common garden study. *Plant Cell Environ.* 38, 448–460
20. Peguero-Pina, J.J. *et al.* (2012) Leaf anatomical properties in relation to differences in mesophyll conductance to CO_2 and

photosynthesis in two related Mediterranean *Abies* species. *Plant Cell Environ.* 35, 2121–2129

21. Zhang, Y.J. *et al.* (2015) Extending the generality of leaf economic design principles in the cycads, an ancient lineage. *New Phytol.* 206, 817–829
22. Xiong, D. *et al.* (2018) Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species. *Plant Cell Environ.* 41, 436–450
23. Wright, I.J. *et al.* (2004) The worldwide leaf economics spectrum. *Nature* 428, 821–827
24. Proctor, M.C.F. (2000) Mosses and alternative adaptation to life on land. *New Phytol.* 148, 1–6
25. Waite, M. and Sack, L. (2010) How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. *New Phytol.* 185, 156–172
26. Wang, Z. *et al.* (2017) The 'plant economic spectrum' in bryophytes, a comparative study in subalpine forest. *Am. J. Bot.* 104, 261–270
27. Popper, Z.A. *et al.* (2011) Evolution and diversity of plant cell walls: from algae to flowering plants. *Annu. Rev. Plant Biol.* 62, 567–590
28. Onoda, Y. *et al.* (2017) Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytol.* 214, 1447–1463
29. Terashima, I. and Ono, K. (2002) Effects of $HgCl_2$ on CO_2 dependence of leaf photosynthesis: evidence indicating involvement of aquaporins in CO_2 diffusion across the plasma membrane. *Plant Cell Physiol.* 43, 70–78
30. Uehlein, N. *et al.* (2003) The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. *Nature* 425, 734–737
31. Uehlein, N. *et al.* (2008) Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability. *Plant Cell* 20, 648–657
32. Hanba, Y.T. *et al.* (2004) Overexpression of the barley aquaporin HvPIP2;1 increases internal CO_2 conductance and CO_2 assimilation in the leaves of transgenic rice plants. *Plant Cell Physiol.* 45, 521–529
33. Flexas, J. *et al.* (2006) Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo. *Plant J.* 48, 427–439
34. Price, D. *et al.* (1994) Specific reduction of chloroplast carbonic anhydrase activity by antisense RNA in transgenic tobacco plants has a minor effect on photosynthetic CO_2 assimilation. *Planta* 193, 331–340
35. Williams, T.G. *et al.* (1996) Photosynthetic gas exchange and discrimination against $^{13}CO_2$, and $C^{18}O^{16}O$ in tobacco plants modified by an antisense construct to have low chloroplastic carbonic anhydrase. *Plant Physiol.* 112, 319–326
36. Gillon, J.S. and Yakir, D. (2000) Internal conductance to CO_2 diffusion and $C^{18}OO$ discrimination in C_3 leaves. *Plant Physiol.* 123, 201–213
37. Perez-Martin, A. *et al.* (2014) Regulation of photosynthesis and stomatal and mesophyll conductance under water stress and recovery in olive trees: correlation with gene expression of carbonic anhydrase and aquaporins. *J. Exp. Bot.* 65, 3143–3156
38. Momayyezi, M. and Guy, R.D. (2017) Substantial role for carbonic anhydrase in latitudinal variation in mesophyll conductance of *Populus trichocarpa* Torr. & Gray. *Plant Cell Environ.* 40, 138–149
39. Flexas, J. *et al.* (2007) Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. *Plant Cell Environ.* 30, 1284–1298
40. Flexas, J. *et al.* (2008) Mesophyll conductance to CO_2: current knowledge and future prospects. *Plant Cell Environ.* 31, 602–621
41. Vrábl, D. *et al.* (2009) Mesophyll conductance to CO_2 transport estimated by two independent methods: effect of variable CO_2 concentration and abscisic acid. *J. Exp. Bot.* 60, 2315–2323
42. von Caemmerer, S. and Evans, J.R. (2015) Temperature responses of mesophyll conductance differ greatly between species. *Plant Cell Environ.* 38, 629–637
43. Nadal, M. and Flexas, J. (2018) Mesophyll conductance to CO_2 diffusion: effects of drought and opportunities for improvement. In *Water Scarcity and Sustainable Agriculture in Semiarid Environment* (García-Tejero, I.F. and Durán-Zuazo, V.H., eds), pp. 404–438, Elsevier
44. Mizokami, Y. *et al.* (2019) Effects of instantaneous and growth CO_2 levels, and ABA on stomatal and mesophyll conductances. *Plant Cell Environ.* 42, 1257–1269
45. Gago, J. *et al.* (2016) Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: a multispecies meta-analysis approach. *Plant Physiol.* 171, 265–279
46. Hassiotou, F. *et al.* (2009) Influence of leaf dry mass per area, CO_2, and irradiance on mesophyll conductance in sclerophylls. *J. Exp. Bot.* 60, 2303–2314
47. Scafaro, A.P. *et al.* (2011) Temperature response of mesophyll conductance in cultivated and wild *Oryza* species with contrasting mesophyll cell wall thickness. *Plant Cell Environ.* 34, 1999–2008
48. Terashima, I. *et al.* (2011) Leaf functional anatomy in relation to photosynthesis. *Plant Physiol.* 155, 108–116
49. Tosens, T. *et al.* (2012) Developmental changes in mesophyll diffusion conductance and photosynthetic capacity under different light and water availabilities in *Populus tremula*: how structure constrains function. *Plant Cell Environ.* 35, 839–856
50. Tosens, T. and Laanisto, L. (2018) Mesophyll conductance and accurate photosynthetic carbon gain calculations. *J. Exp. Bot.* 69, 5315–5318
51. Tomás, M. *et al.* (2013) Importance of leaf anatomy in determining mesophyll diffusion conductance to CO_2 across species: quantitative limitations and scaling up by models. *J. Exp. Bot.* 64, 2269–2281
52. Earles, J.M. *et al.* (2019) Embracing 3D complexity in leaf carbon–water exchange. *Trends Plant Sci.* 24, 15–24
53. Peguero-Pina, J.J. *et al.* (2017) Cell-level anatomical characteristics explain high mesophyll conductance and photosynthetic capacity in sclerophyllous Mediterranean oaks. *New Phytol.* 214, 585–596
54. Xiong, D. *et al.* (2017) Leaf anatomy mediates coordination of leaf hydraulic conductance and mesophyll conductance to CO_2 in *Oryza*. *New Phytol.* 213, 572–583
55. Salisbury, F.B. and Ross, C.W. (1992) *Plant Physiology*. Wadsworth
56. Carins Murphy, M.R. *et al.* (2014) Acclimation to humidity modifies the link between leaf size and the density of veins and stomata. *Plant Cell Environ.* 37, 124–131
57. Carins Murphy, M.R. *et al.* (2016) Cell expansion not cell differentiation predominantly co-ordinates veins and stomata within and among herbs and woody angiosperms grown under sun and shade. *Ann. Bot.* 118, 1127–1138
58. Carins Murphy, M.R. *et al.* (2017) Ferns are less dependent on passive dilution by cell expansion to coordinate leaf vein and stomatal spacing than angiosperms. *PLoS One* 12, e0185648
59. de Boer, H.J. *et al.* (2016) Optimal allocation of leaf epidermal area for gas exchange. *New Phytol.* 210, 1219–1228
60. Brodribb, T.J. *et al.* (2013) Unified changes in cell size permit coordinated leaf evolution. *New Phytol.* 199, 559–570
61. Feild, T.S. and Brodribb, T.J. (2013) Hydraulic tuning of vein cell microstructure in the evolution of angiosperm venation networks. *New Phytol.* 199, 720–726
62. Simonin, K.A. and Roddy, A.B. (2018) Genome downsizing, physiological novelty, and the global dominance of flowering plants. *PLoS Biol.* 16, e2003706
63. Ren, T. *et al.* (2019) Prospects for enhancing leaf photosynthetic capacity by manipulating mesophyll cell morphology. *J. Exp. Bot.* 70, 1153–1165
64. Tholen, D. *et al.* (2008) The chloroplast avoidance response decreases internal conductance to CO_2 diffusion in *Arabidopsis thaliana* leaves. *Plant Cell Environ.* 31, 1688–1700
65. Carriquí, M. *et al.* (2019) Leaf anatomy does not explain apparent short-term responses of mesophyll conductance to light and CO_2 in tobacco. *Physiol. Plantarum.* 165, 604–618
66. Shrestha, A. *et al.* (2019) The temperature response of mesophyll conductance, and its component conductances, varies between species and genotypes. *Photosynth. Res.* 141, 65–82

67. Kwon, H.K. *et al.* (2005) A proteomic approach to apoplastic proteins involved in cell wall regeneration in protoplasts of *Arabidopsis* suspension-cultured cells. *Plant Cell Physiol.* 46, 843–857
68. Moore, J.P. *et al.* (2008) Adaptations of higher plant cell walls to water loss: drought vs desiccation. *Physiol. Plant.* 134, 237–245
69. Bellincampi, D. *et al.* (2014) Plant cell wall dynamics and wall-related susceptibility in plant–pathogen interactions. *Front. Plant Sci.* 228, 1–8
70. Le Gall, H. *et al.* (2015) Cell wall metabolism in response to abiotic stress. *Plants* 4, 112–166
71. Tenhaken, R. (2015) Cell wall remodeling under abiotic stress. *Front. Plant Sci.* 5, 771
72. Carpita, N.C. *et al.* (1979) Determination of the pore size of cell walls of living plants. *Science* 205, 1144–1147
73. Read, S.M. and Bacic, A. (1996) Cell wall porosity and its determination. In *Modern Methods for Plant Analysis*In (Vol. 17) (Linskins, H.F. and Jackson, J.F., eds), pp. 63–80, Springer
74. Evans, J.R. *et al.* (2009) Resistances along the CO_2 diffusion pathway inside leaves. *J. Exp. Bot.* 60, 2235–2248
75. Houston, K. *et al.* (2016) The plant cell wall: a complex and dynamic structure as revealed by the responses of genes under stress conditions. *Front. Plant Sci.* 7, 984
76. Pignochi, C. and Foyer, C. (2003) Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. *Curr. Opin. Plant Biol.* 6, 379–389
77. Popper, Z.A. and Fry, S.C. (2004) Primary cell wall composition of pteridophytes and spermatophytes. *New Phytol.* 164, 165–174
78. Sarkar, P. *et al.* (2009) Plant cell walls throughout evolution: towards a molecular understanding of their design principles. *J. Exp. Bot.* 60, 3615–3635
79. Ellsworth, P.V. *et al.* (2018) Cell wall properties in *Oryza sativa* influence mesophyll CO_2 conductance. *New Phytol.* 219, 66–76
80. Wu, Y. and Cosgrove, D.J. (2000) Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. *J. Exp. Bot.* 51, 1543–1553
81. Choi, J.Y. *et al.* (2011) Constitutive expression of CaXTH3, a hot pepper xyloglucan endotransglucosylase/hydrolase, enhanced tolerance to salt and drought stresses without phenotypic defects in tomato plants (*Solanum lycopersicum* cv. Dotaerang). *Plant Cell Rep.* 30, 867–877
82. Mouden, S. *et al.* (2017) Towards eco-friendly crop protection: natural deep eutectic solvents and defensive secondary metabolites. *Phytochem. Rev.* 16, 935–951
83. Sumbele, S. *et al.* (2012) Photosynthetic capacity is negatively correlated with the concentration of leaf phenolic compounds across a range of different species. *AoB Plants* 2012, pls025
84. Nadal, M. and Flexas, J. (2019) Variation in photosynthetic characteristics with growth form in a water-limited scenario: implications for assimilation rates and water use efficiency in crops. *Agr. Water Manage.* 216, 457–472
85. McElwain, J.C. *et al.* (2016) Using modern plant trait relationships between observed and theoretical maximum stomatal conductance and vein density to examine patterns of plant macroevolution. *New Phytol.* 209, 94–103
86. Brodribb, T.J. *et al.* (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiol.* 144, 1890–1898
87. Flexas, J. *et al.* (2013) Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. *J. Exp. Bot.* 64, 3965–3981
88. Flexas, J. (2016) Genetic improvement of leaf photosynthesis in intrinsic water use efficiency in C3 plants: why so much little success? *Plant Sci.* 251, 155–161
89. Ort, D.R. *et al.* (2015) Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proc. Natl. Acad. Sci. U. S. A.* 112, 8529–8536
90. Escalona, J.M. *et al.* (2003) Distribution of leaf photosynthesis and transpiration within grapevine canopies under different drought conditions. *Vitis* 42, 57–64
91. Flexas, J. *et al.* (2016) Mesophyll conductance to CO_2 and Rubisco as targets for improving intrinsic water use efficiency in C_3 plants. *Plant Cell Environ.* 39, 965–982
92. Granot, D. and Kelly, G. (2019) Evolution of guard-cell theories: the story of sugars. *Trends Plant Sci.* 24, 507–518
93. Lin, M.T. *et al.* (2014) A faster Rubisco with potential to increase photosynthesis in crops. *Nature* 513, 547–550
94. Whitney, S.M. *et al.* (2015) Improving recombinant Rubisco biogenesis, plant photosynthesis and growth by coexpressing its ancillary RAF1 chaperone. *Proc. Natl. Acad. Sci. U. S. A.* 112, 3564–3569
95. South, P.F. *et al.* (2019) Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. *Science* 363, 6422
96. Lehmeier, C. *et al.* (2017) Cell density and airspace patterning in the leaf can be manipulated to increase leaf photosynthetic capacity. *Plant J.* 92, 981–994
97. Yang, Z. *et al.* (2016) Leveraging abscisic acid receptors for efficient water use in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 113, 6791–6796
98. Han, J. *et al.* (2018) Mesophyll conductance in cotton bracts: anatomically determined internal CO_2 diffusion constraints on photosynthesis. *J. Exp. Bot.* 69, 5433–5443
99. Nadal, M. *et al.* (2018) Possible link between photosynthesis and leaf modulus of elasticity among vascular plants: a new player in leaf traits relationships? *Ecol. Lett.* 21, 1372–1379
100. Peguero-Pina, J.J. *et al.* (2017) Ancient cell structural traits and photosynthesis in today's environment. *J. Exp. Bot.* 68, 1389–1392
101. Kaiser, E. *et al.* (2018) Fluctuating light takes crop photosynthesis on a rollercoaster ride. *Plant Physiol.* 176, 977–989
102. Koester, R.P. *et al.* (2016) Has photosynthetic capacity increased with 80 years of soybean breeding? An examination of historical soybean cultivars. *Plant Cell Environ.* 39, 1058–1067
103. Kromdijk, J. *et al.* (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science* 354, 857–886
104. Papanatsiou, M. *et al.* (2019) Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth. *Science* 363, 1456–1459