Letter

Cell wall composition in relation to photosynthesis across land plants' phylogeny: crops as outliers

Introduction and context

The role of cell wall composition in photosynthesis has only recently been proposed. Apparently contradictory results have been reported, but previous studies were often limited to single or closely related species. The aim of the present letter is to combine published and novel data on cell wall composition and photosynthesis limitations, including data for all the major land plant's phylogenetic groups (Supporting Information Methods S1; Dataset S1), to provide novel evidence on the importance of cell wall composition in determining mesophyll conductance to CO_2 diffusion (g_m) across land plants' phylogeny. We address the hypothesis that the pectin fraction of total major cell wall compounds is positively related to g_m and, consequently, to photosynthesis, when pooling species from across the entire phylogeny.

The role of leaf functional traits determining photosynthesis

The *leaf economics spectrum* (LES) represents a complex framework of interconnected leaf traits related to carbon fixation and nutrient use across plant lineages (Wright *et al.*, 2004), with net CO assimilation (A_N) and leaf mass per area (LMA) as key traits (Onoda *et al.*, 2017). Generally, LMA varies among plant species and negatively correlates with physiological traits such as net assimilation (A_N) at a phylogenetic scale (Onoda *et al.*, 2017). Similarly, lower LMA is linked with enhanced mesophyll conductance to CO diffusion (g_m) (Onoda *et al.*, 2017). This parameter, encompassing the CO_2 pathway from substomatal cavities to Rubisco carboxylation sites in chloroplasts stroma, is recognized as a key trait regulating photosynthesis (Flexas *et al.*, 2012, 2018). However, the mechanistic regulation of g_m is not fully understood (Evans *et al.*, 2009; Flexas *et al.*, 2012, 2018; Mizokami *et al.*, 2022).

In addition to LMA, anatomical properties such as the chloroplast surface area exposed to intercellular air spaces per unit leaf area (S_c/S) and cell wall thickness (T_{cw})—often assumed to be positively linked with increased LMA—strongly influence g_m across different plant lineages (Tosens *et al.*, 2016; Peguero-Pina *et al.*, 2017; Veromann-Jurgenson *et al.*, 2017, 2020; Carriquí

et al., 2019; Gago *et al.*, 2019; Flexas & Carriquí, 2020). Thick cell walls limit g_m, suggesting a phylogenetic trend towards increasing g_m by reducing T_{cw} from mosses to angiosperms (Gago *et al.*, 2019; Flexas & Carriquí, 2020). However, while the cell wall conductance to CO_2 (g_{cw}) strongly influences g_m and, consequently, photosynthesis, T_{cw}–representing the apparent CO_2 diffusion path length across the cell wall (ΔL_{cw})—is just one component. The other components are diffusivity (D), which is a function of leaf temperature, cell wall porosity (p_{cw}), and cell wall tortuosity (τ_{cw}) (Evans *et al.*, 2009; Terashima *et al.*, 2011; Flexas *et al.*, 2021):

$$g_{cw} = \frac{D \times p_{cw}}{\Delta L_{cw} \times \tau_{cw}} \qquad \text{Eqn 1}$$

Although leaf temperature is unrelated to cell wall, porosity and tortuosity are theoretically influenced by cell wall composition, and even T_{cw} itself could be indirectly linked to it.

Recently, cell wall compositional traits were shown to be involved in g_m (Ellsworth *et al.*, 2018; Clemente-Moreno *et al.*, 2019; Carriquí *et al.*, 2020; Roig-Oliver *et al.*, 2020a,c, 2021a,b,c; Salesse-Smith *et al.*, 2024). In Flexas *et al.* (2021), we proposed several mechanisms through which cell wall composition might influence g_m, which can be summarized as follows. Although the physical porosity of cell walls is not likely to be a significant barrier for CO_2 diffusion, given that CO_2 molecules are much smaller than the typical pore sizes (Carpita *et al.*, 1979), pectins exhibit hydrocolloid properties that allow them to absorb and retain large amounts of water. Since CO_2 diffuses through aqueous solutions, the presence of hydrophilic regions within the pores could significantly affect its diffusion rate.

Although specific land plants lineages possess specific cell wall compositional characteristics (Sarkar *et al.*, 2009; Sørensen & Willats, 2010; Popper *et al.*, 2011), the cell wall is a three-dimensional framework mainly composed by cellulose microfibrils crosslinked to noncellulosic polysaccharides (hemicelluloses), all embedded within a pectin matrix (Somerville *et al.*, 2004; Cosgrove, 2005; Anderson & Kieber, 2020). This pectin network is believed to be a key structure determining several cell wall properties that could potentially affect CO_2 diffusion, such as porosity, thickness and elasticity (Cosgrove, 2005; Ochoa-Villareal *et al.*, 2012; Schiraldi *et al.*, 2012; Weraduwage *et al.*, 2016; Novakovic *et al.*, 2018; Carriquí *et al.*, 2020; Roig-Oliver *et al.*, 2020a,c, 2021b, 2022; Flexas *et al.*, 2021). For instance, a remarkable correlation was found between A_N and/or g_m and the bulk modulus of elasticity (ε) when pooling together species from different growth forms and phylogenetic groups along vascular plants (Nadal *et al.*, 2018). In fact, recent studies provided empirical relationships between variations in pectin concentration and adjustments in T_{cw} and ε itself. However, these studies were

performed either testing one or few species (Teng *et al.*, 2006; Clemente-Moreno *et al.*, 2019; Roig-Oliver *et al.*, 2020a,c, 2021a, c) or varieties (Ellsworth *et al.*, 2018; Roig-Oliver *et al.*, 2021d, 2022) of angiosperms responding to environmental stresses or having specific genetic modifications, or being comparisons of nonstressed species within the same phylogenetic group (Carriquí *et al.*, 2020; Roig-Oliver *et al.*, 2021b). These studies suggested that either pectin concentration or the proportion between different cell wall components (i.e. the pectin to hemicelluloses and cellulose ratio) are crucial regulators of g_m and other leaf physiological traits, but in a variable manner (Carriquí *et al.*, 2020; Roig-Oliver *et al.*, 2020a, 2022).

To our knowledge, no previous study has addressed the implication of cell wall composition influencing both g_m and T_{cw} along land plants' lineages. Thus, our aims were (1) to perform a meta-analysis using both literature and newly measured species in which cell wall composition, photosynthetic and leaf anatomical properties across species spanning from mosses to angiosperms are considered; and (2) to explore how cell wall compositional traits influence leaf anatomy and photosynthesis, being T_{cw}, cell wall porosity and g_m key traits. Our main hypothesis is that cell wall composition and, particularly, the fraction of pectins over the other two major components (cellulose + hemicellulose)—as a proxy of cell wall porosity (and, perhaps, tortuosity)—contributes to determining g_m variations along land plants' lineages.

Variation along land plants' phylogeny

Despite the modest number of species considered in our dataset, it still covers 70% or more of the published ranges (excluding the first and last decile) for each trait and phylogenetic group in 75% of the cases (Fig. S1). This comprehensive coverage ensures that our findings are representative and robust.

The exploration of the variation in structural and physiological traits, as well as those related to cell wall composition, across several land plant groups reveals significant insights (Figs 1, S2). Contrary to common assumptions, our findings suggest that there is no dependence of T_{cw} on LMA across phylogenetic scales (Figs 1a,b, S2). Instead, the patterns for A_N and g_m show remarkable similarities across different plant lineages, as already highlighted by Gago *et al.* (2019) and Huang *et al.* (2022) (Fig. 1c,d). This observation underscores the notion that limitations imposed by g_m are dominant throughout land plants' phylogeny (Gago *et al.*, 2019).

It has been described that LMA scales positively with T_{cw} in vascular plants (Onoda *et al.*, 2017). The data included here support this view except for mosses (Fig. S2). This finding emphasizes the complexity of plant adaptations, revealing that factors influencing T_{cw} do not necessarily correlate with leaf mass. Instead, the inverse but strong phylogenetic patterns observed in T_{cw} compared to A_N and g_m indicate that these structural and physiological traits are more closely linked, even when mosses are included. Moreover, our analysis shows that there are no major differences among groups in main cell wall compounds contents or in the pectin to hemicellulose and cellulose ratio: intra-group variations appear to be more pronounced than inter-group

variations (Fig. 1e–h). The same applies to the leaf cell wall fraction considered as alcohol-insoluble residues (AIR) (Fig. S3). Interestingly, hemicellulose and cellulose contents correlate independently of pectin content, which further reinforces our findings. This is analogous to the well-documented relationship between LMA and T_{cw} in vascular plants. While this correlation has been thoroughly substantiated and its mechanistic basis is well understood (Onoda *et al.*, 2017; Wuyun *et al.*, 2024), our data show that mosses exhibit a distinct pattern (Fig. S2).

Dependency of photosynthesis on cell wall composition

Contrary to expectations based on Fig. 1, the analysis of the relationships between cell wall composition and g_m shown in Fig. 2 reveals several important insights, particularly when distinguishing between crop and noncrop species. As a first approach, we applied both linear (lm) and nonlineal least square (nls) models to assess correlations, choosing for each pair of parameters the function with the lowest Akaike Information Criterion (AIC). However, to account for potential phylogenetical differences in these relationships, we explored alternative options. One option involved applying robust regressions for each phylogenetic group, following Knauer *et al.* (2022) using the ROBUSTBASE R package (Maechler *et al.*, 2022). However, due to low-sample sizes within each group, the results of this method did not significantly improve those obtained with standard lm and nls models. Indeed, Knauer *et al.* (2022) specifically recommend testing relationships only when the number of measurements is ≥ 12, making further exploration of this approach impractical with our dataset. Another approach was to consider linear (lme) and nonlinear (nlme) mixed-effects models, incorporating phylogenetic group as a random factor. In this case, we performed the same pairwise correlations using both lm/nls and lme/nlme and applied ANOVA to discern which model performed better. Additionally, we evaluated the goodness-of-fit metrics for the most suitable statistical model (Table S1). In the nonlinear correlations, nls models performed better than nlme models – for which they are used in Fig. 2(a) – while in the linear correlations lme models performed better than lm models (Fig. 2b,c), indicating a significant phylogenetic effect in these relationships. The different plots in Fig. 2 show the correlations based on the best performance model, that is nls for nonlinear correlations and lme for linear correlations.

As already shown (Evans, 2021; Flexas *et al.*, 2021), g_m correlates negatively with T_{cw}, which is a proxy for the effective path length across the cell wall, following an exponential decay function $\left(g_m = a \times e^{-b \times T_{cw}}\right)$, as determined by selecting the model with the lowest AIC (Fig. 2a). Additionally, a significant positive linear correlation emerges between g_m and the pectin-to-(cellulose + hemicelluloses) fraction (P/(C + H)), a proposed proxy for porosity and tortuosity (Carriquí *et al.*, 2020; Flexas *et al.*, 2021), thus confirming our hypothesis (Fig. 2b). When analyzing all species together, the relationship had a marginal r_m^2 of 0.06, but a conditional r_c^2 of 0.60, indicating a significant phylogenetic influence. Importantly, the strength of this correlation increased when crops were excluded ($r_m^2 = 0.18$, $r_c^2 = 0.51$), and it improved
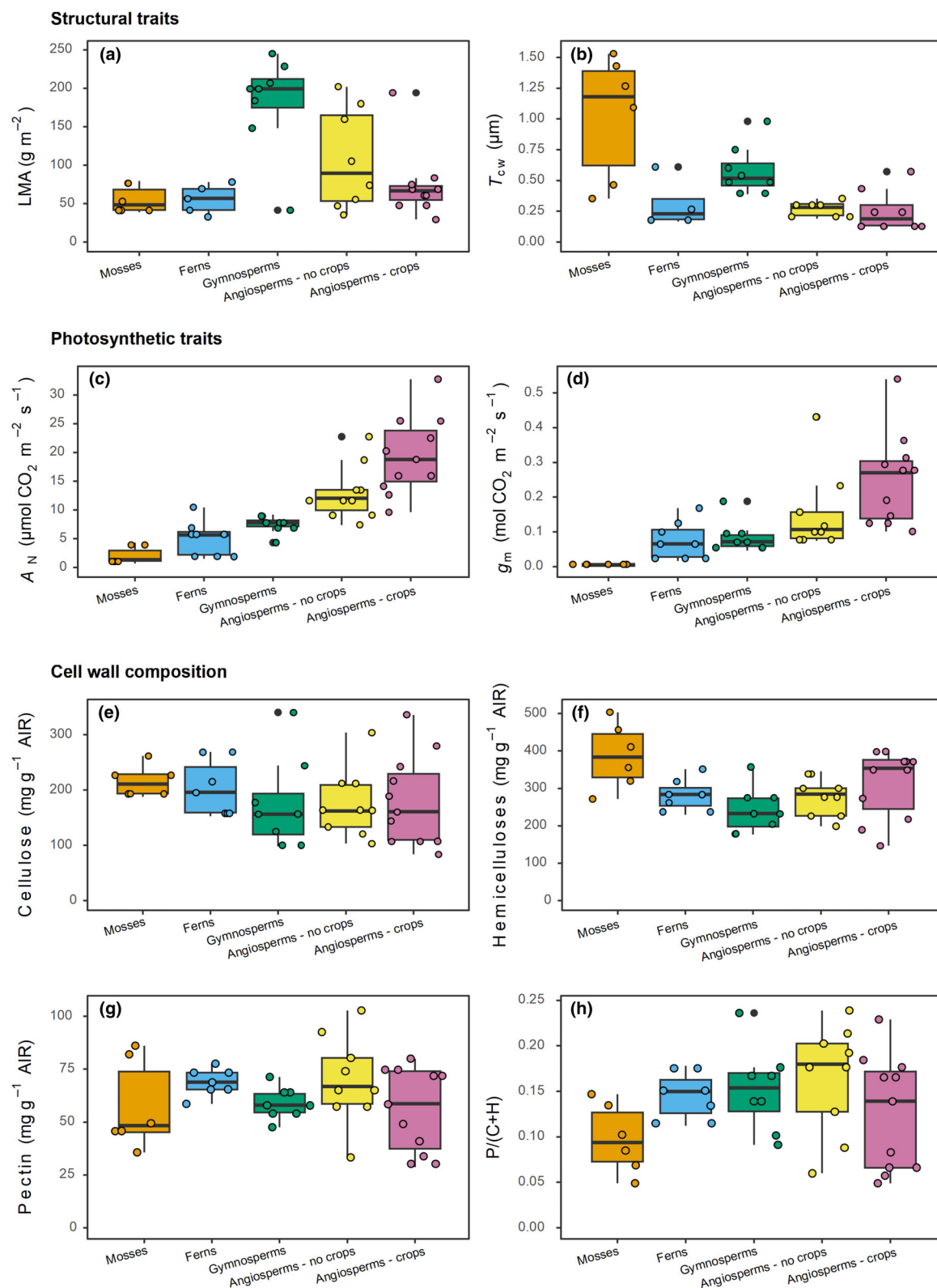
Fig. 1 Phylogenetic trends in structural and physiological traits and in cell wall composition. Dot boxplots showing (a) leaf dry mass per unit area (LMA), (b) cell wall thickness (T_{cw}), (c) net CO_2 assimilation (A_N), (d) mesophyll conductance (g_m), (e) cellulose, (f) hemicelluloses, (g) pectin, and (h) pectin to cellulose and hemicellulose ratio (P/(C + H)) for mosses ($n = 6$), ferns ($n = 8$), gymnosperms ($n = 8$), wild angiosperms ($n = 10$), and angiosperm crops ($n = 11$). Boxplots display the median (horizontal line), interquartile range (box) and whiskers extending to 1.5 times the interquartile range. Outliers are shown as individual black points. Data were compiled from Clemente-Moreno *et al*. (2019), Carriquí *et al*. (2020), Nadal *et al*. (2020, 2023), Roig-Oliver *et al*. (2020b,c, 2021b,c, 2022), and from newly measured species.
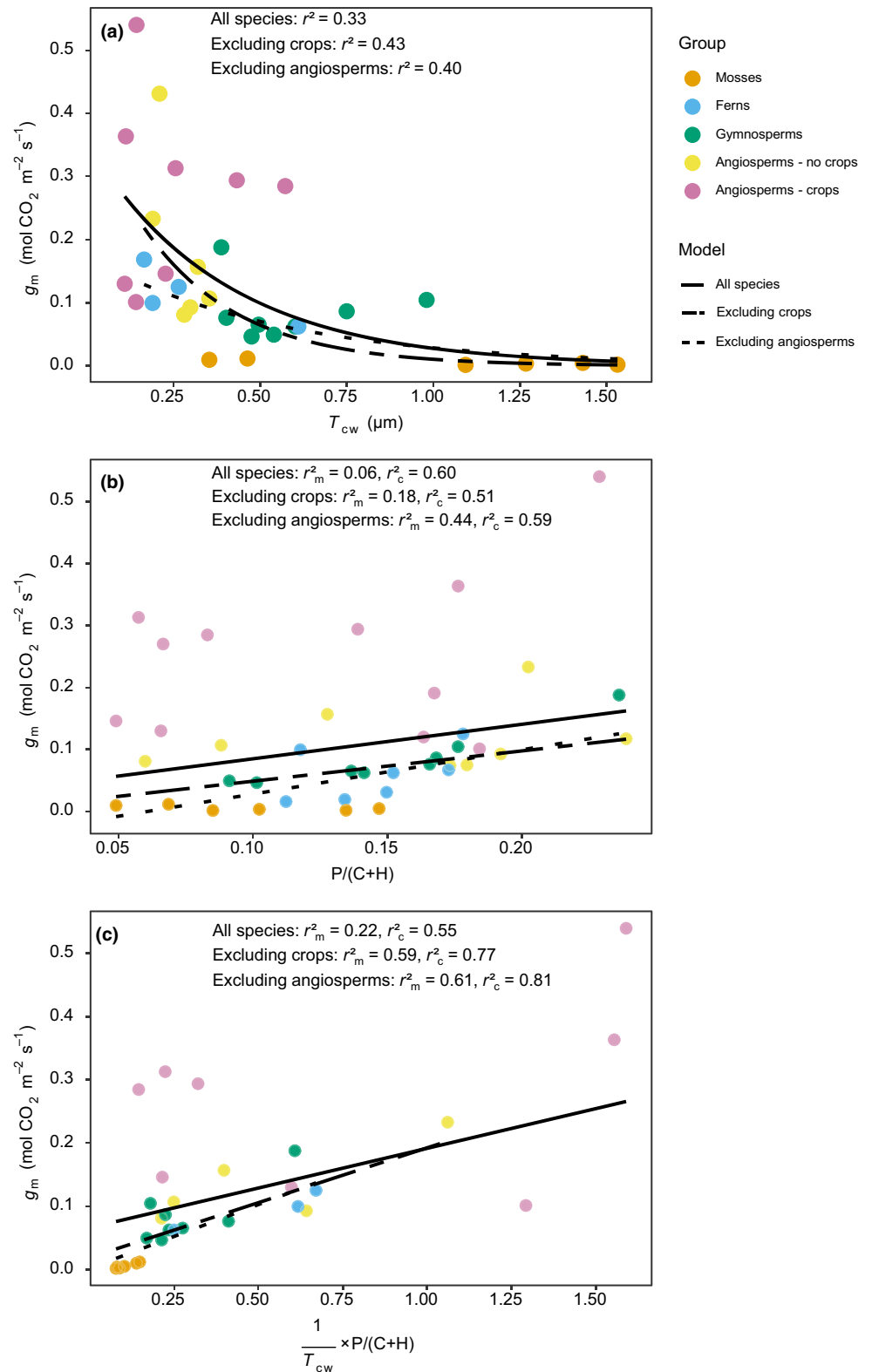
Fig. 2 Relationships between mesophyll conductance (g_m) and (a) cell wall thickness (T_{cw}), (b) pectin to hemicellulose and cellulose ratio (P/(C + H)), and (c) $1/T_{cw} \times$ P/(C + H). Regression lines are based on model predictions (see Supporting Information Table S1) between g_m and cell wall traits. Lines represent exponential decay or linear fittings on data pooled across all phylogenetic groups, excluding crops and excluding all angiosperms. Significant lines in (a) are exponential decay regression lines, and in (b, c) linear mixed-effects models (*lme*) with phylogenetic group as a random effect. Model fits and coefficients of determination (r^2) in (a) and conditional (r_c^2) and marginal (r_m^2) coefficients of determination for mixed-effects models in (b) and (c) are shown where model P-values are < 0.05. Data were compiled from Clemente-Moreno *et al.* (2019), Carriquí *et al.* (2020), Nadal *et al.* (2020, 2023), Roig-Oliver *et al.* (2020b,c, 2021b,c, 2022), and from newly measured species.

further when noncrop angiosperms were also removed ($r_m^2 = 0.44$, $r_c^2 = 0.59$). These results highlight the influence of phylogenetic and functional group-specific effects on the observed relationship. Notably, a much stronger positive correlation between g_m and the ratio of P/(C + H) to T_{cw} was found (Fig. 2c), indicating the combined importance of path length – T_{cw} – and porosity/tortuosity – P/(C + H). This finding supports the notion that both factors – path length and porosity/tortuosity – affect g_m. When

analyzing all species, the r_m^2 was 0.22, and the conditional r_c^2 was 0.55, reflecting moderate explanatory power with notable phylogenetic effects. Again, the relationship in Fig. 2c is especially pronounced when crop species are excluded from our dataset ($r_m^2 = 0.59$, $r_c^2 = 0.77$) but not significantly further improved when noncrop angiosperms were also removed ($r_m^2 = 0.61$, $r_c^2 = 0.81$).

In noncrop species, the pectin fraction in relation to cellulose and hemicellulose plays a crucial role in determining how easily CO can diffuse through the leaf mesophyll, likely because it affects cell wall porosity and tortuosity (Flexas *et al.*, 2021). Conversely, the inclusion of crop species introduces a different dynamic. Please notice that the effect of excluding crops is persistent irrespective of considering or not phylogenetic effects, that is whenever pairwise correlations are better explained by lm/nls or lme/nlme considering the phylogenetic group as a random factor. Crops have indirectly been selectively bred for enhanced photosynthetic efficiency and other agronomically valuable traits (Nadal & Flexas, 2019), which is the consequence of these plants having been derived from wild species with naturally high-photosynthetic capacities (Gomez-Fernandez *et al.*, 2024). They often possess very thin cell walls, which might have weakened the correlation between $P/(C + H)$ and g_m because they are optimized for rapid growth and high yield rather than for natural selection pressures influencing photosynthetic efficiency (Xiong, 2023). Consequently, as shown in Fig. 2 (b), crops can achieve a large g_m despite not necessarily showing a large $P/(C + H)$ ratio. This does not mean that cell wall thickness and/or composition does not affect g_m in crops. Indeed, improved photosynthesis and yield has been recently achieved by genetically manipulating cell walls (Salesse-Smith *et al.*, 2024), and empirical relationships between g_m and cell wall composition have been shown in different studies subjecting crops to stress conditions (Roig-Oliver *et al.*, 2020a,c, 2021a, 2022). However, it implies that the role in crops is lesser than in wild species and/or that crops may have compensating mechanisms for facilitating CO_2 diffusion. Currently, we do not know what these compensatory mechanisms might be. However, it has been suggested that certain aquaporins may play a role in CO_2 diffusion, as seen in studies on broad bean, common bean, rice and tobacco (Terashima & Ono, 2002; Hanba *et al.*, 2004; Flexas *et al.*, 2006). Let's hypothesize that this role was acquired relatively recently and only in specific crops. This idea aligns with findings indicating that aquaporins do not appear to have the same role in Arabidopsis (Kromdijk *et al.*, 2020). If this is indeed the case, an increased CO_2 diffusion through the plasma membrane – and potentially through chloroplasts (Uehlein *et al.*, 2008)—could enhance g_m, despite potential disentangling an impediment of the cell wall composition vs CO_2 diffusion. This scenario is analogous to the relationship between LMA and T_{cw}. While this relationship is well-substantiated and its mechanistic basis is thoroughly understood (Onoda *et al.*, 2017; Wuyun *et al.*, 2024), our study reveals that mosses behave differently. Although this hypothesis is highly speculative, it highlights the critical need for further research in this area. The impact of crop selection on cell wall composition underscores the necessity of focusing on noncrop species to better understand the fundamental connections between cell wall components and photosynthetic traits.

From Eqn 1 the CO conductance across cell walls should depend on ΔL_{cw} and p_{cw}/τ_{cw} as the mixed variable, here approached as $1/T_{cw} \times P/(C + H)$. In fact, the correlation of this parameter with g_m across different plant groups becomes notably more significant, with all species having an $r_m^2 = 0.22$, $r_c^2 = 0.55$, excluding angiosperms $r_m^2 = 0.59$, $r_c^2 = 0.77$, and excluding crops $r_m^2 = 0.61$, $r_c^2 = 0.81$. These results suggest that this combined variable significantly explains the variations in g_m (Fig. 2c), and emphasizes the relevant role that the pectin fraction plays in regulating g_m and, consequently, A_N, in wild, nondomesticated plants. To fully comprehend this dependency, it is essential to explore how pectins influence various aspects of cell wall properties – such as porosity and thickness – that affect gas exchange. While the actual physical porosity should not be a major limitation given that CO molecules are small compared to cell wall pore sizes (Carpita *et al.*, 1979; Flexas *et al.*, 2021), pectins possess hydrocolloid characteristics and can retain several times their volume in water. Since CO diffuses in solution, the hydrophilic fraction of the pores could determine its diffusion (Flexas *et al.*, 2021). Moreover, pectins can be under different methyl-esterification states and may interact with other chemical cell wall compounds, altogether affecting the 'effective' porosity and, perhaps, tortuosity (Flexas *et al.*, 2021). While cell walls are often considered rigid and fixed structures, instead they are dynamic structures that continuously respond to various stimuli in a regulated manner (Forand *et al.*, 2022; Delmer *et al.*, 2024). Beyond these rough approximations, the relationship between these parameters is likely far more complex. For instance, as shown in Fig. 2, mosses display a unique pattern: despite significant variations in T_{cw} and $P/(C + H)$, they all maintain similarly low g_m values. Interestingly, species with higher T_{cw} tend to compensate with a higher $P/(C + H)$ ratio, while those with lower $P/(C + H)$ appear to offset it with reduced T_{cw}. This compensatory mechanism could indicate unique adaptations in mosses, suggesting an area that warrants further investigation. By focusing on these fundamental connections, we can better direct efforts to improve photosynthesis in species with relatively thick cell walls and enhance their physiological performance under contrasting environmental conditions.

Concluding remarks

This is the very first report to show general relationships by considering species spanning the entire phylogeny of land plants. In doing so, we identify a clear biochemical basis – one that can be traced back to specific genes – for a large component of mesophyll conductance and, thus, photosynthetic capacity. It opens new avenues for improving the photosynthesis of terrestrial plants. Additionally, it suggests that current crops are already optimized and even uncoupled from these general relationships, raising questions about the regulation of g_m in crop species.

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Competing interests

None declared.

Author contributions

MC, MR-O and JF designed the research. MR-O measured the new studies presented in this study. MC performed the data analysis. MC, MR-O and JF wrote the manuscript, with contributions from MJC-M. All authors revised and approved the manuscript.

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Data availability

All data are available in the Supporting Information (Dataset S1).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Data used in this study.

Fig. S1 Heatmap of percentage coverage of the published range (excluding the first and last decile) for each trait and phylogenetic group.

Fig. S2 Spearman correlation matrices for pairwise correlations among selected variables for all species or vascular plant species.

Fig. S3 Dot boxplots of dry mass-based cellulose fraction of alcohol-insoluble residues across land plant groups.

Methods S1 Methods used in this study.

Table S1 Goodness-of-fit metrics for statistical models shown in Fig. 2.

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