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Optimal stomatal theory predicts CO₂ responses of stomatal conductance in both gymnosperm and angiosperm trees

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Summary

- Optimal stomatal theory predicts that stomata operate to maximise photosynthesis (A_net) and minimise transpirational water loss to achieve optimal intrinsic water-use efficiency (iWUE). We tested whether this theory can predict stomatal responses to elevated atmospheric CO₂ (eCO₂), and whether it can capture differences in responsiveness among woody plant functional types (PFTs).
- We conducted a meta-analysis of tree studies of the effect of eCO₂ on iWUE and its components A_net and stomatal conductance (g_s). We compared three PFTs, using the unified stomatal optimisation (USO) model to account for confounding effects of leaf–air vapour pressure difference (D). We expected smaller g_s, but greater A_net, responses to eCO₂ in gymnosperms compared with angiosperm PFTs.
- We found that iWUE increased in proportion to increasing eCO₂ in all PFTs, and that increases in A_net had stronger effects than reductions in g_s. The USO model correctly captured stomatal behaviour with eCO₂ across most datasets. The chief difference among PFTs was a lower stomatal slope parameter (g_s) for the gymnosperm, compared with angiosperm, species.
- Land surface models can use the USO model to describe stomatal behaviour under changing atmospheric CO₂ conditions.

Introduction

Stomata are small pores in vascular plant leaves that open and close to allow the passive exchange of gases between the atmosphere and a plant’s internal surfaces. It has long been recognised that stomatal behaviour represents a fundamental compromise between carbon dioxide (CO₂) diffusion into the plant and simultaneous water loss (Cowan, 1982; Jones & Sutherland, 1991) such that maintaining stomatal opening, to allow CO₂ to diffuse into the sub-stomatal cavity, implies substantial diffusive water loss via transpiration. Stomatal opening is sensitive to both endogenous and external drivers (Brodrribb et al., 2009). Predicting stomatal responses to the environment is crucial as stomatal aperture determines both photosynthetic carbon gain and the rate of water loss in plants (Cowan & Farquhar, 1977), thus affecting intrinsic water-use efficiency (iWUE; the ratio of net photosynthetic CO₂ assimilation, A_net, to stomatal conductance to water vapour, g_s). Understanding the magnitude and drivers of iWUE in our changing climate is key to modelling and predicting global carbon and water cycles under a future climate (Walker et al., 2020).

Atmospheric CO₂ concentration (C_a) directly affects stomatal aperture (Morison, 1987; Franks et al., 2012). For example, Medlyn et al. (2001) reported that g_s was reduced by an average...
of 21% in European forest trees with exposure to elevated CO₂ (eCO₂: +350 μmol mol⁻¹) across four types of CO₂ exposure facilities. Similarly, Ainsworth & Rogers (2007) reported sₕ was reduced by an average of 19% in response to eCO₂ (+208 μmol mol⁻¹) in tree species growing in free-air CO₂ enrichment (FACE) experiments. This reduction in water use with rising CO₂ could have large-scale consequences, including increased resilience of vegetation to drought (Jiang et al., 2021) and global increases in streamflow (Gedney et al., 2006) contributing to increased greening in water-limited regions (Keenan & Williams, 2018).

However, not all species respond to eCO₂ to the same extent (Saxe et al., 1998), and there are species-specific stomatal responses to eCO₂ that are apparent among eCO₂ experiments (Saxe et al., 1998; Medlyn et al., 2001). For tree species, it has been observed that eCO₂-induced reductions in sₕ are small or absent in gymnosperm species especially when compared with those in angiosperm species (Saxe et al., 1998; Medlyn et al., 2001; Klein & Ramon, 2019), which suggests that stomatal sensitivity to eCO₂ may vary with phylogeny (Brodrrib et al., 2009; Lammutsera et al., 2011; Hasper et al., 2017). For example, in a meta-analysis of field-based chamber experiments on forest trees, Medlyn et al. (2001) found that the C₄ response of sₕ was significantly stronger in deciduous broadleaved angiosperm trees than in coniferous species (all gymnosperms), in the set of experiments examined, although they also noted that this difference was confounded with tree age. Recently, a meta-analysis conducted by Klein & Ramon (2019) also found a significant difference in stomatal responses to eCO₂ between gymnosperm and angiosperm species. In addition to sₕ responses in isolation, there are also indications that iWUE (Aₙet/gₙ) may respond differentially to rising CO₂ between angiosperm and gymnosperm species. For example, it has been suggested that iWUE in gymnosperms may have a stronger positive response to eCO₂, compared with angiosperms, as a result of differences in leaf structure (Niinemets et al., 2011).

If there are indeed differences between angiosperm and gymnosperm, or more specifically between plant functional types (PFTs), in their stomatal responses to rising CO₂, then it is important to capture these differences in vegetation models. Many vegetation models still employ a stomatal model of the form proposed by Ball et al. (1987), which was shown by Medlyn et al. (2011) to be consistent with the optimal stomatal theory put forward by Cowan & Farquhar (1977). This theory hypothesises that stomata behave in a manner that maximises CO₂ fixation through photosynthesis while minimising water loss via transpiration, by maximising the expression, Aₙet – λE, where Aₙet is photosynthesis (μmol CO₂ m⁻² s⁻¹),  E is transpiration (mol H₂O m⁻² s⁻¹), and λ (μmol CO₂ mol⁻¹ H₂O) represents the marginal ‘carbon cost of water use’ to the plant (cf. Cowan & Farquhar, 1977). That is, the optimal stomatal conductance is that which maximises

\[ \max(A_{net} - \lambda E) \]  

Eqn 1

Medlyn et al. (2011) showed that this theory, when coupled to a biochemical model of photosynthesis (Farquhar & von Caemmerer, 1982), results in an expression for gₙ (mol H₂O m⁻² leaf s⁻¹) that can be approximated as follows, also known as the unified stomatal optimisation (USO) model:

\[ g_{n} \approx 1.6 \left(1 + \frac{g_{1}}{\sqrt{D}}\right) \frac{A_{net}}{C_{a}} \]  

Eqn 2

where \( g_{1} \) (kPa 0.5) is a collection of constant terms including an inverse dependence on \( \lambda \), \( D \) is the leaf–air vapour pressure difference (kPa), 1.6 is the apparent ratio of the diffusion coefficients of water vapour and CO₂, and \( g_{1} \) and \( A_{net} \) are the functions of \( D \) and air temperature, \( T_{air} \), as well as other physiological parameters. Notably that here \( \lambda \) is defined according to Hari et al. (1986), and is the inverse of the original definition in Cowan & Farquhar (1977) and Medlyn et al. (2011). In addition, the empirical intercept term, \( g_{1} \), added by Medlyn et al. (2011) is unnecessary here and is omitted for simplicity. As the marginal cost of water \( \lambda \) is unknown, the parameter \( g_{1} \) is obtained in practice by fitting to data. Assuming the model parameter \( g_{1} \) is constant, and under environmental conditions producing constant \( D \), the USO predicts that the \( A_{net}/g_{n} \) ratio, that is the intrinsic WUE (iWUE = \( A_{net}/g_{n} \)) should be proportional to the atmospheric CO₂ concentration (Barton et al., 2012). This model has been used to predict stomatal responses successfully in several CO₂ manipulation experiments (e.g. Barton et al., 2012; De Kauwe et al., 2013; Gimeno et al., 2016).

There are several ways in which a differential response of \( g_{n} \) to \( C_{a} \) could be captured by the USO model. First, there may be a difference in the \( T_{air} \) or \( D \) at which measurements were made. The relative responsiveness of \( A_{net} \) and \( g_{n} \) can vary within an experiment depending on \( T_{air} \) or \( D \). For example, \( A_{net} \) is more responsive to eCO₂ at high leaf temperatures (Long, 1991). Additional complications may occur if \( D \) differs between treatments. Barton et al. (2012) showed that iWUE was proportional to eCO₂ in Eucalyptus saligna, once treatment differences in \( D \) were taken into account. They also showed that the responsiveness of \( g_{n} \) to eCO₂ decreased at high \( D \), when high \( T_{air} \) meant that photosynthesis was more responsive for biochemical reasons (Long, 1991). These mechanisms will be captured in the USO model, which incorporates an effect of \( D \), especially when coupled to a photosynthetic biochemical model that captures interactions with \( T_{air} \) and \( C_{a} \) (e.g. Duursma, 2015).

Second, a substantial increase in iWUE in response to eCO₂ can occur as a strong increase in \( A_{net} \), with a marginal reduction in \( g_{n} \), or a marginal increase in \( A_{net} \) and a strong reduction in \( g_{n} \). Photosynthetic responses may be stronger in species that have a low \( g_{1} \) and consequently a low intercellular CO₂. There is strong evidence that gymnosperm trees have a lower \( g_{1} \) than angiosperm trees (e.g. Lin et al., 2015), so this is a plausible mechanism for species or PFT differences. In addition, photosynthetic responses to eCO₂ are expected to be larger in species with thicker or denser leaves because thicker leaves have greater diffusive limitation of the transport of CO₂ to the sites of carboxylation, and so respond more positively to eCO₂ than species with thinner leaves (Niinemets et al., 2011). This mechanism would explain a
difference between angiosperms and gymnosperm PFTs, but would also suggest a difference in responsiveness between evergreen and deciduous angiosperms, since evergreen species typically have thicker leaves (Wright et al., 2004; Poorter et al., 2009; Kattge et al., 2011). These mechanisms would be captured in the model by specifying a different value of $g_1$ across species or PFTs.

Finally, it is also possible that the USO model does not accurately capture responses to eCO$_2$ in gymnosperm PFTs unless it is assumed that the slope parameter $g_1$ changes with treatment (i.e. $g_1$ is a function of $C_a$). For example, Uddling & Wallin (2012) found no decrease in $g_s$ at eCO$_2$ in *Picea abies*. They found that the response of iWUE was considerably less than predicted from the optimal stomatal theory (Eqn 2) and that the slope parameter $g_1$ increased from 1.45 to 2.44 (kPa$^{-0.5}$) between aCO$_2$ and eCO$_2$ treatments. This mechanism could potentially be captured in the model by specifying $g_1$ as a function of $C_a$.

In this paper, we examined the effect of eCO$_2$ on iWUE, $g_s$ and $A_{net}$ responses across a diverse set of field-based forest and tree plantation experiments of deciduous and evergreen angiosperms and evergreen gymnosperms. We focused on experiments with sufficient data available to evaluate the USO model (Eqn 2). A series of FACE and whole-tree chamber (WTC) experiments over the last two decades have contributed detailed datasets across a variety of species that include larger and older trees and ecosystem-scale experiments which provide the opportunity to re-examine stomatal responses to eCO$_2$ (Ellsworth, 1999; Gunderson et al., 2002; Bernacchi et al., 2003; Uddling et al., 2009; Bader et al., 2010, 2016; Gimeno et al., 2016). We used these data to test whether there are differences in responsiveness of $g_s$ and iWUE between different PFTs, and to utilise the optimal stomatal theory to explore the reasons for these differences. We also use the data to test a number of other hypotheses arising from optimal stomatal theory. For example, the theory implies that the parameter $g_1$ will be lowest in plants with a relatively high marginal carbon cost of water and therefore low hydraulic conductance (Medlyn et al., 2011). Previous research shows that branch-level hydraulic conductance is relatively low in gymnosperms (Becker et al., 1999), compared with angiosperms, and may decline with tree age (Hubbard et al., 1999), suggesting that gymnosperms and older trees are likely to have a relatively high marginal carbon cost of water.

We hypothesised that (1) following USO, iWUE would increase in proportion to the CO$_2$ increase similarly across all species; (2) on average, $g_s$ would decrease and $A_{net}$ would increase in response to eCO$_2$, with $A_{net}$ being the principal driver for increased iWUE; (3) the magnitude of change in $A_{net}$ to eCO$_2$ would depend on $D$, with the response of $A_{net}$ to eCO$_2$ larger at high $D$ and (4) the magnitude of change in both $A_{net}$ and $g_s$ to eCO$_2$ would depend on PFT, with $A_{net}$ being more responsive and $g_s$ being less responsive in evergreen gymnosperms, when compared with angiosperm PFT. We separate angiosperms into evergreen and deciduous groups to further test for differences in responsiveness associated with leaf morphology. We anticipated that differences in stomatal responses between PFTs (evergreen and deciduous angiosperm and evergreen gymnosperm) could be captured through the USO model, which accounts for variability in $D$ and can be parameterised with different values of $g_1$ for different PFTs. We predicted that the optimal stomatal theory predicts that $g_1$ values will be lower in gymnosperms (Lloyd & Farquhar, 1994; Medlyn et al., 2011; Lin et al., 2015), compared with angiosperms, and older trees, compared with younger trees.

### Materials and Methods

#### Datasets

To fit the optimal stomatal model, we need detailed individual leaf gas exchange measurements with supporting environmental data such as photosynthetic photon flux density (PPFD), leaf temperature ($T_{leaf}$), $T_{air}$ and $D$. Some datasets, particularly those using open top chambers or branch bag (BB) approaches, were also used by Medlyn et al. (2001). We added available datasets from more recent major field-based eCO$_2$ experiments on field-grown plantation or forest trees, which largely used FACE or WTC approaches. Overall, 20 distinct datasets were obtained, covering 17 tree species at 16 separate field-based eCO$_2$ experiments across 13 study sites (Table 1).

The experiments differed in a number of ways, including but not limited to site climate, CO$_2$ fumigation treatment and experimental duration (Tables 1, 2). We selected data for which CO$_2$ concentration was the only treatment variable, and we excluded data collected under additional manipulative treatments such as nutrient, drought, temperature or ozone treatments. There were two factors common to all experiments: the experiments were conducted on freely rooted trees and all studies included at least two growing seasons. Medlyn et al. (2001) demonstrated high variability in stomatal responses in experiments running for less than half a year, particularly for those in pots. The one exception to this was the second Swiss Canopy Crane experiment, with *P. abies*, for which suitable data were only available from the initial 2 months after the start of CO$_2$ fumigation (Table 1). All experimental approaches were given equal weight in the meta-analysis.

The 17 study species were split into three PFTs: evergreen gymnosperm forest (EGF; $n = 3$), evergreen angiosperm forest (EAF; $n = 3$) and deciduous angiosperm forest (DAF; $n = 11$). The EAF PFT only included species of the *Eucalyptus* genus. In total, 3661 leaf gas exchange measurements were obtained. In each experiment, plants were grown at two atmospheric CO$_2$ concentrations which, across the experiments, had means of 383 and 628 μmol mol$^{-1}$, respectively (Table 2; Fig. S1). The experiments took place between 1993 and 2019 (or, in global average ambient $C_a$ terms, from 357 to 411 μmol mol$^{-1}$), with the experimental duration ranging from 1 to 12 yr. All datasets consisted of either instantaneous spot measurements of $A_{net}$ and $g_s$ or CO$_2$ response curves (i.e. $A$-$C_a$ curves), from which the initial point at treatment CO$_2$ level was taken. Measurements were made using several gas exchange systems, with the Li-6400 as the most common instrument (Li-Cor Inc., Lincoln, NE, USA). The datasets were split into three discrete categories of tree age: aged ($> 80$ yr old; $n = 5$), mature ($10 < age < 80$ yr old; $n = 6$) and sapling ($< 10$ yr old; $n = 9$) in addition to two categories of water condition (i.e. irrigated ($n = 6$) or non-irrigated ($n = 14$)).
Table 1 Details of experiments from which data were obtained.

<table>
<thead>
<tr>
<th>Name</th>
<th>Species</th>
<th>PFT</th>
<th>Year(s) of sampling</th>
<th>Experimental set up</th>
<th>Longitude</th>
<th>Latitude</th>
<th>MAT (°C)</th>
<th>Plant age</th>
<th>Plant age category</th>
<th>Water condition category</th>
<th>Year(s) of CO₂ exposure</th>
<th>Number of replicates</th>
<th>Primary reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glencorse_1, UK*</td>
<td>Picea sitchensis</td>
<td>EGF</td>
<td>1993</td>
<td>BB</td>
<td>−3.12°W</td>
<td>55.31°N</td>
<td>8.3</td>
<td>16</td>
<td>Mature</td>
<td>Not irrigated</td>
<td>4</td>
<td>6</td>
<td>Barton &amp; Jarvis (1999)</td>
</tr>
<tr>
<td>Flakaliden_1, Sweden*</td>
<td>Picea abies</td>
<td>EGF</td>
<td>1994–1995</td>
<td>BB</td>
<td>19.27°E</td>
<td>64.07°N</td>
<td>2.4</td>
<td>29</td>
<td>Mature</td>
<td>Not irrigated</td>
<td>4</td>
<td>6</td>
<td>Roberntz &amp; Stockfors (1998)</td>
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<td>2009</td>
<td>webFACE</td>
<td>7.30°E</td>
<td>47.28°N</td>
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<td>100</td>
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<td>2 months</td>
<td>5</td>
<td>Bader et al. (2016)</td>
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<td>2003–2004</td>
<td>WTC</td>
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<td>64.07°N</td>
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<td>45</td>
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<td>&gt; 2</td>
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<td>1994</td>
<td>OTC</td>
<td>−3.12°W</td>
<td>55.31°N</td>
<td>8.3</td>
<td>0–4</td>
<td>Sapling</td>
<td>Not irrigated</td>
<td>4</td>
<td>6</td>
<td>Rey &amp; Jarvis (1997)</td>
</tr>
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<td>ORNL, USA (TN)</td>
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<td>DAF</td>
<td>1999,</td>
<td>FACE</td>
<td>−84.20°W</td>
<td>35.54°N</td>
<td>14.8</td>
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<td>19 and 10</td>
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<td>webFACE</td>
<td>7.30°E</td>
<td>47.28°N</td>
<td>11.7</td>
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<td>52.80°N</td>
<td>9</td>
<td>175</td>
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<td>8–9</td>
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<td>7–8</td>
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<td>Uddling et al. (2009)</td>
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<td>FACE</td>
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<td>45.06°N</td>
<td>6</td>
<td>8–9</td>
<td>Sapling</td>
<td>Not irrigated</td>
<td>7–8</td>
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<td>FACE</td>
<td>11.48°E</td>
<td>42.22°N</td>
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<td>WTC</td>
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<td>−33.36°S</td>
<td>17.2</td>
<td>2</td>
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<td>15 months</td>
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<td>15 months</td>
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<td>80</td>
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</tbody>
</table>

DAF, deciduous angiosperm; EAF, evergreen angiosperm; EGF, evergreen gymnosperm; PFT, plant functional type. Experimental set-up: branch bag (BB), open top chamber (OTC), whole-tree chamber (WTC) and free-air CO₂ enrichment (FACE). Mean annual temperature (MAT; °C). Plant age and length of CO₂ exposure are given in years unless otherwise stated. Number of replicates refers to the number of ‘true’ replicates in each study (number of trees in BB, OTC, WTC and webFACE studies; number of rings in FACE studies).

* Experiments for which data were included in Medlyn et al. (2001).
Table 2 Measurement details.

<table>
<thead>
<tr>
<th>Experiment name</th>
<th>Species</th>
<th>Equipment</th>
<th>Measurement type</th>
<th>Total no. data points</th>
<th>PPFD (μmol m(^{-2}) s(^{-1}))</th>
<th>Temperature (°C)</th>
<th>D (kPa)</th>
<th>Mean CO(_2) (μmol mol(^{-1}))</th>
<th>aCO(_2)</th>
<th>eCO(_2)</th>
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<tbody>
<tr>
<td>Glencorse_1, UK*</td>
<td>Picea sitchensis</td>
<td>ADC LCA3 + light source</td>
<td>A-C(_i)</td>
<td>116</td>
<td>800</td>
<td>18–35</td>
<td></td>
<td>326</td>
<td>343</td>
<td>703</td>
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<tr>
<td>Flakaliden_1, Sweden*</td>
<td>Picea abies</td>
<td>Li-Cor 6200 + light source</td>
<td>A-C(_i)</td>
<td>63</td>
<td>1000</td>
<td>5–24 (T(_{air}))</td>
<td>0–1.1</td>
<td>344</td>
<td>356</td>
<td>563</td>
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<tr>
<td>Duke FACE, USA (NC)</td>
<td>Pinus taeda</td>
<td>Li-Cor 6400 + light source</td>
<td>A-C(_i) and spot measurements</td>
<td>152</td>
<td>1500</td>
<td>10–31</td>
<td>0.69–2.95</td>
<td>364</td>
<td>566</td>
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<tr>
<td>SCC_2, Switzerland</td>
<td>Picea abies</td>
<td>Li-Cor 6400XT + light source</td>
<td>A-C(_i)</td>
<td>83</td>
<td>663–1804</td>
<td>−</td>
<td>0.4–7.1</td>
<td>369</td>
<td>527</td>
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<tr>
<td>Flakaliden_2, Sweden</td>
<td>Picea abies</td>
<td>Custom made gas exchange system</td>
<td>Automated cuvette</td>
<td>2010</td>
<td>1000–1995</td>
<td>8.5–37</td>
<td>0.26–6.3</td>
<td>342</td>
<td>565</td>
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<td>Glencorse_2, UK*</td>
<td>Betula pendula</td>
<td>Li-Cor 6200 + home made light source</td>
<td>Spot measurements</td>
<td>135</td>
<td>1200</td>
<td>18.4–32.8</td>
<td>0.8–3.0</td>
<td>344</td>
<td>701</td>
<td></td>
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<tr>
<td>Gribskov</td>
<td>Fagus sylvatica</td>
<td>Ciras-1 + light source</td>
<td>Spot measurements</td>
<td>16</td>
<td>1000</td>
<td>25.6–29</td>
<td>1.7–3.2</td>
<td>347</td>
<td>700</td>
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<td>ORNL, USA (TN)</td>
<td>Liquidambar styraciflua</td>
<td>Li-Cor 6400</td>
<td>A-C(_i)</td>
<td>115</td>
<td>1800</td>
<td>−</td>
<td>0.8–3.4</td>
<td>372</td>
<td>554</td>
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<tr>
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<td>Quercus petraea</td>
<td>Li-Cor 6400 + light source</td>
<td>Spot measurements</td>
<td>68</td>
<td>1000</td>
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<td>0.7–1.86</td>
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<td>Li-Cor 6800 + light source</td>
<td>A-C(_i)</td>
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<td>1500</td>
<td>24–28</td>
<td>1.07–2.3</td>
<td>383</td>
<td>527</td>
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<td>Rhinelander, USA (WI)</td>
<td>Betula papyrifera</td>
<td>Li-Cor 6400 + light source</td>
<td>Spot measurements</td>
<td>75</td>
<td>1800</td>
<td>23–38</td>
<td>0.9–3.8</td>
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<td>557</td>
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<tr>
<td>POPFACE, Italy</td>
<td>Populus tremuloides</td>
<td>Li-Cor 6400</td>
<td>Spot measurements</td>
<td>74</td>
<td>1800</td>
<td>22–37</td>
<td>0.59–4.1</td>
<td>366</td>
<td>560</td>
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<tr>
<td>POPFACE, Italy</td>
<td>Populus nigra</td>
<td>Li-Cor 6400</td>
<td>Spot measurements</td>
<td>35</td>
<td>870–2000</td>
<td>21–34</td>
<td>0.84–2.88</td>
<td>368</td>
<td>565</td>
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<td>POPFACE, Italy</td>
<td>Populus euramericana</td>
<td>Li-Cor 6400</td>
<td>Spot measurements</td>
<td>55</td>
<td>847–2000</td>
<td>21–35</td>
<td>0.57–2.7</td>
<td>355</td>
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<tr>
<td>POPFACE, Italy</td>
<td>Populus alba</td>
<td>Li-Cor 6400</td>
<td>Spot measurements</td>
<td>51</td>
<td>834–2000</td>
<td>25–35</td>
<td>0.68–3.4</td>
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<tr>
<td>Gunnarsholt</td>
<td>Populus trichocarpa</td>
<td>Li-Cor 6200 or Ciras-1</td>
<td>Spot measurements</td>
<td>44</td>
<td>1200</td>
<td>6–26</td>
<td>0.38–1.4</td>
<td>352</td>
<td>702</td>
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<td>Richmond_1, Australia</td>
<td>Eucalyptus saligna</td>
<td>Li-Cor 6400XT + light source</td>
<td>Spot measurements</td>
<td>197</td>
<td>1000–1800</td>
<td>15–43</td>
<td>0.5–6.47</td>
<td>384</td>
<td>625</td>
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<tr>
<td>Richmond_2, Australia</td>
<td>Eucalyptus globulus</td>
<td>Li-Cor 6400XT + light source</td>
<td>Spot measurements</td>
<td>103</td>
<td>1800</td>
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<td>0.8–4.9</td>
<td>390</td>
<td>627</td>
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<td>EucFACE, Australia</td>
<td>Eucalyptus tereticornis</td>
<td>Li-Cor 6400XT</td>
<td>Spot measurements</td>
<td>132</td>
<td>1800</td>
<td>18–39</td>
<td>0.7–6.2</td>
<td>398</td>
<td>544</td>
<td></td>
</tr>
</tbody>
</table>

* Experiment name refers to the facility and location of the dataset. Equipment refers to the gas exchange equipment used to collect the data. Measurement type refers to the measurements conducted in each dataset. PPFD refers to photosynthetic photon flux density. Temperature refers to leaf temperature (T\(_{leaf}\)) unless otherwise stated and is shown as the range at which measurements were taken. D refers to the leaf–air vapour pressure difference and is shown as the range at which measurements were taken. Mean CO\(_2\) refers to the mean CO\(_2\) treatment in either the ambient CO\(_2\) (aCO\(_2\)) or elevated CO\(_2\) (eCO\(_2\)) plots at each site. 

* Experiments for which data were included in Medlyn et al. (2001).
Details of each experiment and measurement conditions are given in Tables 1, 2 respectively. Further information on each experiment can also be found in the individual references provided in Table 1.

The datasets were collected across prevailing environmental conditions which included a range of light (PPFD) and D conditions. As our hypotheses centre on D and not PPFD, we filtered out data measured at PPFD < 800 μmol m⁻² s⁻¹. The majority of our datasets were measured at a controlled value of saturating light; only two studies used variable light and were therefore subject to filtering (Bernacchi et al., 2003; Uddling et al., 2009). In the one dataset where D was not reported (Roberts & Stocke, 1998), we calculated a lower bound to D using data on air temperature (T_air) and relative humidity. In all other cases, leaf-to-air-D was used.

Data analysis

We first conducted a standard meta-analysis of the data. We then separated data into D bins to conduct a second meta-analysis accounting for differences in D among treatments and experiments. Finally, we fit the USO model to the data and explored model fits and fitted parameters.

For each experiment, we compiled the A_sat, g_s, iWUE, C_a, D and T_leaf data and calculated the means, standard deviation and sample size under elevated and ambient (control) CO₂ plots using the experiment replicates stated by each individual study. We calculated overall effects of eCO₂ in a weighted, mixed-effects model using the rma.mv function from the R package METAFORE (Viechtbauer, 2010). Plant functional type was a fixed factor and the potential dependency of studies within the same site (e.g. different species, different treatments) was accounted for by including study site and species as random effects. All response variables were log-transformed before analysis so that statistics investigate a potential power law relation. To quantify and standardise the CO₂ response ratios across varying ranges of CO₂ manipulation, we report data as a relativised value of CO₂ response, that is a CO₂ normalised response ratio (rc) as follows:

\[
rc = \frac{\log(X_e/X_s)}{\log(C_e/C_s)} \tag{Eqn 3}
\]

where \(X_e\) and \(X_s\) represent the values of the response variables (iWUE, g_s and A_sat) at ambient or lower CO₂ (C_s) and elevated or higher CO₂ (C_e) respectively. A value of \(rc = 1\) represents direct (linear) proportionalitity between a variable’s CO₂ response and the change in CO₂. Values of \(rc\neq1\) indicate sub- or super-linear responses, depending on whether \(rc < 1\) or \(rc > 1\) respectively. As \(rc\rightarrow0\), the treatment effect approaches zero (i.e. \(X_e = X_s\)). We report uncertainties as 95% confidence intervals (CI) on \(rc\) as calculated by the rma.rv function and significant responses were recognised if the CI did not overlap zero. Effect size measurements from individual studies in the meta-analysis were weighted by the inverse of the variance (Eqn 4).

\[
\text{var} = \frac{SD_{C_e}^2}{n_{C_e}X_{C_e}^{-2}} + \frac{SD_{C_s}^2}{n_{C_s}X_{C_s}^{-2}} \tag{Eqn 4}
\]

where SD represents the standard deviation, \(n\) represents the number of experimental replicates, \(X\) represents the mean, and \(C_e\) and \(C_s\) represent elevated and ambient \(C_a\). To examine the effect of D on the response ratios, we binned observations into 0.5 kPa bins of D for each species and dataset within measurement campaigns. The bin width was chosen such that it allowed sufficient data per bin (\(n = 3\)) to calculate a mean response within each D bin without losing resolution of the D response. For this analysis, we only included D bins that overlap among experiments (0.75–2.75 kPa). We constructed mixed-effects models for each PFT with sensitivity to CO₂ as the dependent variable. The D bin was taken as a fixed effect and study site and species as random effects. We report the statistically significant predictors.

We fitted the USO model (Eqn 2) to the measurements of gas exchange for each tree species and dataset within each measurement campaign. Note that we here do not use an intercept (\(g_0\)) in the model as it can bias the \(g_1\) value (Duursma et al., 2019). We obtained \(R^2\) values for each study site and species by comparing the fitted predicted values to the observed values. To test for significant differences in \(g_1\) (Eqn 2) between CO₂ treatments, we fitted the model using a dummy variable for treatment level. To test for significant differences in \(g_1\) among PFT, tree age and water condition (as defined in Table 1), we constructed mixed-effects models using the rma.mv function from the R package METAFORE (Viechtbauer, 2010). Plant functional type, tree age category and water condition category were fixed effects and study site and species were random effects.

All analyses in this study were conducted using R v.4.0.2 (R Core Team, 2022). The datasets are publicly available (see ‘Data availability’).

Results

Mean responses to eCO₂

We found that eCO₂ significantly increased plant iWUE for all vegetation types (\(P < 0.001\), Fig. 1a–c showing mean ± 95% CIs). We could not disprove the hypothesis that the magnitude of the iWUE response is proportional to increase in CO₂; the mean \(rc\) was not significantly different from 1 (overall mean \(rc\) = 0.89 (95% CI: 0.79, 1.01)). This proportionality did not differ significantly among vegetation types (Table 3, 95% CIs = 0.78–1.27 for EAF, 0.73–1.02 for DAF, and 0.68–1.08 for EGP), supporting the prediction of the USO model that iWUE increases in proportion to the CO₂ increase, across all PFTs.

Across the range of eCO₂ experiments we analysed, stomatal response to eCO₂ had an overall mean response that was not significantly different from zero (mean \(rc\) = −0.09 (95% CI: −0.26, 0.08)), indicating no mean reduction in \(g_s\). Looking at the PFTs individually, there was a significant reduction in \(g_s\) with eCO₂ in EAF trees (\(P < 0.05\), mean \(rc\) = −0.46, 95% CI: −0.85,
Fig. 1 Estimated effect of eCO₂ on the mean response ratios, that is, a CO₂ normalised response ratio ($r_c$) of intrinsic water-use efficiency (IWUE; a–c), stomatal conductance ($g_s$; d–f) and photosynthesis ($A_{\text{net}}$; g–i) across different species and dataset (see Table 1). Each data point represents the mean effect size (overall log response, Eqn 3) of an individual study and species; error bars are the 95% confidence intervals (CI) across the true replicates ($n = 2–12$). In each plot, the mean (summary) effect size of all the studies for each plant functional type (PFT) is depicted at the bottom. We interpret the CO₂ effect at the 1.0 line (vertical dashed line in a–c) to be in proportion to the CO₂ increase (see main text) and the zero line (vertical dotted line in d–i) represents no significant CO₂ effect (i.e. no response reference line). Arrow in (i) represents data point that extend beyond the limits of the plot. Colours refer to PFT with green as evergreen angiosperm (EAF), blue as deciduous angiosperm (DAF) and orange as evergreen gymnosperm (EGF). The mean response ratios represent, on average, an increase in CO₂ from 383 to 628 μmol mol⁻¹.

Table 3 Between-group heterogeneity for the effect of eCO₂ treatment on IWUE, $A_{\text{net}}$ and $g_s$.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$Q_M$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IWUE</td>
<td>0.77</td>
<td>0.67</td>
</tr>
<tr>
<td>$g_s$</td>
<td>4.24</td>
<td>0.12</td>
</tr>
<tr>
<td>$A_{\text{net}}$</td>
<td>1.99</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Plant functional type (PFT; i.e. DAF, EAF and EGF) was used as a moderator in the multivariate linear mixed-effects model. Results were based on log-transformed response ratios. We tested whether plant responses to IWUE, $g_s$ and $A_{\text{net}}$ under eCO₂ were different between PFT. $Q_M$ refers to the test statistic for the omnibus test of coefficients of PFT group effect, with $P < 0.05$ indicating significant differences between the PFTs. Response variables are as follows: intrinsic water-use efficiency (IWUE, mol CO₂ mol⁻¹ H₂O), stomatal conductance ($g_s$, mol H₂O m⁻² s⁻¹) and photosynthetic rate ($A_{\text{net}}$, μmol m⁻² s⁻¹) response ratios.

However, a heterogeneity test (Viechtbauer, 2010) did not indicate a significant difference among PFTs in stomatal responses to eCO₂ (Table 3). As can be seen in Fig. 1, and in the CI ranges reported earlier, there is considerable variability in stomatal response across experiments within PFTs, meaning that there is relatively little power to detect differences among PFTs. Overall, however, this analysis does not confirm the hypothesised pattern of stronger $g_s$ responses in angiosperm species than gymnosperm species.

Across all experiments, photosynthesis increased strongly under eCO₂ (overall response of $r_c = 0.82$, with 95% CI of 0.64–1.01; $P < 0.001$, Fig. 1g–i). The largest average photosynthetic response to eCO₂ occurred in the EGF (mean $r_c = 1.1$ (95% CI: 0.39, 1.79)), whereas this response in EAF and DAF was marginally lower (mean $r_c = 0.63$, (95% CI: 0.26, 1.00) and 0.81, (95% CI: 0.64, 0.97), respectively). Similar to $g_s$, however, statistical analysis did not indicate significant differences in the photosynthetic response to eCO₂ among PFTs (Table 3).
Did D affect responses to eCO₂?

Variability across experiments in stomatal responses to eCO₂ may potentially be related to differences in the range of D in each experiment (Fig. S2). We tested how responses to eCO₂ vary with D (Table 4; Fig. 2). Our expectation was that the response of iWUE to eCO₂ would not vary with D, whereas the eCO₂ response of Aₜₑₙ would increase, and the eCO₂ response of gₛ would decrease, with increasing D. Our results differed from these expectations in several ways. Fig. 2a shows the CO₂-induced increase in iWUE generally increased with D across all PFTs (Table 4). More specifically, the effect of D on the iWUE response to eCO₂ was statistically significant for DAF (P < 0.001) and EGF (P < 0.05), and only EAF behaved in the same way we expected.

We also found that the effect of D on the responses of Aₜₑₙ and gₛ to eCO₂ differed among the three PFTs (P < 0.05; Fig. 2b,c; Table 4). For EAF, we found a significant effect of D on the gₛ response to eCO₂ (P < 0.05; i.e. increase in D led to a smaller reduction in gₛ in response to eCO₂), and a significant effect of D on the CO₂ response of Aₜₑₙ (P < 0.001). These responses cancelled each other out to yield no change in the CO₂ response of iWUE (Fig. 2a). For DAF, we found a significant positive effect of D on the CO₂ response of Aₜₑₙ (P < 0.001) but no effect on the response of gₛ, yielding an overall positive eCO₂ effect on iWUE. In contrast, for EGF, the effect of D on the CO₂ response of iWUE resulted from a significant effect of D on the gₛ response to eCO₂ (P < 0.05), and no effect on the Aₜₑₙ response to CO₂.

Optimal stomatal model

To facilitate the interpretation of these differences among PFTs, we fitted the USO model (Eqn 2) to data from aCO₂ and eCO₂ treatments in all experiments (Figs 3, S3). The fitted model generally provided good explanatory power (median R² of 0.70). Fitted values of the gₛ parameter ranged from 1.4 to 7.9 kPa⁻¹. The gₛ parameter was highest in the poplar species Populus nigra (7.9 kPa⁻¹ with 95% CI of 6.3–9.4) and 6.7 kPa⁻¹ (with 95% CI of 5.6–7.9) for eCO₂ and aCO₂ respectively) and lowest in P. abies (1.4 kPa⁻¹ with 95% CI of 1.3–1.49) and 1.45 kPa⁻¹ (with 95% CI of 1.42–1.49) both aCO₂ treatments in Flakaliden_1 and Flakaliden_2 respectively). We found that gₛ was significantly lower in EGF species than in DAF and EAF species (P < 0.0001; Fig. 3; Table S1). A lower gₛ value is suggested to lead to a larger response of Aₜₑₙ. In support of this, we found that the EGF species had both the lowest gₛ and the highest photosynthetic response to eCO₂.

For 17 of the 20 datasets, there was no significant difference between the gₛ parameters estimated for aCO₂ and eCO₂. However, we found that for all three datasets from boreal sites (P. abies trees measured at the Flakaliden site in Northern Sweden and Populus trichocarpa measured at the Gunnarsholt site in Iceland), estimated gₛ parameters were significantly higher under eCO₂. In addition, we found that gₛ was significantly higher in species that received supplemental water (P < 0.001) and decreased significantly with tree age (P < 0.05; Table S1).

Discussion

We quantified the relative responses of iWUE, gₛ, and Aₜₑₙ across a set of manipulative experiments on large trees (Table 1). We found that the mean response of iWUE was approximately proportional to eCO₂, as predicted by the USO model, and this response was similar across the three woody PFTs examined in the study. The results also showed that D had a significant effect on these responses to eCO₂ in angiosperms but not in gymnosperms, suggesting differing sensitivities to D between these PFTs. The use of the USO model allows these differences to be taken into account. Consistent with previous analyses (Medlyn et al., 2011; Gimeno et al., 2016), we found gₛ was not altered by eCO₂ in most experiments, implying that the optimal stomatal theory is largely successful in predicting the coupling of carbon uptake and water loss in field and forest grown trees under eCO₂.

Differences in the response of gₛ to CO₂ among PFTs were not statistically significant, in contrast to previous studies, but the tendency for a smaller response in gymnosperm species could be captured in the USO model through the lower gₛ values, which drive a larger Aₜₑₙ response.

Does iWUE respond in proportion to eCO₂?

The increases in iWUE with eCO₂ are comparable to those reported in meta-analyses of FACE experimental data in Ainsworth & Long (2005; mean rₛ = 1.2 ± 0.33). The results are also consistent with a meta-analysis of tree-ring isotopic data consisting of 422 tree species (Adams et al., 2020), although experimental data of tree-ring isotopic analyses in two FACE experiments suggest much higher iWUE responses (Battaglia et al., 2013; mean rₛ = 1.4 and 1.3 for Duke University and Oak Ridge National Laboratory, ORNL, respectively). A general review, compiling several different strands of evidence, concluded that most supported an rₛ for iWUE close to one (Walker et al., 2020). We conclude that the proportional increase in iWUE with eCO₂ is a reliable response which supports
optimisation theory that proposes $A_{\text{net}}$ and $g_s$ are well coupled to maximise C gain and minimise water loss to the plant (Medlyn et al., 2011; De Kauwe et al., 2013; Walker et al., 2020). This result can therefore allow the prediction of the response of $g_s$ to eCO$_2$ from the $A_{\text{net}}$ response and vice versa.

Fig. 2 The effect of $D$ on the mean log response ratios for the CO$_2$ effect ($r_c$) on (a) intrinsic water-use efficiency (iWUE), (b) stomatal conductance ($g_s$) and (c) net photosynthesis ($A_{\text{net}}$) across the datasets. Each data point shows the mean response for each 0.5 kPa bin ($D$ range of 0.75–2.75) per species per dataset. Symbols refer to dataset and colours refer to plant functional type (PFT) with green as evergreen angiosperm (EAF), blue as deciduous angiosperm (DAF) and orange as evergreen gymnosperm (EGF). Shaded areas are 95% confidence intervals.

Which is the primary response variable, $A_{\text{net}}$ or $g_s$?

We found increases in iWUE with eCO$_2$ were predominantly due to increased $A_{\text{net}}$ rather than reductions in $g_s$. This conclusion is consistent with results from several previous meta-analyses of...
physiological responses of tree species, although the statistical significance of the reduction in $g_\text{i}$ varies across studies. Curtis & Wang (1998) found an increase in $A_{\text{net}}$ of +54% compared with a non-significant reduction in $g_\text{i}$ of −11%. Medlyn et al. (2001) found a statistically significant reduction of −14% in $g_\text{i}$ compared to an increase in $A_{\text{net}}$ of 51% (Medlyn et al., 1999). Similarly, Ainsworth & Rogers (2007) found a statistically significant reduction of −20% in $g_\text{i}$ compared with an increase in $A_{\text{net}}$ of +45% in tree species. Our results, which include many additional high-quality datasets, show an even stronger influence of $A_{\text{net}}$. We express our findings as a relative response ratio to standardise for the difference in treatment CO2 concentrations used in different experiments. We find a mean increase ($r_\text{g}$) of 0.82 in $A_{\text{net}}$ and no statistical change in $g_\text{i}$ (mean reduction of −0.09).

However, we also found the responses of both $A_{\text{net}}$ and $g_\text{i}$ to eCO2 can be variable within an experiment, as a result of differences in environmental variables such as $D$ and light. Barton et al. (2012) confirmed this finding experimentally at both leaf and whole-canopy scales in *E. saligna* trees and found photosynthetic responses to eCO2 increased with increases in $D$. We observed differences in $D$ sensitivity among PFTs for the responses of $A_{\text{net}}$ and $g_\text{i}$ to eCO2 (Fig. 2). Changes in $A_{\text{net}}$ due to eCO2 were more responsive to $D$ in angiosperms than in gymnosperms, suggesting a higher sensitivity of physiological processes to $D$ in angiosperms compared with gymnosperms. Our findings show that $D$ needs to be considered when comparing studies where $D$ differs, supporting the use of the USO model to account for differences. It also highlights the limitations on inferences drawn in previous research regarding gymnosperm vs angiosperm responses, as previous studies have generally not accounted for either differences or sensitivity in $D$ when assessing the response of stomata to eCO2. A further environmental variable that can alter the responses of both $A_{\text{net}}$ and $g_\text{i}$ is light. A limitation of our study is that we analysed only data with high PPFD (i.e. > 800 PPFD) to allow for comparisons between datasets. Under these conditions, we found strong photosynthetic responses to eCO2, which may be diminished under lower PPFD (Bernacchi et al., 2003; Uddling et al., 2009; Badet et al., 2016).

To avoid generalisations based on the mean responses of iWUE, $A_{\text{net}}$, and $g_\text{i}$ due to the effects of differences in $D$, we recommend using the USO model to normalise iWUE to account for any effect of $D$. Consistent with the study hypotheses, we found that the optimal stomatal model successfully predicted $g_\text{i}$ under both aCO2 and eCO2 and the $g_\text{i}$ parameter remained unchanged in response to eCO2 (Gimeno et al., 2016), with three exceptions (Fig. 3). The three cases where CO2 treatment significantly affected $g_1$ were the two *P. abies* datasets at the Flakaliden site (northern Sweden) and *P. trichocarpa* at the Gunnarsholt site (Iceland). The two Flakaliden datasets came from two very different experiments (BB and WTC) carried out a decade apart, suggesting that although the response differs from other experiments, it may be a real effect at the Flakaliden site. The forest soil at Flakaliden is nutrient impoverished (Bergh et al., 1999), so nutrient limitations at the site may have had an effect on leaf physiology at the Flakaliden site and may explain the unusual response observed. This explanation is corroborated by the fact the $g_\text{i}$ parameter remained unchanged with eCO2 in *P. abies* growing on fertile soil at the Swiss Canopy Crane site.
consistent with other reviews of species differences in \( g_t \) (Lin et al., 2015). A low \( g_t \) implies a low intercellular CO\(_2\) concentration and thus a higher responsiveness of photosynthesis to rising CO\(_2\). As \( A_{\text{net}} \) and \( g_s \) are coupled in the USO model, this will also result in a smaller responsiveness of \( g_s \).

**Conclusion**

This study provides a large body of data collected over the last few decades that incorporates a number of tree species and experiments, including recent forest FACE experiments, which is a robust base to evaluate \( g_s \) under elevated CO\(_2\). It resolves some of the long-standing questions regarding stomatal behaviour in elevated CO\(_2\) in woody species. The results show that the eCO\(_2\) responses of iWUE, \( g_s \) or \( A_{\text{net}} \) were not significantly different among three key PFTs (Table 3), which has importance to how large-scale models depict these responses (Kleidon, 2004). The results suggest that increases in \( A_{\text{net}} \) rather than reductions in \( g_s \) drove the increases in iWUE that occur with eCO\(_2\) in all vegetation types. However, the results highlighted the importance of incorporating environmental factors, such as \( D \), when comparing vegetation type. Our analysis of CO\(_2\) responses suggests that angiosperms were more responsive to \( D \) than gymnosperms (Fig. 2; Table 4).

With the exception of data from boreal study sites, the optimal stomatal model was successful in describing the stomatal behaviour with changes in atmospheric variables, such as with eCO\(_2\) and \( D \) (Fig. 3). We highlight the areas where data representation needs improvement but conclude that a stomatal parameter such as \( g_t \) can be implemented in ecosystem to land surface models as a reliable way to characterise the key aspects of stomatal behaviour that affect hydrology and land surface energy exchange will remain important as atmospheric CO\(_2\) increases into the future.

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**Author contributions**

AG, BEM and DSE conceived the paper, whilst AG was undertaking doctoral research supervised by ARMK and JP. AG wrote the paper with major contributions from BEM, DSE and MJ. AG, MK-FB, CVMB, CB, CC, DSE, KYC, MED, TEG, MH, SLamba, SLeuzinger, JU, JW and GW collected the data for this manuscript. AG organized the datasets under the supervision of BEM, DSE and MJ. MJ and AG designed and performed the statistical analyses, with input from BEM and DSE. AG and BEM wrote the first draft of the paper. AG, MJ, DSE, RMK, JP,
MK-FB, CVMB, CB, CC, KYC, MED, TEG, MH, SLamba, SLeuizinger, JU, JW, GW, BEJ contributed to the interpretation of the results and intellectual input of this manuscript revision. All authors read and approved the submitted version.

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**Data availability**

The data that support the findings of this study are publicly available via FigShare (doi: 10.6084/m9.figshare.21252609.v1).

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Supporting Information

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

Fig. S1 Mean ambient CO2 (aCO2; blue) and elevated CO2 (eCO2; red) for each of the studies in the dataset.

Fig. S2 Histograms showing the counts of binned D (0.5 kPa) across the ambient CO2 (blue) and elevated CO2 (red) treatments for each of the studies in the dataset.

Fig. S3 Relationship between stomatal conductance (g) and an index based on the USO formulation calculated from D, Anet and CO2 for each dataset (cf. Medlyn et al., 2011).

Table S1 Between-group heterogeneity for the effect of plant functional type (PFT) (i.e. DAF, EAF and EGF), Tree age (i.e. Old, Mature and Sapling) and Water condition (i.e. Irrigated and Not irrigated) on g (kPa−0.5) values.

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