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Anna Gardner<sup>1,2</sup> , Mingkai Jiang<sup>3,4</sup> , David S. Ellsworth<sup>3</sup> , A. Robert MacKenzie<sup>1,5</sup> , Jeremy Pritchard<sup>1,2</sup>,  
Martin Karl-Friedrich Bader<sup>6</sup> , Craig V. M. Barton<sup>3</sup> , Carl Bernacchi<sup>7</sup>, Carlo Calfapietra<sup>8</sup>, Kristine Y. Crous<sup>3</sup> ,  
Mirindi Eric Dusenge<sup>9</sup>, Teresa E. Gimeno<sup>10,11</sup> , Marianne Hall<sup>12</sup> , Shubhangi Lamba<sup>13</sup> ,  
Sebastian Leuzinger<sup>14</sup> , Johan Uddling<sup>13</sup> , Jeffrey Warren<sup>15</sup>, Göran Wallin<sup>13,16</sup>  and Belinda E. Medlyn<sup>3</sup>

<sup>1</sup>Birmingham Institute of Forest Research, University of Birmingham, Edgbaston, B15 2TT, UK; <sup>2</sup>School of Biological Sciences, University of Birmingham, Edgbaston, B15 2TT, UK; <sup>3</sup>Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia; <sup>4</sup>College of Life Sciences, Zhejiang University, 866 Yuhangtang Rd, Hangzhou, Zhejiang Province, China; <sup>5</sup>School of Geography, Earth & Environmental Sciences, University of Birmingham, Edgbaston, B15 2TT, UK; <sup>6</sup>Department of Forestry and Wood Technology, Linnaeus University, Växjö 351 95, Sweden; <sup>7</sup>USDA-ARS Global Change and Photosynthesis Research Unit, Urbana, IL 61801, USA; <sup>8</sup>Institute of Research on Terrestrial Ecosystems (IRET), National Research Council of Italy (CNR), Porano 05010, Italy; <sup>9</sup>Western Centre for Climate Change, Sustainable Livelihoods and Health, Department of Geography, The University of Western Ontario, London, ON N6A 5C2, Canada; <sup>10</sup>CREAF, Bellaterra (Cerdanyola del Vallès), Catalonia 08193, Spain; <sup>11</sup>Basque Centre for Climate Change (BC3), Leioa 48940, Spain; <sup>12</sup>Centre for Environmental and Climate Science, Lund University, Sölvegatan 37, Lund 223 62, Sweden; <sup>13</sup>Department of Biological and Environmental Sciences, University of Gothenburg, PO Box 461, Gothenburg 40530, Sweden; <sup>14</sup>Auckland University of Technology, Institute of Earth and Oceanic Sciences, School of Applied Sciences, Private Bag 92006 (Mail No C-43), Auckland 1142, New Zealand; <sup>15</sup>Environmental Sciences Division and Climate Change Science Institute, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA; <sup>16</sup>Environmental Change Institute, School of Geography and the Environment, University of Oxford, South Parks Road, Oxford, OX1 3QY, UK

## Summary

Author for correspondence:

A. Robert MacKenzie

Email: a.r.mackenzie@bham.ac.uk

Received: 18 July 2022

Accepted: 17 October 2022

*New Phytologist* (2023) **237**: 1229–1241

doi: 10.1111/nph.18618

**Key words:** climate change, deciduous, evergreen, free-air CO<sub>2</sub> enrichment, photosynthesis, water-use efficiency.

- Optimal stomatal theory predicts that stomata operate to maximise photosynthesis ( $A_{\text{net}}$ ) 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 CO<sub>2</sub> (eCO<sub>2</sub>), 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 eCO<sub>2</sub> on iWUE and its components  $A_{\text{net}}$  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  $A_{\text{net}}$ , responses to eCO<sub>2</sub> in gymnosperms compared with angiosperm PFTs.
- We found that iWUE increased in proportion to increasing eCO<sub>2</sub> in all PFTs, and that increases in  $A_{\text{net}}$  had stronger effects than reductions in  $g_s$ . The USO model correctly captured stomatal behaviour with eCO<sub>2</sub> across most datasets. The chief difference among PFTs was a lower stomatal slope parameter ( $g_1$ ) for the gymnosperm, compared with angiosperm, species.
- Land surface models can use the USO model to describe stomatal behaviour under changing atmospheric CO<sub>2</sub> 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 (CO<sub>2</sub>) diffusion into the plant and simultaneous water loss (Cowan, 1982; Jones & Sutherland, 1991) such that maintaining stomatal opening, to allow CO<sub>2</sub> 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 CO<sub>2</sub> assimilation,  $A_{\text{net}}$ , 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 CO<sub>2</sub> 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 CO<sub>2</sub> (eCO<sub>2</sub>; +350 μmol mol<sup>-1</sup>) across four types of CO<sub>2</sub> exposure facilities. Similarly, Ainsworth & Rogers (2007) reported  $g_s$  was reduced by an average of 19% in response to eCO<sub>2</sub> (+208 μmol mol<sup>-1</sup>) in tree species growing in free-air CO<sub>2</sub> enrichment (FACE) experiments. This reduction in water use with rising CO<sub>2</sub> 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 eCO<sub>2</sub> to the same extent (Saxe *et al.*, 1998), and there are species-specific stomatal responses to eCO<sub>2</sub> that are apparent among eCO<sub>2</sub> experiments (Saxe *et al.*, 1998; Medlyn *et al.*, 2001). For tree species, it has been observed that eCO<sub>2</sub>-induced reductions in  $g_s$  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 eCO<sub>2</sub> may vary with phylogeny (Brodribb *et al.*, 2009; Lammertsma *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 C<sub>a</sub> response of  $g_s$  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 eCO<sub>2</sub> between gymnosperm and angiosperm species. In addition to  $g_s$  responses in isolation, there are also indications that iWUE ( $A_{\text{net}}/g_s$ ) may respond differently to rising CO<sub>2</sub> between angiosperm and gymnosperm species. For example, it has been suggested that iWUE in gymnosperms may have a stronger positive response to eCO<sub>2</sub>, 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 C<sub>a</sub>, 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 CO<sub>2</sub> fixation through photosynthesis while minimising water loss via transpiration, by maximising the expression,  $A_{\text{net}} - \lambda E$ , where  $A_{\text{net}}$  is photosynthesis (μmol CO<sub>2</sub> m<sup>-2</sup> leaf s<sup>-1</sup>),  $E$  is transpiration (mol H<sub>2</sub>O m<sup>-2</sup> leaf s<sup>-1</sup>) and  $\lambda$  (μmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O) 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

$$\max(A_{\text{net}} - \lambda E) \quad \text{Eqn 1}$$

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  $g_s$  (mol H<sub>2</sub>O m<sup>-2</sup> leaf s<sup>-1</sup>) that can be approximated as follows, also known as the unified stomatal optimisation (USO) model:

$$g_s \approx 1.6 \left( 1 + \frac{g_1}{\sqrt{D}} \right) A_{\text{net}} / C_a \quad \text{Eqn 2}$$

where  $g_1$  (kPa<sup>0.5</sup>) 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 CO<sub>2</sub>, and  $g_s$  and  $A_{\text{net}}$  are the functions of  $D$  and air temperature,  $T_{\text{air}}$ , as well as other physiological parameters. Note 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,  $g_0$ , 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  $A_{\text{net}}/g_s$  ratio, that is the intrinsic WUE (iWUE =  $A_{\text{net}}/g_s$ ) should be proportional to the atmospheric CO<sub>2</sub> concentration (Barton *et al.*, 2012). This model has been used to predict stomatal responses successfully in several CO<sub>2</sub> 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  $g_s$  to C<sub>a</sub> could be captured by the USO model. First, there may be a difference in the  $T_{\text{air}}$  or  $D$  at which measurements were made. The relative responsiveness of  $A_{\text{net}}$  and  $g_s$  can vary within an experiment depending on  $T_{\text{air}}$  or  $D$ . For example,  $A_{\text{net}}$  is more responsive to eCO<sub>2</sub> 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 eCO<sub>2</sub> in *Eucalyptus saligna*, once treatment differences in  $D$  were taken into account. They also showed that the responsiveness of  $g_s$  to eCO<sub>2</sub> decreased at high  $D$ , when high  $T_{\text{air}}$  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 biochemistry model that captures interactions with  $T_{\text{air}}$  and C<sub>a</sub> (e.g. Duursma, 2015).

Second, a substantial increase in iWUE in response to eCO<sub>2</sub> can occur as a strong increase in  $A_{\text{net}}$ , with a marginal reduction in  $g_s$ , or a marginal increase in  $A_{\text{net}}$  and a strong reduction in  $g_s$ . Photosynthetic responses may be stronger in species that have a low  $g_1$  and consequently a low intercellular CO<sub>2</sub>. 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 eCO<sub>2</sub> are expected to be larger in species with thicker or denser leaves because thicker leaves have greater diffusive limitation of the transport of CO<sub>2</sub> to the sites of carboxylation, and so respond more positively to eCO<sub>2</sub> 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  $e\text{CO}_2$  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  $e\text{CO}_2$  in *Picea abies*. They found that the response of  $i\text{WUE}$  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 ( $\text{kPa}^{0.5}$ ) between  $a\text{CO}_2$  and  $e\text{CO}_2$  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  $e\text{CO}_2$  on  $i\text{WUE}$ ,  $g_s$  and  $A_{\text{net}}$  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  $e\text{CO}_2$  (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  $i\text{WUE}$  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,  $i\text{WUE}$  would increase in proportion to the  $\text{CO}_2$  increase similarly across all species; (2) on average,  $g_s$  would decrease and  $A_{\text{net}}$  would increase in response to  $e\text{CO}_2$ , with  $A_{\text{net}}$  being the principal driver for increased  $i\text{WUE}$ ; (3) the magnitude of change in  $A_{\text{net}}$  to  $e\text{CO}_2$  would depend on  $D$ , with the response of  $A_{\text{net}}$  to  $e\text{CO}_2$  larger at high  $D$ ; and (4) the magnitude of change in both  $A_{\text{net}}$  and  $g_s$  to  $e\text{CO}_2$  would depend on PFT, with  $A_{\text{net}}$  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 ( $T_{\text{leaf}}$ ),  $T_{\text{air}}$  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  $e\text{CO}_2$  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  $e\text{CO}_2$  experiments across 13 study sites (Table 1).

The experiments differed in a number of ways, including but not limited to site climate,  $\text{CO}_2$  fumigation treatment and experimental duration (Tables 1, 2). We selected data for which  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  concentrations which, across the experiments, had means of 383 and 628  $\mu\text{mol mol}^{-1}$ , 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  $\mu\text{mol mol}^{-1}$ ), with the experimental duration ranging from 1 to 12 yr. All datasets consisted of either instantaneous spot measurements of  $A_{\text{net}}$  and  $g_s$  or  $\text{CO}_2$  response curves (i.e.  $A-C_i$  curves), from which the initial point at treatment  $\text{CO}_2$  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 ( $10 < \text{age} < 80$  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.

Name	Species	PFT	Year(s) of sampling	Experimental set up	Longitude	Latitude	MAT (°C)	Plant age	Plant age category	Water condition category	Year(s) of CO <sub>2</sub> exposure	Number of replicates	Primary reference
Glencorse_1, UK*	<i>Picea sitchensis</i>	EGF	1993	BB	-3.12°W	55.31°N	8.3	16	Mature	Not irrigated	4	6	Barton & Jarvis (1999)
Flakaliden_1, Sweden*	<i>Picea abies</i>	EGF	1994–1995	BB	19.27°E	64.07°N	2.4	29	Mature	Not irrigated	4	6	Robertz & Stockfors (1998)
Duke FACE, USA (NC)	<i>Pinus taeda</i>	EGF	1998–2006	FACE	-79.09°W	35.97°N	14.8	15–23	Mature	Not irrigated	4–12	3	Ellsworth et al. (2012)
Swiss Canopy Crane_2, Switzerland	<i>Picea abies</i>	EGF	2009	webFACE	7.30°E	47.28°N	11.7	100	Old	Not irrigated	2 months	5	Bader et al. (2016)
Flakaliden_2, Sweden	<i>Picea abies</i>	EGF	2003–2004	WTC	19.27°E	64.07°N	2.4	45	Mature	Not irrigated	> 2	3	Uddling & Wallin (2012)
Glencorse_2, UK*	<i>Betula pendula</i>	DAF	1994	OTC	-3.12°W	55.31°N	8.3	0–4	Sapling	Not irrigated	4	6	Rey & Jarvis (1997)
Gribskov, Denmark*	<i>Fagus sylvatica</i>	DAF	1996	BB	12.16°E	55.59°N	8.8	36	Mature	Not irrigated	2	4	Sigurdsson et al. (2002)
ORNL, USA (TN)	<i>Liquidambar styraciflua</i>	DAF	1999, 2008–2009	FACE	-84.20°W	35.54°N	14.8	20	Mature	Not irrigated	1, 9 and 10	2	Warren et al. (2015)
Swiss Canopy Crane_1, Switzerland	<i>Quercus petraea</i>	DAF	2001	webFACE	7.30°E	47.28°N	11.7	100	Old	Not irrigated	1	3	Bader et al. (2010)
BIFoR FACE, UK	<i>Fagus sylvatica</i>	DAF	2019	FACE	-2.03°W	52.80°N	9	175	Old	Not irrigated	4	3	Gardner et al. (2021)
Rhinelander, USA (WI)	<i>Quercus robur</i>	DAF	2005–2006	FACE	-89.05°W	45.06°N	6	8–9	Sapling	Not irrigated	7–8	3	Uddling et al. (2009)
	<i>Betula papyrifera</i>	DAF										3	
	<i>Populus tremuloides</i>	DAF										6	Bernacchi et al. (2003)
POPFACE, Italy	<i>Populus nigra</i>	DAF	2000	FACE	11.48°E	42.22°N	14	1	Sapling	Irrigated	1	12	
	<i>Populus euramericana</i>	DAF										12	
Gunnarsholt, Iceland	<i>Populus alba</i>	DAF	1996	WTC	-20.13°W	63.51°N	5.2	4	Sapling	Irrigated	3	4	Sigurdsson et al. (2002)
Richmond_1, Australia	<i>Populus trichocarpa</i>	EAF	2008–2009	WTC	150.44°E	-33.36°S	17.2	2	Sapling	Irrigated	2	3	Barton et al. (2012)
Richmond_2, Australia	<i>Eucalyptus saligna</i>	EAF	2010–2011	WTC	150.44°E	-33.36°S	17.2	15 months	Sapling	Irrigated	15 months	3	Quentin et al. (2015)
EucFACE, Australia	<i>Eucalyptus globulus</i>	EAF	2013	FACE	150.44°E	-33.37°S	17.2	80	Old	Not irrigated	6 months	3	Gimeno et al. (2016)
	<i>Eucalyptus tereticornis</i>	EAF										3	

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 CO<sub>2</sub> enrichment (FACE). Mean annual temperature (MAT; °C). Plant age and length of CO<sub>2</sub> 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.

Experiment name	Species	Equipment	Measurement type	Total no. data points	Measurement conditions				Mean CO <sub>2</sub> (μmol mol <sup>-1</sup> )
					PPFD (μmol m <sup>-2</sup> s <sup>-1</sup> )	Temperature (°C)	D (kPa)	aCO <sub>2</sub> eCO <sub>2</sub>	
Glencorse_1, UK*	<i>Picea sitchensis</i>	ADC LCA3 + light source	A-C <sub>i</sub>	116	800	18–35	0–1.1	326	657
Flakaliden_1, Sweden*	<i>Picea abies</i>	Li-Cor 6200 + light source	A-C <sub>i</sub>	63	1000	5–24 ( <i>T</i> <sub>air</sub> )	0.69–2.95	343	703
Duke FACE, USA (NC)	<i>Pinus taeda</i>	Li-Cor 6400 + light source	A-C <sub>i</sub> and spot measurements	152	1500	10–31	0.69–2.95	364	563
SCC_2, Switzerland	<i>Picea abies</i>	Li-Cor 6400XT + light source	A-C <sub>i</sub>	83	663–1804	–	0.4–7.1	369	527
Flakaliden_2, Sweden	<i>Picea abies</i>	Custom made gas exchange system	Automated cuvette	2010	1000–1995	8.5–37	0.26–6.3	342	665
Glencorse_2, UK*	<i>Betula pendula</i>	Li-Cor 6200 + home made light source	Spot measurements	135	1200	18.4–32.8	0.8–3.0	344	701
Gribskov	<i>Fagus sylvatica</i>	Ciras-1 + light source	Spot measurements	16	1000	25.6–29	1.7–3.2	347	700
ORNL, USA (TN)	<i>Liquidambar styraciflua</i>	Li-Cor 6400XT	A-C <sub>i</sub>	115	1800	–	0.8–3.4	372	554
SCC_1, Switzerland	<i>Quercus petraea</i>	Li-Cor 6400 + light source	Spot measurements	68	1000	23–27	0.7–1.86	380	550
	<i>Quercus sylvatica</i>			98	1000	23–28	0.5–1.7	380	550
BIFoR FACE, UK	<i>Quercus robur</i>	Li-Cor 6800 + light source	A-C <sub>i</sub>	43	1500	24–28	1.07–2.3	383	527
Rhinelander, USA (WI)	<i>Betula papyrifera</i>	Li-Cor 6400 + light source	Spot measurements	75	1800	23–38	0.9–3.8	365	557
	<i>Populus tremuloides</i>			74	1800	22–37	0.59–4.1	366	560
POPFACE, Italy	<i>Populus nigra</i>	Li-Cor 6400	Spot measurements	35	870–2000	21–34	0.84–2.88	368	565
	<i>Populus euramericana</i>			55	847–2000	21–35	0.57–2.7	355	562
	<i>Populus alba</i>			51	834–2000	25–35	0.68–3.4	356	566
Gunnarsholt	<i>Populus trichocarpa</i>	Li-Cor 6200 or Ciras-1	Spot measurements	44	1200	6–26	0.38–1.4	352	702
Richmond_1, Australia	<i>Eucalyptus saligna</i>	Li-Cor 6400XT + light source	Spot measurements	197	1000–1800	15–43	0.5–6.47	384	625
Richmond_2, Australia	<i>Eucalyptus globulus</i>	Li-Cor 6400XT + light source	Spot measurements	103	1800	19–39	0.8–4.9	390	627
EucFACE, Australia	<i>Eucalyptus tereticornis</i>	Li-Cor 6400XT + light source	Spot measurements	132	1800	18–39	0.7–6.2	398	544

\* '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*<sub>leaf</sub>) 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<sub>2</sub> refers to the mean CO<sub>2</sub> treatment in either the ambient CO<sub>2</sub> (aCO<sub>2</sub>) or elevated CO<sub>2</sub> (eCO<sub>2</sub>) 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  $PPFD < 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ . 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 (Robertz & Stockfors, 1998), we calculated a lower bound to  $D$  using data on air temperature ( $T_{\text{air}}$ ) 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  $A_{\text{net}}$ ,  $g_s$ ,  $iWUE$ ,  $C_a$ ,  $D$  and  $T_{\text{leaf}}$  data and calculated the means, standard deviation and sample size under elevated and ambient (control)  $\text{CO}_2$  plots using the experiment replicates stated by each individual study. We calculated overall effects of  $e\text{CO}_2$  in a weighted, mixed-effects model using the `rma.mv` function from the R package METAFOR (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  $\text{CO}_2$  response ratios across varying ranges of  $\text{CO}_2$  manipulation, we report data as a relativised value of  $\text{CO}_2$  responses, that is a  $\text{CO}_2$  normalised response ratio ( $r_c$ ) as follows:

$$r_c = \frac{\log_e\left(\frac{X_e}{X_a}\right)}{\log_e\left(\frac{C_e}{C_a}\right)} \quad \text{Eqn 3}$$

where  $X_e$  and  $X_a$  represent the values of the response variables ( $iWUE$ ,  $g_s$  and  $A_{\text{net}}$ ) at ambient or lower  $\text{CO}_2$  ( $C_a$ ) and elevated or higher  $\text{CO}_2$  ( $C_e$ ) respectively. A value of  $r_c = 1$  represents direct (linear) proportionality between a variable's  $\text{CO}_2$  response and the change in  $\text{CO}_2$ . Values of  $r_c \neq 1$  indicate sub- or super-linear responses, depending on whether  $r_c < 1$  or  $r_c > 1$  respectively. As  $r_c \rightarrow 0$ , the treatment effect approaches zero (i.e.  $X_e = X_a$ ). We report uncertainties as 95% confidence intervals (CI) on  $r_c$  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).

$$\text{var} = \frac{SD_{C_e}^2}{n_{C_e} \bar{X}_{C_e}^2} + \frac{SD_{C_a}^2}{n_{C_a} \bar{X}_{C_a}^2} \quad \text{Eqn 4}$$

where SD represents the standard deviation,  $n$  represents the number of experimental replicates,  $\bar{X}$  represents the mean, and  $C_e$  and  $C_a$  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  $\text{CO}_2$  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  $\text{CO}_2$  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 METAFOR (Viechtbauer, 2010). Plant functional type, tree age category and water condition category were fixed effects and study site and species were random effects.

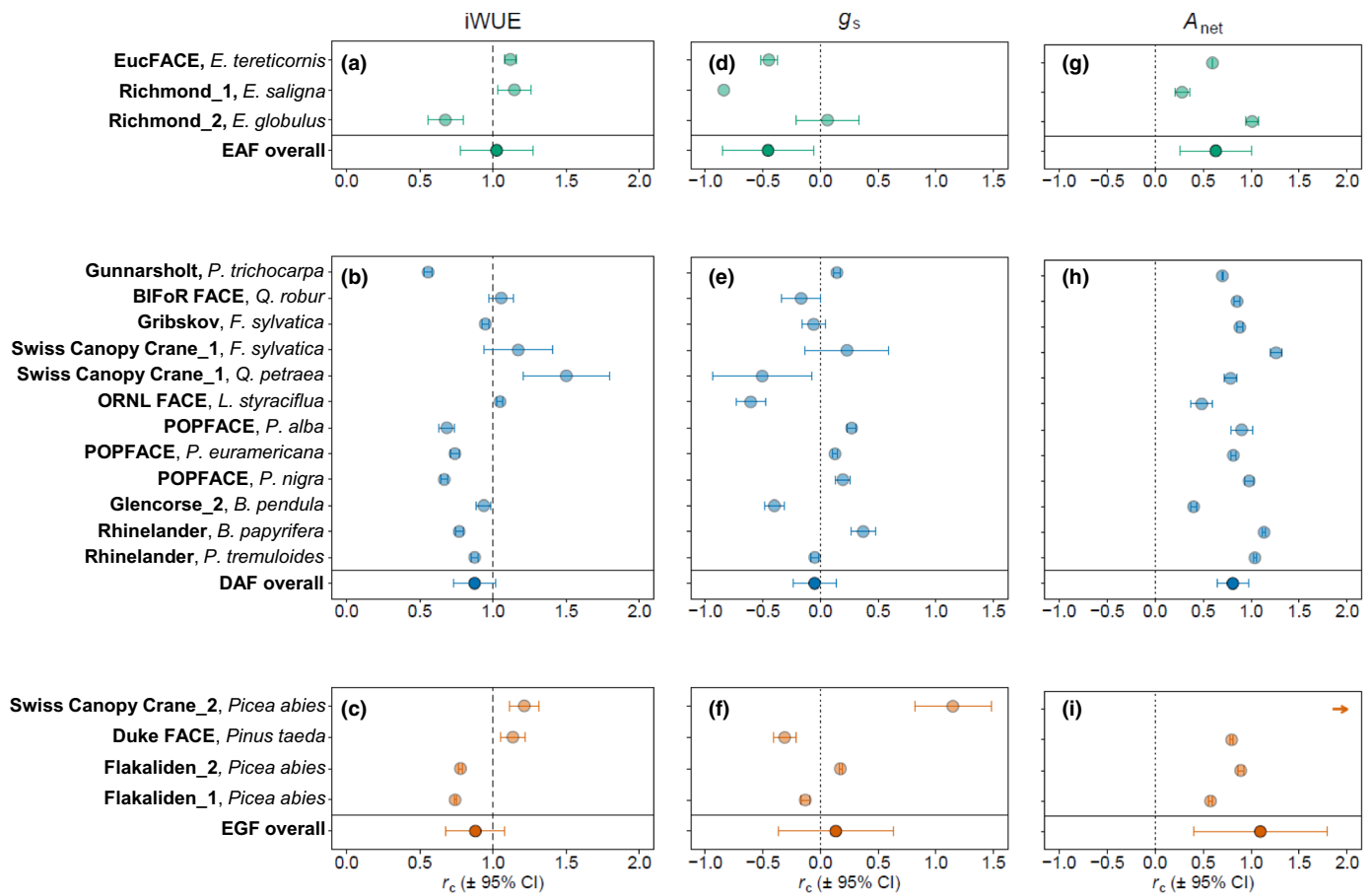
All analyses in this study were conducted using R v.4.0.2 (R Core Team, 2022). The datasets are publicly available (see 'Data availability').

## Results

### Mean responses to $e\text{CO}_2$

We found that  $e\text{CO}_2$  significantly increased plant  $iWUE$  for all vegetation types ( $P < 0.001$ , Fig. 1a–c showing mean  $\pm$  95% CIs). We could not disprove the hypothesis that the magnitude of the  $iWUE$  response is proportional to increase in  $\text{CO}_2$ ; the mean  $r_c$  was not significantly different from 1 (overall mean  $r_c = 0.89$  (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 EGF), supporting the prediction of the USO model that  $iWUE$  increases in proportion to the  $\text{CO}_2$  increase, across all PFTs.

Across the range of  $e\text{CO}_2$  experiments we analysed, stomatal response to  $e\text{CO}_2$  had an overall mean response that was not significantly different from zero (mean  $r_c = -0.09$  (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  $e\text{CO}_2$  in EAF trees ( $P < 0.05$ , mean  $r_c = -0.46$ , (95% CI: -0.85,



**Fig. 1** Estimated effect of eCO<sub>2</sub> on the mean response ratios, that is, a CO<sub>2</sub> normalised response ratio ( $r_c$ ) of intrinsic water-use efficiency (iWUE; a–c), stomatal conductance ( $g_s$ ; d–f) and photosynthesis ( $A_{net}$ ; 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 CO<sub>2</sub> effect at the 1.0 line (vertical dashed line in a–c) to be in proportion to the CO<sub>2</sub> increase (see main text) and the zero line (vertical dotted line in d–i) represents no significant CO<sub>2</sub> 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 CO<sub>2</sub> from 383 to 628  $\mu\text{mol mol}^{-1}$ .

**Table 3** Between-group heterogeneity for the effect of eCO<sub>2</sub> treatment on iWUE,  $A_{net}$  and  $g_s$ .

Variable	$Q_M$	$P$ -value
iWUE	0.77	0.67
$g_s$	4.24	0.12
$A_{net}$	1.99	0.37

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  $A_{net}$  under eCO<sub>2</sub> 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,  $\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ), stomatal conductance ( $g_s$ ;  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and photosynthetic rate ( $A_{net}$ ;  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) response ratios.

–0.06); Fig. 1g), but neither DAF nor EGF showed significant decreases in  $g_s$  with eCO<sub>2</sub> (mean  $r_c = -0.05$  (95% CI: –0.23, 0.14) and 0.13 (95% CI: –0.37, 0.63) respectively; Fig. 1h,i).

However, a heterogeneity test (Viechtbauer, 2010) did not indicate a significant difference among PFTs in stomatal responses to eCO<sub>2</sub> (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 eCO<sub>2</sub> (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 eCO<sub>2</sub> 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 eCO<sub>2</sub> among PFTs (Table 3).

**Table 4** Statistical output from the meta-analysis of the effect of leaf–air vapour pressure difference,  $D$ , on the intrinsic water-use efficiency (iWUE,  $\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ), stomatal conductance ( $g_s$ ;  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and photosynthetic rate ( $A_{\text{net}}$ ;  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) response ratios.

PFT	iWUE ( $\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ )		$g_s$ ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )		$A_{\text{net}}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	
	Slope	<i>P</i> -value	Slope	<i>P</i> -value	Slope	<i>P</i> -value
DAF	0.22	<b>&lt;0.001</b>	0.14	0.38	0.41	<b>&lt;0.001</b>
EAF	0.22	0.075	0.50	<b>0.006</b>	0.72	<b>&lt;0.001</b>
EGF	0.13	<b>0.021</b>	−0.18	<b>0.03</b>	−0.10	0.24

Table shows the slope response to  $D$  and respective *P*-values among plant functional type (PFT) that are shown in Fig. 2. Significant *P*-values ( $P < 0.05$ ) are shown in bold.

### Did $D$ affect responses to $e\text{CO}_2$ ?

Variability across experiments in stomatal responses to  $e\text{CO}_2$  may potentially be related to differences in the range of  $D$  in each experiment (Fig. S2). We tested how responses to  $e\text{CO}_2$  vary with  $D$  (Table 4; Fig. 2). Our expectation was that the response of iWUE to  $e\text{CO}_2$  would not vary with  $D$ , whereas the  $e\text{CO}_2$  response of  $A_{\text{net}}$  would increase, and the  $e\text{CO}_2$  response of  $g_s$  would decrease, with increasing  $D$ . Our results differed from these expectations in several ways. Fig. 2a shows the  $\text{CO}_2$ -induced increase in iWUE generally increased with  $D$  across all PFTs (Table 4). More specifically, the effect of  $D$  on the iWUE response to  $e\text{CO}_2$  was statistically significant for DAF ( $P < 0.001$ ) and EGF ( $P < 0.05$ ), and only EAF behaved in the way we expected.

We also found that the effect of  $D$  on the responses of  $A_{\text{net}}$  and  $g_s$  to  $e\text{CO}_2$  differed among the three PFTs ( $P < 0.05$ ; Fig. 2b,c; Table 4). For EAF, we found a significant effect of  $D$  on the  $g_s$  response to  $e\text{CO}_2$  ( $P < 0.05$ ; i.e. increase in  $D$  led to a smaller reduction in  $g_s$  in response to  $e\text{CO}_2$ ), and a significant effect of  $D$  on the  $\text{CO}_2$  response of  $A_{\text{net}}$  ( $P < 0.001$ ). These responses cancelled each other out to yield no change in the  $\text{CO}_2$  response of iWUE (Fig. 2a). For DAF, we found a significant positive effect of  $D$  on the  $\text{CO}_2$  response of  $A_{\text{net}}$  ( $P < 0.001$ ) but no effect on the response of  $g_s$ , yielding an overall positive  $e\text{CO}_2$  effect on iWUE. In contrast, for EGF, the effect of  $D$  on the  $\text{CO}_2$  response of iWUE resulted from a significant effect of  $D$  on the  $g_s$  response to  $e\text{CO}_2$  ( $P < 0.05$ ), and no effect on the  $A_{\text{net}}$  response to  $\text{CO}_2$ .

### Optimal stomatal model

To facilitate the interpretation of these differences among PFTs, we fitted the USO model (Eqn 2) to data from  $a\text{CO}_2$  and  $e\text{CO}_2$  treatments in all experiments (Figs 3, S3). The fitted model generally provided good explanatory power (median  $R^2$  of 0.70). Fitted values of the  $g_1$  parameter ranged from 1.4 to 7.9  $\text{kPa}^{0.5}$ . The  $g_1$  parameter was highest in the poplar species *Populus nigra* (7.9  $\text{kPa}^{0.5}$  (with 95% CI of 6.3–9.4) and 6.7  $\text{kPa}^{0.5}$  (with 95%

CI of 5.6–7.9) for  $e\text{CO}_2$  and  $a\text{CO}_2$  respectively) and lowest in *P. abies* (1.4  $\text{kPa}^{0.5}$  (with 95% CI of 1.3–1.49) and 1.45  $\text{kPa}^{0.5}$  (with 95% CI of 1.42–1.49) both  $a\text{CO}_2$  treatments in Flakaliden\_1 and Flakaliden\_2 respectively). We found that  $g_1$  was significantly lower in EGF species than in DAF and EAF species ( $P < 0.0001$ , Fig. 3; Table S1). A lower  $g_1$  value is suggested to lead to a larger response of  $A_{\text{net}}$ . In support of this, we found that the EGF species had both the lowest  $g_1$  and the highest photosynthetic response to  $e\text{CO}_2$ .

For 17 of the 20 datasets, there was no significant difference between the  $g_1$  parameters estimated for  $a\text{CO}_2$  and  $e\text{CO}_2$ . 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  $g_1$  parameters were significantly higher under  $e\text{CO}_2$ . In addition, we found that  $g_1$  was significantly higher in species that received supplemental water ( $P < 0.001$ ) and decreased significantly with tree age ( $P < 0.05$ ; Table S1).

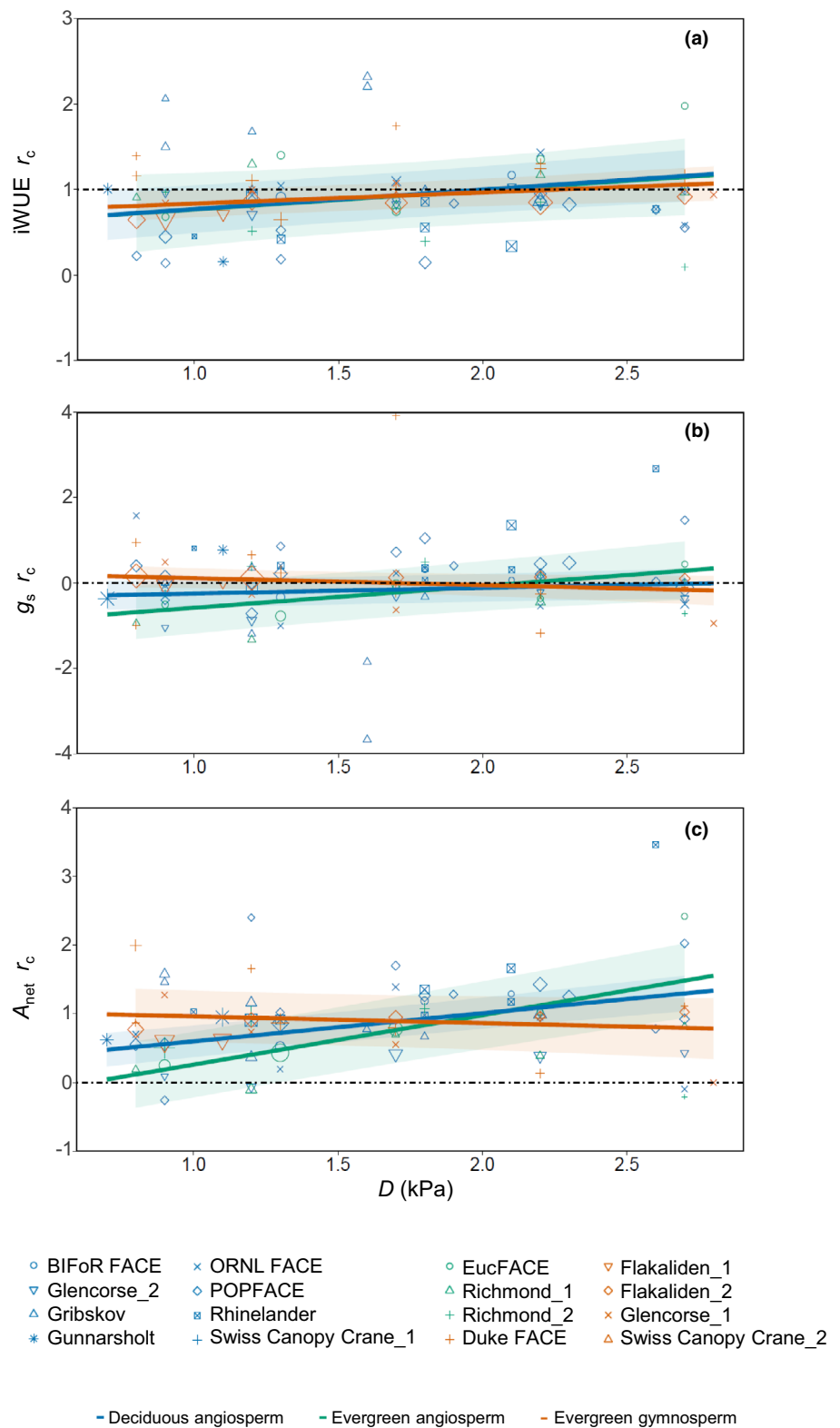
### Discussion

We quantified the relative responses of iWUE,  $g_s$  and  $A_{\text{net}}$  across a set of manipulative experiments on large trees (Table 1). We found that the mean response of iWUE was approximately proportional to  $e\text{CO}_2$ , 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  $e\text{CO}_2$  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  $g_1$  was not altered by  $e\text{CO}_2$  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  $e\text{CO}_2$ . Differences in the response of  $g_s$  to  $\text{CO}_2$  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  $g_1$  values, which drive a larger  $A_{\text{net}}$  response.

### Does iWUE respond in proportion to $e\text{CO}_2$ ?

The increases in iWUE with  $e\text{CO}_2$  are comparable to those reported in meta-analyses of FACE experimental data in Ainsworth & Long (2005; mean  $r_c = 1.2 \pm 0.33$ ). 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 (Battipaglia *et al.*, 2013; mean  $r_c = 1.4$  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  $r_c$  for iWUE close to one (Walker *et al.*, 2020). We conclude that the proportional increase in iWUE with  $e\text{CO}_2$  is a reliable response which supports the



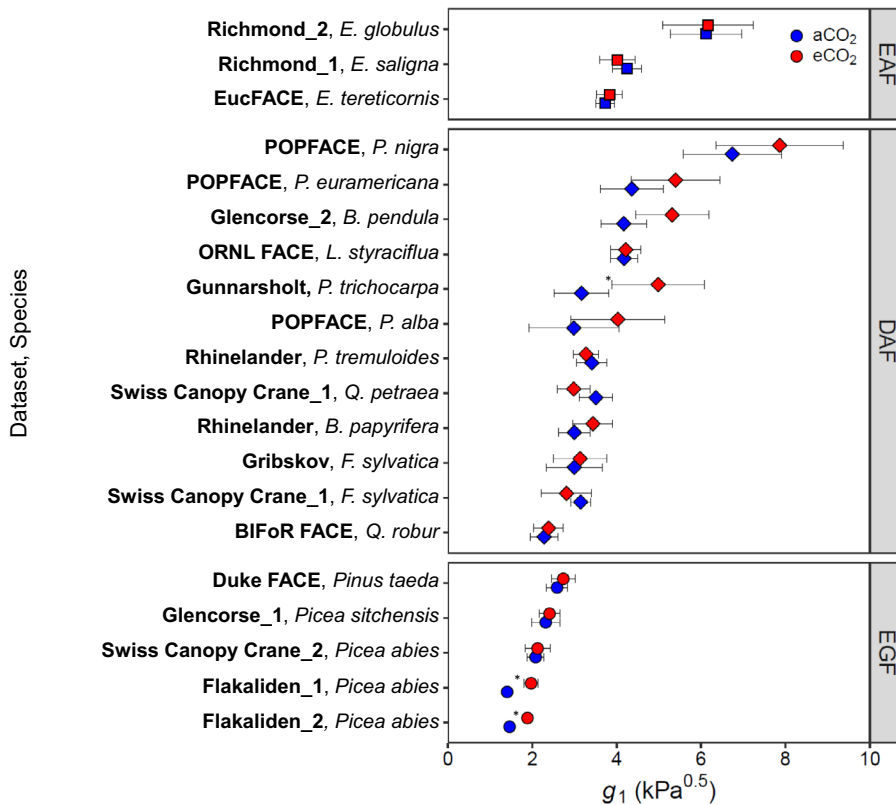


**Fig. 2** The effect of  $D$  on the mean log response ratios for the  $CO_2$  effect ( $r_c$ ) on (a) intrinsic water-use efficiency ( $iWUE$ ), (b) stomatal conductance ( $g_s$ ) and (c) net photosynthesis ( $A_{net}$ ) across the datasets. Each data point shows the mean response for each 0.5 kPa 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.

optimisation theory that proposes  $A_{net}$  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  $A_{net}$  response and vice versa.

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

We found increases in  $iWUE$  with  $eCO_2$  were predominantly due to increased  $A_{net}$  rather than reductions in  $g_s$ . This conclusion is consistent with results from several previous meta-analyses of



**Fig. 3** Fitted parameter with 95% confidence intervals for the unified stomatal optimisation (USO) model ( $g_1$ ) for each CO<sub>2</sub> treatment per species and dataset, grouped by plant functional type (PFT). Colours indicate CO<sub>2</sub> treatment, with blue for ambient CO<sub>2</sub> (aCO<sub>2</sub>) and red for elevated CO<sub>2</sub> (eCO<sub>2</sub>). Symbols refer to the PFT with diamonds as deciduous angiosperm (DAF), squares as evergreen angiosperm (EAF) and circles as evergreen gymnosperm (EGF). \*, Significant differences in  $g_1$  between CO<sub>2</sub> treatments.

physiological responses of tree species, although the statistical significance of the reduction in  $g_s$  varies across studies. Curtis & Wang (1998) found an increase in  $A_{\text{net}}$  of +54% compared with a non-significant reduction in  $g_s$  of -11%. Medlyn *et al.* (2001) found a statistically significant reduction of -14% in  $g_s$  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  $g_s$  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 CO<sub>2</sub> concentrations used in different experiments. We find a mean increase ( $r_c$ ) of 0.82 in  $A_{\text{net}}$  and no statistical change in  $g_s$  (mean reduction of -0.09).

However, we also found the responses of both  $A_{\text{net}}$  and  $g_s$  to eCO<sub>2</sub> 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 eCO<sub>2</sub> increased with increases in  $D$ . We observed differences in  $D$  sensitivity among PFTs for the responses of  $A_{\text{net}}$  and  $g_s$  to eCO<sub>2</sub> (Fig. 2). Changes in  $A_{\text{net}}$  due to eCO<sub>2</sub> 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 eCO<sub>2</sub>. A further environmental variable that can alter the responses of both  $A_{\text{net}}$  and  $g_s$  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 eCO<sub>2</sub>, which may be diminished under lower PPFD (Bernacchi *et al.*, 2003; Uddling *et al.*, 2009; Bader *et al.*, 2016).

To avoid generalisations based on the mean responses of iWUE,  $A_{\text{net}}$  and  $g_s$  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  $g_s$  under both aCO<sub>2</sub> and eCO<sub>2</sub> and the  $g_1$  parameter remained unchanged in response to eCO<sub>2</sub> (Gimeno *et al.*, 2016), with three exceptions (Fig. 3). The three cases where CO<sub>2</sub> 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  $g_1$  parameter remained unchanged with eCO<sub>2</sub> in *P. abies* growing on fertile soil at the Swiss Canopy Crane site

(Bader *et al.*, 2013). However, other sites also have highly nutrient-limited soils, including the EucFACE experiment. Alternatively, it is notable that all three exceptions were from boreal study sites, suggesting that trees in boreal climates may deviate in responses of stomata to  $e\text{CO}_2$ . However, we only had three suitable datasets from boreal trees; it would be valuable to test this discrepancy in other boreal sites.

Other limitations of the database available for this study are that we only had one genus within the EAF group, all from one geographical location, and no data at all from the tropics. This motivates future experiments with a focus on a wider range of boreal species in addition to more EAF species, from different regions such as in tropical forests, to confirm the stomatal responsiveness with  $e\text{CO}_2$  of these PFTs.

Overall, this study found that the optimal stomatal model was successful in characterising the response to  $e\text{CO}_2$  across a wide range of experiments and suggests that the coupling between  $A_{\text{net}}$  and  $g_s$  remained unchanged to  $e\text{CO}_2$ . Hence, we can recommend that land surface models would benefit from using the  $g_1$  parameter to characterise the behaviour of stomata to a changing environment. However, it is also important to recognise that we limited our dataset to conditions of  $PPFD > 800 \mu\text{mol}$  and  $D < 3 \text{ kPa}$  to maximise comparability among datasets. It is possible that responses to  $\text{CO}_2$  will differ at the leaf scale compared to canopy scale (Keenan *et al.*, 2016) as different light and  $D$  conditions prevail in the lower canopy (Crous *et al.*, 2020). Further work will be required to assess the model under different conditions, and test scalability to the canopy.

### How should we model differences among PFTs?

Previously, research has suggested that physiological responses to  $e\text{CO}_2$  differ among woody PFTs, with smaller  $i\text{WUE}$  and  $g_s$  responses observed in gymnosperms than in angiosperms (Saxe *et al.*, 1998; Medlyn *et al.*, 2001; Niinemets *et al.*, 2011; Klein & Ramon, 2019). One goal of this work was to evaluate how this difference among PFTs could be captured in the USO or similar stomatal models. In contrast to previous research, we found that the increases in  $i\text{WUE}$  with  $e\text{CO}_2$  were broadly similar across the three PFTs examined in the study. When examining the analysis of this study with that of previous analyses, the main difference lies in the calculation of the responses. Previous analyses predominantly examined absolute responses to  $e\text{CO}_2$ , in contrast to relative responses in this study. Therefore, starting stomatal values in previous analyses were generally lower in EGF than in both DAF and EAF species and likely influenced the conclusions (Klein & Ramon, 2019). The strength of this analysis is that we examined the relative responses using replicates, as stated by each individual dataset, as well as including the capacity to correct for  $D$ .

We also found no significant differences among PFTs in the  $e\text{CO}_2$  responses of  $g_s$  (Fig. 1; Table 3). However, we did observe that the  $g_s$  response in the evergreen gymnosperms was least sensitive to  $e\text{CO}_2$  and  $g_s$  was significantly reduced with  $e\text{CO}_2$  in evergreen angiosperms. We also found that there was a tendency for EGF species to have a higher  $A_{\text{net}}$  response. This outcome may be a result of the fact that this PFT also had the lowest  $g_1$  values,

consistent with other reviews of species differences in  $g_1$  (Lin *et al.*, 2015). A low  $g_1$  implies a low intercellular  $\text{CO}_2$  concentration and thus a higher responsiveness of photosynthesis to rising  $\text{CO}_2$ . 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  $\text{CO}_2$ . It resolves some of the long-standing questions regarding stomatal behaviour in elevated  $\text{CO}_2$  in woody species. The results show that the  $e\text{CO}_2$  responses of  $i\text{WUE}$ ,  $g_s$  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  $i\text{WUE}$  that occur with  $e\text{CO}_2$  in all vegetation types. However, the results highlighted the importance of incorporating environmental factors, such as  $D$ , when comparing vegetation type. Our analysis of  $\text{CO}_2$  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  $e\text{CO}_2$  and  $D$  (Fig. 3). We highlight the areas where data representation needs improvement but conclude that a stomatal parameter such as  $g_1$  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  $\text{CO}_2$  increases into the future.

### Acknowledgements

AG gratefully acknowledges a studentship provided by the John Horseman Trust and the University of Birmingham. The BIFoR FACE facility is supported by the JABBS Foundation, the University of Birmingham and the John Horseman Trust. ARMK acknowledges support from the UK Natural Environment Research Council through grant NE/S015833/1. MJ and BEM acknowledge funding from the Australian Research Council (DE210101654, FL190100003).

### 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, SLeuzinger, JU, JW, GW, BEM contributed to the interpretation of the results and intellectual input of this manuscript revision. All authors read and approved the submitted version.

## ORCID

Martin Karl-Friedrich Bader  <https://orcid.org/0000-0002-3742-9762>  
 Craig V. M. Barton  <https://orcid.org/0000-0003-0085-0534>  
 Kristine Y. Crous  <https://orcid.org/0000-0001-9478-7593>  
 David S. Ellsworth  <https://orcid.org/0000-0002-9699-2272>  
 Anna Gardner  <https://orcid.org/0000-0002-4625-9769>  
 Teresa E. Gimeno  <https://orcid.org/0000-0002-1707-9291>  
 Marianne Hall  <https://orcid.org/0000-0001-8430-6880>  
 Mingkai Jiang  <https://orcid.org/0000-0002-9982-9518>  
 Shubhangi Lamba  <https://orcid.org/0000-0003-0438-6134>  
 Sebastian Leuzinger  <https://orcid.org/0000-0001-9306-5281>  
 A. Robert MacKenzie  <https://orcid.org/0000-0002-8227-742X>  
 Johan Uddling  <https://orcid.org/0000-0003-4893-1915>  
 Göran Wallin  <https://orcid.org/0000-0002-5359-1102>

## Data availability

The data that support the findings of this study are publicly available via FigShare (doi: [10.6084/m9.figshare.21252609.v1](https://doi.org/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 CO<sub>2</sub> (aCO<sub>2</sub>; blue) and elevated CO<sub>2</sub> (eCO<sub>2</sub>; red) for each of the studies in the dataset.

**Fig. S2** Histograms showing the counts of binned *D* (0.5 kPa) across the ambient CO<sub>2</sub> (blue) and elevated CO<sub>2</sub> (red) treatments for each of the studies in the dataset.

**Fig. S3** Relationship between stomatal conductance (*g<sub>s</sub>*) and an index based on the USO formulation calculated from *D*, *A<sub>net</sub>* and CO<sub>2</sub> 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<sub>1</sub>* (kPa<sup>0.5</sup>) values.

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