RESEARCH ARTICLE

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Stomatal sensitivity to CO_2 diverges between angiosperm and gymnosperm tree species

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Abstract

1. The response of tree leaf gas exchange to elevated CO_2 concentrations has been investigated in numerous experiments along the past 30 years. Stomatal regulation is a major plant control over leaf gas exchange, and the response to the increasing CO_2 will shape the biological activity of forests in the future.

2. Here, we collected 144 records from 57 species on stomatal conductance in CO_2 manipulation experiments on trees (340–980 ppm CO_2). CO_2-induced stomatal downregulation was calculated as the slope of the linear regression between stomatal conductance and $[CO_2]$.

3. Among tree species, the slope (a) of change in stomatal conductance per 100 ppm CO_2 increase ranged between 0 and −151, indicating stomatal downregulation, and only four species showed upregulation. There was a significant divergence between evergreen gymnosperms (a = −3.6 ± 1.0), deciduous angiosperms (a = −16.3 ± 3.1) and evergreen angiosperms (a = −32.8 ± 7.1). Gymnosperms were less sensitive to CO_2 changes than deciduous angiosperms even when considering only field experiments. The significant role of tree functional group in predicting CO_2-induced stomatal downregulation was detected in multiple mixed-effect models, with p values ranging between 0.0002 and 0.0295.

4. The significantly higher stomatal sensitivity to CO_2 of angiosperms versus gymnosperms might be related to the overall higher stomatal conductance of angiosperms; their thinner leaves, in turn losing water faster; and the decreasing atmospheric $[CO_2]$ at the time of their taxa diversification. We conclude that species differences must be taken into account in forecasting future forest fluxes.

KEYWORDS
biome, deciduous, evergreen, leaf thickness, stomatal conductance, stomatal downregulation

1 | INTRODUCTION

Atmospheric CO_2 concentration is increasing by more than 1.5 ppm/year and will continue to increase due to human activity. Direct effects on plants have been the subject of numerous experiments over the past decades, mostly focusing on photosynthesis and growth rate (Ainsworth & Long, 2005; Medlyn et al., 1999; van der Sleen et al., 2015) and on crop yield (Long, Ainsworth, Leakey, Nösberger, &

Ort, 2006 and references therein). Experiments on trees have particular importance due to their dominant role as ecosystem designers and drivers of biogeochemical cycles. Small-scale greenhouse experiments led the way to field experiments, first using open-top chambers and branch bags, and later with free-air CO_2 enrichment (FACE). Creating an artificial atmosphere with an elevated CO_2 level has been a major technical and financial challenge. Synthesis of CO_2 experiments on trees has been offered in the past (Ainsworth &

Long, 2005; Curtis & Wang, 1998; Medlyn et al., 1999, 2001). Since new studies are being published year by year, the option of comparison among a large number of species, leaf habits and biomes has now become possible.

Many of the CO_2 experiments on trees reported a decrease in stomatal conductance (g_s) under elevated CO_2, that is, stomatal downregulation at the stomatal aperture level (Medlyn et al., 2001 and references therein). Stomata open to uptake carbon, in turn driven by tree growth, among other carbon sinks (Fatichi, Leuzinger, & Körner, 2014). Considering that water is lost during carbon uptake, and since both processes are diffusive, stomatal downregulation at increasing ambient CO_2 is expected. This is not to be mixed with reduction in the number of stomata per leaf area, that is, stomatal density, which has also been observed to occur in parallel in some cases (Berryman, Eamus, & Duff, 1994; Eamus, Berryman, & Duff, 1993; Goodfellow, Eamus, & Duff, 1997; Haworth, Killi, Materassi, Raschi, & Centritto, 2015) but not in others (Barton & Jarvis, 1999; Herrick, Maherali, & Thomas, 2004; Tricker et al., 2005; Yu-Mei, Shi-jie, Ying, & Xia, 2005). The two responses have been suggested to act at different temporal scales, that is, g_s reduction at the short-term and stomatal density reduction at the long-term (Berryman et al., 1994). However, reverse patterns have also been observed (Tricker et al., 2005). Alternatively, the two responses were interpreted as being complementary at the species level (e.g. in Mango trees; Goodfellow et al., 1997) or at the phylogenetic group level (i.e. serving different strategies by different species of the same taxonomic group; Haworth et al., 2015).

Studying the sensitivity of stomatal conductance to atmospheric CO_2 concentration, that is, $g_s(CO_2)$, a lower response in conifers versus broadleaves has already been suspected, yet not directly demonstrated (Medlyn et al., 2001; Saxe, Ellsworth, & Heath, 1998). Due to the relatively small number of species tested, differences between the plant groups were confounded with tree age. In the dataset that was used in Medlyn et al. (2001), most experiments on conifers were on mature trees, whereas most experiments on broadleaves were on saplings. In conclusion, the authors were unable to determine whether there was a clear divergence in $g_s(CO_2)$ between gymnosperms and angiosperms. Earlier studies on smaller sets of species have also reported lower $g_s(CO2)$ sensitivity in conifers versus broadleaves, but no speculation on group-based divergence has been made. For example, g_s reductions under elevated CO_2 were 32%, 29% and 26% in the broadleaved *Cercis canadensis*, *Quercus rubra* and *Populus deltoides* × *P. nigra*, respectively; and only 11% in *Pinus taeda* (Will & Teskey, 1997). In another study, the lower $g_s(CO_2)$ sensitivity in the conifers *Pinus banksiana*, *Picea mariana* and *Larix laricinia* compared to that of the broadleaved *Populus tremuloides* and *Betula papyrifera* (Tjoelker, Oleksyn, & Reich, 1998) was attributed to growth rate rather than taxonomy. Nevertheless, differences in $g_s(CO_2)$ sensitivity between species were acknowledged in most studies, especially when higher than other effects (e.g. temperature; Tjoelker et al., 1998).

Interspecific differences in $g_s(CO_2)$ might be related to morphological, physiological and evolutionary processes. Leaves are surface organs designed to maximize photosynthesis, while minimizing water loss (Reich, 2014). Maximizing photosynthesis means increasing light absorbance and CO_2 availability (Buckley, Sack, & Farquhar, 2017; Reich, 2014), whereas minimizing water loss entails the use of hydrophobic coating in the cuticle, and forming a thick boundary layer (Maseda & Fernández, 2006; Tanny & Cohen, 2003). Trade-offs between photosynthetic and hydration requirements do exist: for example, a thick boundary layer reduces water loss but in turn also reduces CO_2 concentration in the proximity of the leaf (Buckley et al., 2017). Simple leaves and needle leaves represent contrasting solutions to these design requirements. Simple leaves increase surface area by forming a thin slab, while needles increase surface area by forming a finger-like structure, which must be thicker due to mechanical constraints. Leaf architecture differences in guard cell and epidermis cell structures also exist (Rockwell & Holbrook, 2017), but cross-species data are still limited. Recently, an evolutionary perspective was added to the interpretation of interspecific differences in $g_s(CO_2)$. Based on the comparison of seven plant species, it has been suggested that past atmospheric CO_2 concentration during the time of taxa diversification influences stomatal response time: low atmospheric $[CO_2]$ during taxa diversification may have placed a selection pressure on plants to accelerate stomatal closing to maintain adequate internal CO_2 and optimize water-use efficiency (Elliott-Kingston et al., 2016). Two conifer species and a *Gingko* tree were included in that study, but the other four species were not woody plants, and hence, a conifer/broadleaf comparison was missing.

Typically, stomatal responses bear consequences on tree carbon uptake rates. Yet, despite the CO_2-induced stomatal downregulation, rates of photosynthesis were usually higher under elevated CO_2. For example, in *Betula pendula*, both carboxylation efficiency decreased and g_s decreased (g_s by 21%), but photosynthesis increased by 33%, due to passive diffusion under elevated CO_2 (Rey & Jarvis, 1998). In turn, this meant an increase in water-use efficiency, which has been reported in the majority of tree-CO_2 studies (e.g. Leuzinger & Körner, 2007, Klein et al., 2016, Paudel et al., 2018 for deciduous angiosperm, evergreen gymnosperm and evergreen angiosperm species, respectively). Overall, across species, although maximum Rubisco activity, potential electron transport rate and leaf nitrogen concentration are typically reduced by $c.10\%$, photosynthesis is still enhanced (Medlyn et al., 1999).

Here, we complied a species-rich dataset of stomatal conductance data from CO_2 experiments on trees. Using these data, we test for patterns among biomes and functional groups of phylogeny and leaf habit. We also consider variations between experimental setups, treatments and additional parameters such as tree age and length of exposure. Ultimately, we test hypotheses regarding underlying mechanisms of divergence in CO_2-induced stomatal downregulation among functional groups. Specifically, we used auxiliary data to test whether stomatal sensitivity to CO_2 scales with the average level of stomatal conductance, that is, that higher baseline g_s offers a wider range for stomatal closure (H1); the decrease in leaf thickness, since the thinner the leaf, the larger the water savings (H2); and the decrease in atmospheric CO_2 level at the time of taxa diversification,

an evolutionary driver of high stomatal sensitivity (H3, as formulated in Elliott-Kingston et al. (2016)).

2 | MATERIALS AND METHODS

2.1 | Data collection

Our data collection and analysis followed the guidelines detailed in the handbook of meta-analysis in ecology and evolution (Koricheva, Gurevitch, Mengersen, and (Eds.)., 2013). Scientific papers reporting CO_2 experiments on trees were obtained from Google Scholar using the search term ('stomatal conductance' 'elevated CO_2' 'tree'). Among the search results, studies on non-tree plants were filtered out. Studies included in the analysis were those reporting all of the following parameters: the tree species being tested (in one case there was a mixture of three species; Keel, Pepin, Leuzinger, & Körner, 2007), the experiment place and time, tree age, length of exposure, the experimental design, the number of replicates, the g_s measurement method, the CO_2 levels applied and the mean g_s at each CO_2 concentration. We also extracted the trees' growth conditions, including the light levels, temperatures and water vapour deficit (VPD). In case VPD was not reported, we estimated it using data on temperatures and relative humidity.

In total, 144 experimental records were used, spanning over 57 species under different treatments (including seasonal changes). These records were extracted from 37 scientific papers, published between 1985 and 2018, with the majority published between 1990 and 2010. The data represented the wide range of tree functional groups and biomes, as well as the range of experimental designs and measurement methods (Table 1). While permitting a robust meta-analysis, our dataset was not an exhaustive one, and hence, some important studies are still missing. Specifically, since we needed as many different tree species as possible, reports related to the same study on the same species were of lesser importance to the dataset. The majority of the species were forest trees, with the exception of the fruit trees mango, apple and lemon. The constructed dataset, while not being an exhaustive collection, offers a good mix of tree functional groups from all key biomes, grown in both field and greenhouse conditions (Table 1). The main body of the results is based on the synthesis of case- and species-specific calculated stomatal downregulations. To test the consequences of stomatal response to elevated [CO_2], transpiration and photosynthesis data were taken from two experiments conducted by the authors, which have been published (Klein et al., 2016; Paudel et al., 2018). Auxiliary data of leaf thickness, maximum rate of carboxylation, specific leaf area and wood density were obtained through the plant trait database (TRY; Kattge et al., 2011; trait IDs 46, 186, 11 and 4, respectively).

2.2 | Data analysis

In CO_2 experiments on trees, which measured stomatal conductance, CO_2-induced stomatal downregulation was usually expressed as the per cent change in g_s at the elevated relative to the ambient [CO_2].

TABLE 1 Distribution of the data and species across eco-physiological groups and experimental procedure types

Functional group	n	Biome	n	Setup	n	Measurement method	n	Experimental design	n
Evergreen Gymnosperm	43 (7)	Tropical	13 (11)	Field	101	IRGA	129	Branch bag	18
Deciduous Gymnosperm	1 (1)	Subtropical	12 (9)	Greenhouse	43	Sap flow-based	8	FACE	37
Evergreen Angiosperm	39 (31)	Semi-arid	6 (6)			Porometer	7	Greenhouse	12
Deciduous Angiosperm	61 (18)	Mediterranean	5 (3)					Growth chamber	16
		Temperate	96 (21)					Mini-ecosystem	15
		Boreal	7 (7)					Open-top chamber	46
Total	144 (57)		144 (57)		144		144		144

Note: The number of records and species, *n* (*n*), respectively, are given for each group. FACE, free-air CO_2 enrichment; IRGA, infrared gas analyser.

While such approach is valid for a single study, using it to compare across studies with different ranges of CO_2 treatments is problematic. For example, Klein et al. (2016) applied a treatment of 550 ppm CO_2, whereas Paudel et al. (2018) used the three CO_2 concentrations 400, 650 and 850 ppm. The latter example presents an additional challenge since there is more than one option to calculate percent change for three levels, which was the case in 9 of the 144 records. Based on these considerations, we defined CO_2-induced stomatal downregulation as the linear slope (a) of the $g_s(CO_2)$ regression, in mmol m^{-2} s^{-1} 100 ppm^{-1}. Among the 9 three-point [CO_2] experiments, linear regressions produced correlation coefficients >0.95 in most of the cases, suggesting that the slope parameter was a good representation of the $g_s(CO_2)$ dependence. Across the different studies, the ambient [CO_2] was between 340 and 400 ppm, and the elevated [CO_2] was between 500 and 980 ppm. Within this range of [CO_2], g_s typically declines in a quasi-linear fashion, as demonstrated by both empirical and optimal approaches (Medlyn et al., 2011). This was further confirmed by four different models of optimal stomatal behaviour under mild light (Buckley et al., 2017). Notably, under saturating light, two of the four models predicted quasi-linear decline in g_s only at [CO_2] >600 ppm (Buckley et al., 2017). The case-specific responses are detailed in Table S1, along with the additional species and experiment parameters. Nevertheless, to allow for comparison with earlier meta-analyses, we include a calculation of % change per 100 ppm [CO_2] (Table S1 and text below).

As often observed in meta-analysis studies in plant ecology, experiments included in the analysis diverge in their growth conditions, potentially affecting the studied responses. For example, light levels, temperatures and VPD had contrasting ranges among studies. Earlier synthesis studies on the topic have partly solved this problem by adjusting the reported values to saturating light (e.g. photosynthetically active radiation, PAR, of 1,000 μmol m^{-2} s^{-1}; although higher values exist for species from high-light regions) and a standard low VPD of 1.0 kPa (Medlyn et al., 1999). This was permitted by the application of typical response curves of g_s to each of the environmental parameters. While such approach was successful in smaller datasets, multiple assumptions were involved (e.g. homogeneous behaviour across species; extrapolation at low and high parameter values) and potential interactions were not sufficiently regarded. Instead, we used the values of g_s as originally reported and examined the dependence of these values in light, temperatures and VPD, across all the measurements of the dataset. Particularly, we checked for potential biases of specific species groups (phylogeny and leaf habit) towards specific conditions (e.g. high light, temperature or VPD). To allow for quantitative analysis, we extracted the minimum PAR, the minimum and maximum temperature, and the maximum VPD of each experiment and correlated between each parameter and the measured stomatal sensitivity. To this end, we estimated saturating light, where not specifically reported, as 1,000 μmol m^{-2} s^{-1}, and disregarded night temperatures for estimation the effects of minimum and maximum temperatures. In addition to these considerations, the method of g_s measurement was also recorded, with 90% of records (129 experiments) obtained using an infrared gas analyser

(IRGA), applied directly on the leaf surface. Seven records were obtained using a leaf porometer, and the remaining eight records were calculated from whole-tree sap flow measurements. These eight records are from the large FACE experiments on *Pinus taeda* and *Liquidamber styraciflua*, in Duke University and ORNL, respectively. In both cases, IRGA measurements for these species were also available, and hence, comparison between these measurements is included in our analysis.

2.3 | Statistical analysis

We constructed a number of mixed-effect models with stomatal sensitivity to CO_2 level ($gs(CO_2)$) as the dependent variable. Discrete models were built for the full dataset, the dataset restricted to field experiments and the dataset restricted to high-latitude biomes, to account for the under-representation of gymnosperm species in low-latitude biomes. Parameters tested as fixed and random effects were all factors identified by previous research. Functional group, biome, tree age and exposure length were taken as fixed effects and measurement method and experimental design as random effects. The single-species group 'deciduous gymnosperm' was removed from these analyses in sake of a more balanced representation of functional groups. For each model, we calculated the corrected Akaike's information criteria (AICc). Model and fixed effects were tested by F test and t test, respectively. Random effects were tested by Wald p test. All analyses were performed using the software JMP.

Additional statistical analyses included the following: (a) A histogram was constructed to test the distribution of the variability of stomatal sensitivity to CO_2 across the 144 experiments. To fully represent the range of responses, we used 16 bins of 20 mmol m^{-2} s^{-1} 100 ppm^{-1} each. To further characterize the distribution of stomatal sensitivity to CO_2 across the 144 experiments, we fitted normal and Weibull distribution curves and tested the goodness-of-fit using Shapiro–Wilk W test and Cramer-von Mises W test (respectively), and p-values are reported. (b) Parameter effects on $g_s(CO_2)$ were tested using ANOVA at significance level, α = 0.05. When a significant difference was found, multiple mean comparison was performed by Tukey–Kramer HSD test. (c) The effect of continuous parameters on $g_s(CO_2)$ was tested using correlations, and r^2 values are reported. All analyses were performed using the software JMP.

3 | RESULTS

3.1 | CO_2-induced stomatal downregulation among tree species

Among 57 species in 144 experimental treatments (Table S1), CO_2-induced stomatal downregulation ranged in a continuum of responses between a = 0 and a = −103 (Figure 1). The tropical evergreen angiosperm *Cecropia* species had the minimum value at a = −151. Four tree species exhibited upregulation: *Eucalyptus grandis*, *Trichospermum mexicanum*, *Acacia tetragonophylla* and *Acacia*
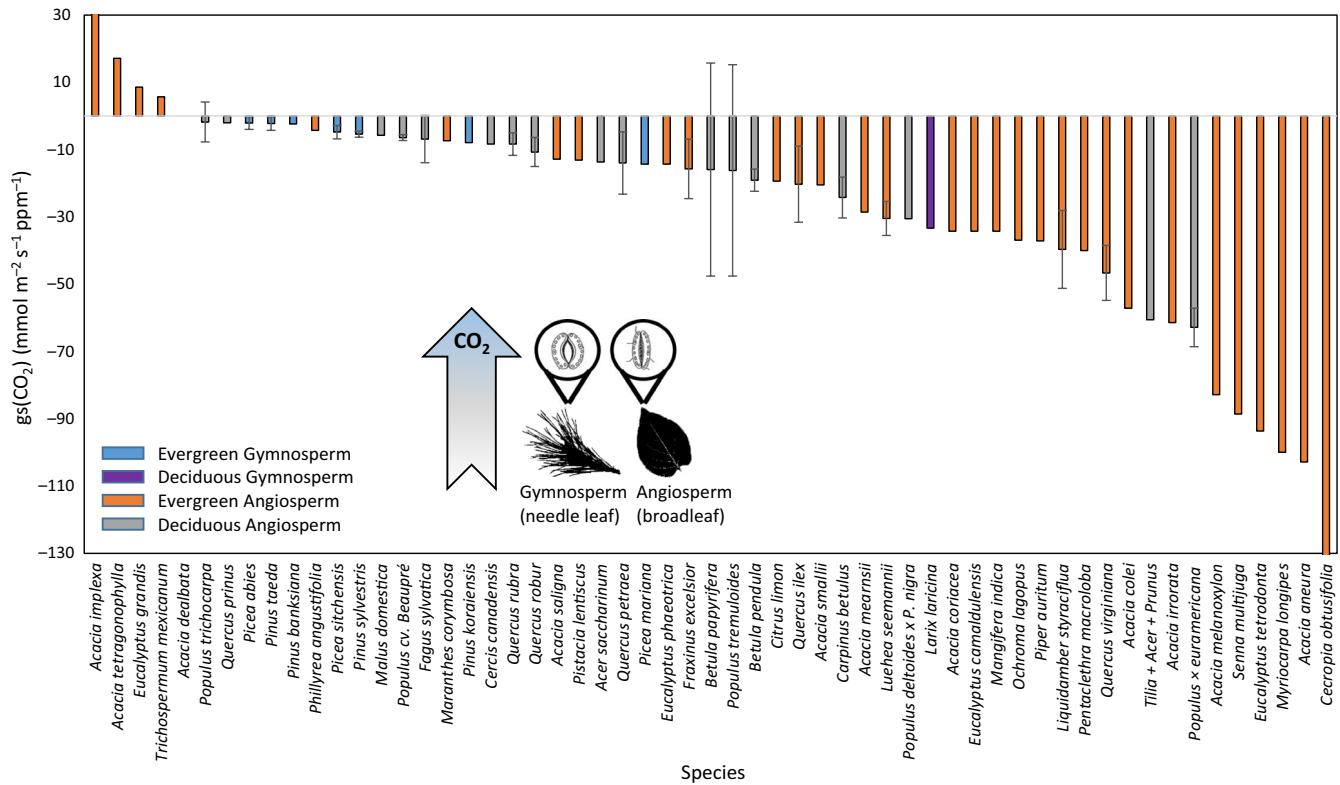
FIGURE 1 The variability of stomatal sensitivity to CO_2 across 57 tree species. Values are slopes of species-specific $g_s(CO_2)$ regression curves, expressing the change in stomatal conductance (mmol m^{-2} s^{-1}) per 100 ppm CO_2 increase (means ± standard error, where possible). To improve visibility, bars for the species representing minimum (−151.4) and maximum (134.3) responses were truncated. The inset illustrates the divergence in stomatal sensitivity to elevated CO_2 between gymnosperms and angiosperms

implexa, the latter showing an extreme value of a = 134. Three of these species (except for *Acacia tetragonophylla*) are among the fastest-growing trees, and hence, their exceptional carbon sink demand might be related to the $g_s(CO_2)$ deviation. Notably, the seven evergreen gymnosperms were among the tree species with mild stomatal response (Figure 1). The five highest and six lowest species were evergreen angiosperms, while deciduous angiosperms mostly populated the moderate downregulation section of the graph. The distribution of the 144 cases peaked at the relatively mild downregulation between a = 0 and a = −20 (Figure 2a). Fitted normal distribution and Weibull distribution curves were both rejected with low goodness-of-fit (p < 0.0001 and p = 0.0100, respectively). This might be related to the few extreme values in the dataset.

3.2 | Changes in stomatal downregulation across biomes, experimental setups and functional groups

CO_2-induced stomatal downregulation diverged significantly across biomes, experimental setups and functional groups (Table 2; Figure 2b–d). Among the models tested for the full dataset, both functional group and biome produced significant effects on $g_s(CO_2)$. The random effects, namely measurement method and experimental design, had no significant effect. Notably, a model based on both group and biome did not yield significant effects, and a model incorporating tree age and exposure length showed no effect for these

parameters either, but a significant effect of group (Table 2). The latter model also produced the minimum AIC term. When considering field experiments alone, models based on group, biome and both factors together produced significant effects. The minimum AIC was found for group effect alone. When considering high-latitude biomes alone, only group had a significant effect (Table 2). Based on the effects detected in the mixed-effect models, we further analysed the influence of biome and functional group, as well as experimental setup, on $g_s(CO_2)$. Stomatal sensitivity to $[CO_2]$ decreased moving from low- to high-latitude biomes (Figure 2b). Tropical tree species showed stronger stomatal downregulation (a = −51.9 ± 7.7) than that of trees in subtropical, Mediterranean, temperate and boreal sites. Conversely, temperate species had the mildest stomatal downregulation (a = −10.8 ± 2.8), diverging significantly from tropical species. Experiments in the field showed milder responses than greenhouse trials: a = −11.0 ± 2.8 versus −a = 31.4 ± 4.4, respectively (Figure 2c). This difference might indicate the existence of interactions with additional factors, such as competition over light, water and nutrients, which play a bigger role in the field than in the greenhouse. Field experiments were typically much longer than greenhouse trials (49.4 ± 2.9 vs. 10.4 ± 2.7 months) and hence minimize the effects of short-term responses, which tended to level off gradually (but see below).

There was a significant divergence among gymnosperms (a = −3.6 ± 4.3), deciduous angiosperms (a = −16.3 ± 3.6) and evergreen
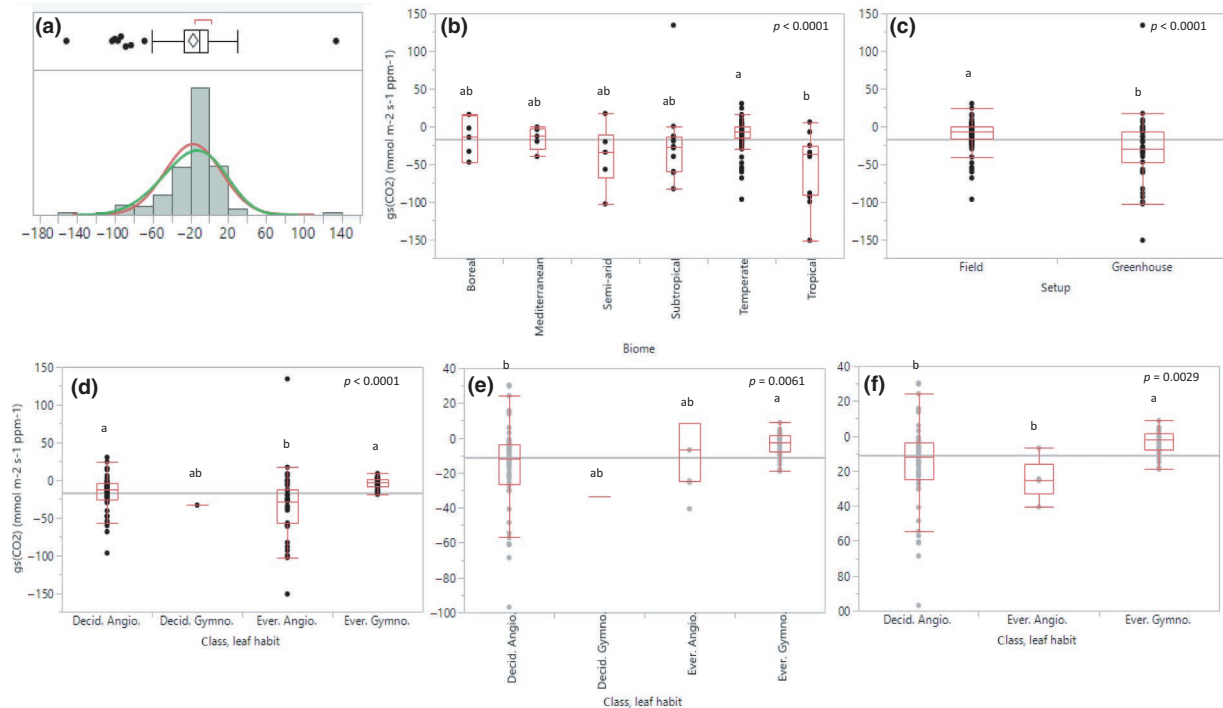
FIGURE 2 The distribution of the variability of stomatal sensitivity to CO_2 across 144 experiments on 57 tree species (a). The red and green curves denote the expected normal and Weibull distributions, both rejected. Boxplots for categorical factors affecting tree stomatal sensitivity to CO_2: biome (b); experimental setup (c); and functional group (d–f). The effect of functional group was also tested considering boreal and temperate biomes alone (e), or field experiments alone (f). Different letters on boxes within the same graph denote significant difference according to Tukey–Kramer HSD test. *p* values are from ANOVA

angiosperms (*a* = −32.8 ± 4.5) (Figure 2d; Table 2). There was not one single case of downregulation by a gymnosperm more negative than *a* = −20.0, except for the deciduous *Larix* species (*a* = −33; Figure 1, Table S1). Responses of the three fruit tree species were typical of their functional groups, with mild stomatal downregulation in the deciduous apple (*a* = −5.7) and stronger downregulation in the evergreen lemon and mango (*a* = −19.4 and −34.3, respectively). Differences among functional groups prevailed also when calculating stomatal sensitivity to [CO_2] as % change 100 ppm^{-1}: from low-sensitivity gymnosperms (−2.2% ± 1.3) to high-sensitivity deciduous angiosperms (−6.2% ± 1.0), and evergreen angiosperms (−8.3% ± 2.3). When considering high-latitude biomes alone (Figure 2e), deciduous angiosperms showed a milder response to CO_2 than evergreen angiosperms. Removing the results of greenhouse experiments yielded a significant difference between gymnosperms and angiosperms (Figure 2f). In both cases, evergreen gymnosperms were consistently and significantly less sensitive to CO_2 than deciduous angiosperms.

3.3 | Processes underlying the variation in stomatal sensitivity to CO_2 among gymnosperms and angiosperms

The significant difference in stomatal sensitivity to CO_2 between gymnosperms and angiosperms should relate to fundamental physiological differences between the groups. Among major leaf traits, specific leaf area (SLA) and the maximum rate of carboxylation

($V_{c\,max}$) have been widely measured, including 27 and 30 of the species in this study, respectively. Correlating the CO_2-induced stomatal downregulation to these traits shows the relatively low SLA of most gymnosperms, but no relationship was identified (Figure S1a,b). Considering the 'plant economics spectrum', we also added a correlation with wood density, a frequently measured trait (with data available for 47 of our species) that is strongly linked to tree growth rate (Chave et al., 2009; Reich, 2014). Among the softwood gymnosperms and among the (mostly) hardwood angiosperms (more so in the evergreen angiosperms), there was no visible pattern (Figure S1c). In the introduction, we presented three hypotheses to test for processes underlying the variation in stomatal sensitivity to CO_2 among gymnosperms and angiosperms. Specifically, we used auxiliary data to test whether stomatal sensitivity to CO_2 scales with the average level of stomatal conductance, that is, that higher baseline g_s offers a wider range for stomatal closure (H1); the decrease in leaf thickness, since the thinner the leaf, the larger the water savings (H2); and the decrease in atmospheric CO_2 level at the time of taxa diversification, an evolutionary driver of high stomatal sensitivity (H3).

(H1) Differences in stomatal function between broadleaves and conifers go beyond the CO_2 level context. In general, g_s was significantly higher in broadleaves, and more so in evergreen broadleaves (*p* < 0.0001 for g_s of the three groups; mean comparison by LSD test for each pair *p* < 0.0001 except for g_s[CO_2]elevated in evergreen angiosperms vs. deciduous angiosperms, *p* = 0.067; Table 3). This means that the ambient g_s across experiments (at

TABLE 2 Mixed-effect model results for stomatal sensitivity to CO_2 level ($gs(CO_2)$; the dependent variable) based on the full dataset (A), the dataset restricted to field experiments (B) and the dataset restricted to high-latitude biomes, to account for the under-representation of gymnosperm species in low-latitude biomes (C)

Dependent variable	Fixed effects	AICc	F ratio	p > F	Effect	Estimate	SE	t ratio	p > |t|
Full Dataset									
$g_s(CO_2)$	Group	1,369.3	9.024	**0.0002**	Intercept	−18.389	12.497	−1.47	0.5047
					Decid. Angio.	0.753	3.733	0.20	0.8406
					Ever. Angio.	−16.341	4.305	−3.80	**0.0003**
					Measurement method	27.458	145.249		0.8501
					Experimental design	91.902	90.887		0.3119
	Biome	1,371.3	4.599	**0.0011**	Intercept	−27.740	11.452	−2.42	0.1368
					Boreal	18.343	8.007	2.29	**0.0237**
					Mediterranean	8.888	11.161	0.80	0.4272
					Semi-arid	−18.120	11.151	−1.62	0.1098
					Subtropical	−0.911	8.031	−0.11	0.9099
					Temperate	14.889	5.349	2.78	**0.0082**
					Measurement method	204.379	314.635		0.5160
					Experimental design	52.961	78.205		0.4983
	Group	1,370.6	2.227	0.1127	Intercept	−33.688	13.746	−2.45	0.0837
	Biome		2.049	0.0788	Decid. Angio.	−10.466	6.549	−1.60	0.1124
					Ever. Angio.	8.549	12.226	0.70	0.4857
					Boreal	25.196	14.680	1.72	0.0885
					Mediterranean	4.691	12.412	0.38	0.7060
					Semi-arid	−22.737	12.767	−1.78	0.0790
					Subtropical	−5.402	10.037	−0.54	0.5915
					Temperate	24.746	12.665	1.95	0.0530
					Measurement method	185.176	305.848		0.5449
					Experimental design	57.329	84.199		0.4959
	Group	1,362.9	6.144	**0.0030**	Intercept	−23.441	12.159	−1.93	0.2495
	Tree age	1.173	0.2819	Decid. Angio.	2.271	3.949	0.58	0.5667	
	Exposure	1.153	0.2992	Ever. Angio.	−15.477	4.564	−3.40	**0.0009**	
					Tree age	0.117	0.108	1.08	0.2819
					Exposure length	0.760	0.708	1.07	0.2992
					Measurement method	13.844	85.258		0.8710
					Experimental design	165.963	148.839		0.2648

(Continues)

TABLE 2 (Continued)

Dependent variable	Fixed effects	AICc	F ratio	p > F	Effect	Estimate	SE	t ratio	p > |t|
Restricted to field experiments									
$g_s(CO_2)$	Group	884.8	6.234	**0.0029**	Intercept	-12.882	7.374	-1.75	0.2215
					Decid. Angio.	-3.094	3.973	-0.78	0.4382
					Ever. Angio.	-8.394	6.327	-1.33	0.1880
					Measurement method	53.001	117.531		0.6520
					Experimental design	57.398	63.190		0.3637
	Biome	890.2	3.800	**0.0258**	Intercept	-14.900	10.524	-1.42	0.2551
					Boreal	16.619	6.206	2.68	**0.0087**
					Temperate	3.933	4.500	0.87	0.3843
					Measurement method	207.303	259.899		0.4251
					Experimental design	37.981	43.684		0.3846
	Group	886.6	3.675	**0.0295**	Intercept	-15.535	10.221	-1.52	0.2319
	Biome		1.732	0.1825	Decid. Angio.	-8.799	5.440	-1.62	0.1092
					Ever. Angio.	5.881	10.677	0.55	0.5833
					Boreal	15.839	8.507	1.86	0.0658
					Temperate	8.507	6.857	1.07	0.2887
					Measurement method	171.028	239.812		0.4757
					Experimental design	50.145	55.381		0.3652
Restricted to high-latitude biomes									
$g_s(CO_2)$	Group	936.4	6.207	**0.0029**	Intercept	-9.142	9.445	-0.97	0.4199
					Decid. Angio.	-10.494	4.250	-2.47	**0.0152**
					Ever. Angio.	7.332	7.982	0.92	0.3606
					Measurement method	173.618	233.268		0.4567
					Experimental design	52.284	53.047		0.3243
	Biome	946.9	0.214	0.6447	Intercept	-10.993	10.288	-1.07	0.3830
					Boreal	1.482	3.205	0.46	0.6447
					Measurement method	219.036	274.516		0.4249
					Experimental design	40.799	47.019		0.3855
	Group	938.7	5.941	**0.0037**	Intercept	-9.430	9.900	-0.95	0.4144
	Biome		0.010	0.9222	Decid. Angio.	-10.499	4.277	-2.45	**0.0158**
					Ever. Angio.	7.245	8.054	0.90	0.3706
					Boreal	-0.308	3.151	-0.10	0.9222

(Continues)

TABLE 2 (Continued)

Dependent variable	Fixed effects	AICc	F ratio	p > F	Effect	Estimate	SE	t ratio	p > |t|
					Measurement method	172.626	232.861		0.4585
					Experimental design	52.722	54.125		0.3300
	Group	930.2	2.989	0.0552	Intercept	-15.167	9.783	-1.55	0.1944
					Decid. Angio.	4.439		-1.95	0.0543
					Ever. Angio.	8.164		0.81	0.4208
	Tree age	1.913	0.1706		Tree age	0.107	0.077	1.38	0.1706
	Exposure	1.625	0.2103		Exposure length	0.679	0.533	1.28	0.2103
					Measurement method	82.446	146.432		0.5734
					Experimental design	114.427	103.182		0.2674

Note: Functional group (Group), biome, tree age and exposure length (Exposure) were taken as fixed effects, and measurement method and experimental design as random effects. Estimates and standard errors (SE) are reported for each effect. For each model, we report the corrected Akaike's information criteria (AICc). Model and fixed effects were tested by F test and t test, respectively. Random effects were tested by Wald p test. Significant effects at the 0.05 threshold appear in bold. Tree age and exposure are not reported for (B) due to imbalance in the data for that specific dataset and parameters.

TABLE 3 ANOVA for the effect of tree group (phylogeny and leaf habit) on g_s

Response	df	MS	F	p
g_s[CO_2]ambient	125	17982.88	31.14	<0.0001
g_s[CO_2]elevated	125	17030.87	17.59	<0.0001

CO_2 of 350–400 ppm) in conifers is already low, and hence, there is lesser leeway for reduction than in broadleaves (Figure 3a). The observation that evergreen angiosperms had the highest mean g_s and the strongest downregulation also support this hypothesis. Also, among species within each group, there was a fair correlation between g_s[CO_2]ambient and g_s(CO_2) (Figure 3b). r^2 for evergreen and deciduous angiosperms were significant at 0.05 and 0.025 levels, respectively.

(H2) Correlating between g_s(CO_2) and leaf thickness (available for 13 of the studied species), there was a good correlation among five deciduous broadleaves, with lower sensitivity in thicker leaves (Figure 3c). Needle leaves were all both thicker and less responsive to the CO_2 increase. From simple physical considerations, the thinner the leaf, the more readily it loses water to the atmosphere. Therefore, thinner leaves can be expected to close stomata when having sufficient CO_2 availability. At the same time, the thinner the leaf, the higher its light absorption. The observation of photosynthetic enhancement in leaves of deciduous angiosperms, at similar level as in an evergreen gymnosperm, and despite the stronger stomatal downregulation in the angiosperms (Figure S2a,b), is another line of evidence supporting this hypothesis. Last, the single deciduous gymnosperm in our dataset, *Larix laricinia*, which showed strong downregulation ($a = -33$), is characterized by thinner needles than almost any other conifer.

(H3) Our analysis can also test the hypothesis that low atmospheric [CO_2] during taxa diversification may have placed a selection pressure on plants to accelerate stomatal closing to maintain adequate internal CO_2 and optimize water-use efficiency. Atmospheric [CO_2] has been decreasing since the beginning of the Cretaceous (145 mya), from >2,000 ppm to the pre-industrial level of 280 ppm. The fact that almost all angiosperms have diversified during this time frame (Pires & Dolan, 2012) means that the relatively high g_s(CO_2) sensitivity could have been expected. On the contrary, conifer trees (the gymnosperm division pinophyta) have diversified from Ginkgoaceae 343 mya, with most families diversifying between 236 and 197 mya (Lu, Ran, Guo, Yang, & Wang, 2014). Atmospheric [CO_2] has been increasing from c.300 to c.2,400 ppm, with values fluctuating around c.1,400 ppm during part of this period, from 255 to 160 mya, that is, during the time of diversification of most conifer families. Our results seem to support this hypothesis, and even considering more recent conifer taxa, it can be generally assumed that most angiosperm taxa evolved at a later geological time, and, since the [CO_2] decrease since the Cretaceous has been mostly monotonous, angiosperms evolved under lower [CO_2] than have gymnosperms.
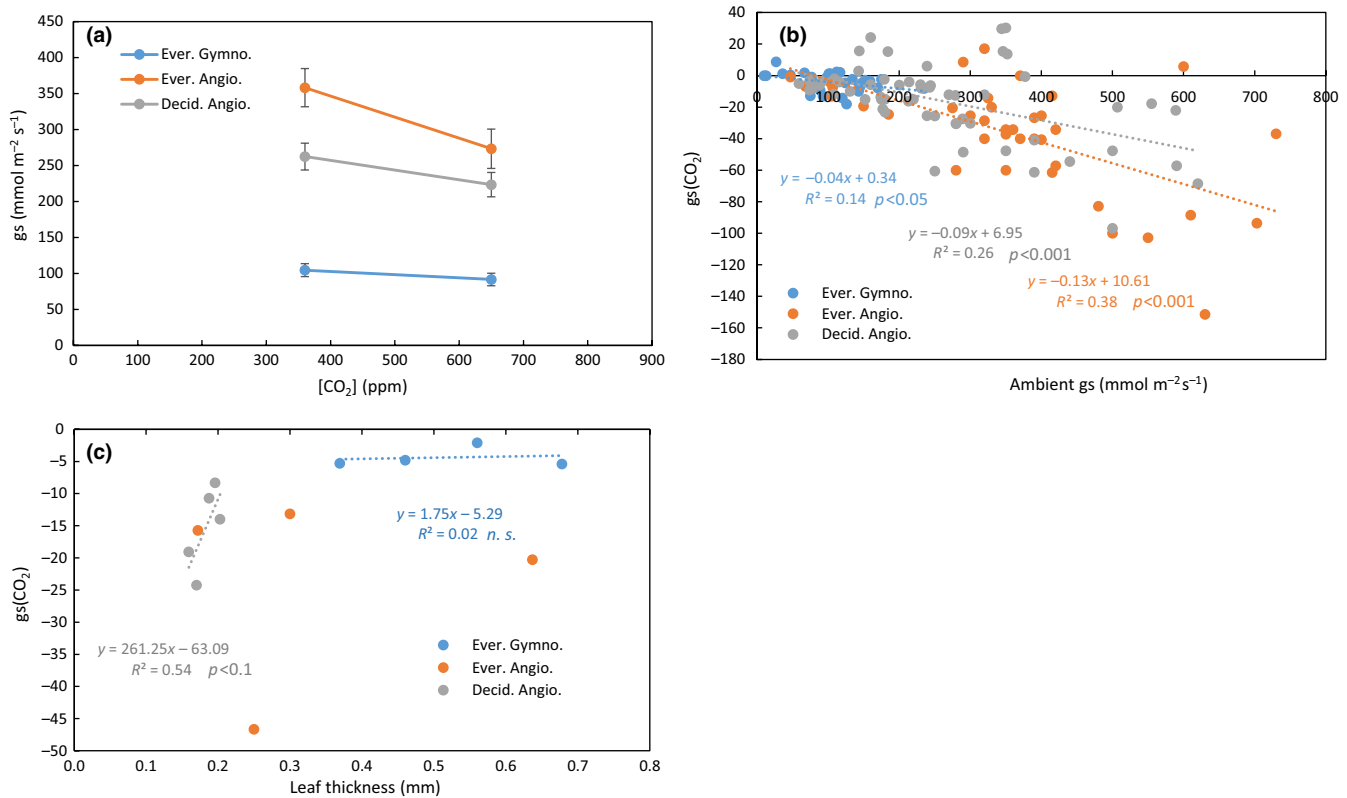
FIGURE 3 Hypothesis testing for the divergence of CO_2-induced stomatal downregulation among functional groups. Stomatal downregulation increases with the rate of stomatal conductance, among the groups (a) and among species-specific experiments within each group (b). Stomatal downregulation decreases with leaf thickness among deciduous angiosperms and is lower in the thicker needles of gymnosperms (c). Dashed lines are linear fits

3.4 | Relationships between stomatal downregulation and experimental determinants

Stomatal sensitivity to CO_2 was plotted against tree age, length of exposure and number of replicates. In these analyses, the outlier response of *Acacia implexa* (upregulation of a = 134) was removed. None of the three correlations yielded an effect (Figure S3), yet a few trends were exposed. Downregulation responses of a = −100 or less were restricted to experiments shorter than 5 months on seedlings, having four replicates or less. But stomatal sensitivities larger than a = −50 were registered in experiments spanning over >90 months, on 100-year-old trees, and having 40 replicates. Functional groups were equally distributed across experiments varying by tree age, exposure length and number of replicates. Therefore, the chance that differences among functional groups were surrogated by any of these parameters was very low.

Did environmental conditions affect the stomatal downregulation across experiments? Although none of the relationships was significant, experiments conducted at higher light, lower VPD and higher temperature generally showed higher stomatal sensitivity (Figure S4). However, these effects reversed at minimum PAR >1,000 μmol m^{-2} s^{-1}, maximum VPD < 1 kPa and maximum temperature >30°C. Importantly, functional groups were equally distributed across these parameters as well. In eight of the records, in two of the

tree species, g_s was not measured directly, but rather indirectly estimated based on whole-tree sap flow measurements. In *Liquidamber styraciflua*, sap flow-derived stomatal downregulation values were significantly milder than those measured directly (a = −11.5% ± 0.5 vs. a = −50.9% ± 13.1, respectively). Yet in *Pinus taeda*, with a higher number of observations of each type, stomatal sensitivities did not differ by measurement method (a = −0.1% ± 1.2 vs. a = −4.1% ± 3.6 for indirect and direct measurement, respectively).

Our species-rich dataset offered a rare opportunity to test interactions of $g_s(CO_2)$ with additional factors, where applied as experiment treatments in combination with the CO_2 treatment. In 45 out of the 144 records, there was a treatment applied (Table S1), 29 of which were on the interaction of elevated CO_2 with N fertilization. In *Pinus taeda*, *Quercus ilex* and *Q. virginiana*, N fertilization decreased stomatal downregulation; But in *Picea abies*, *Liquidamber styraciflua*, *Populus euroamericana* and more so *in Populus trichocarpa*, N fertilization rather increased downregulation. Other treatments did not produce consistent patterns either: leaf age had no effect on stomatal downregulation in *Pinus sylvestris* and *Quercus virginiana*, but in *Picea sitchensis*, 1-year-old needles showed downregulation which was not observed in current-year needles; supplemented irrigation increased downregulation in *Fraxinus excelsior* and had no effect in *Quercus petraea*. Temperature interactions were tested in *Pinus sylvestris* alone and showed no effect.

3.5 | Intra-specific variation in stomatal downregulation

Seven of the 57 species in our dataset were represented in 3–4 studies, thereby allowing for testing for intraspecific variation in stomatal downregulation. The evergreen gymnosperm *Pinus sylvestris* showed downregulation values between *a* = −4.6 and *a* = −8.1 in three different experiments (Figure S5). The deciduous angiosperm *Quercus petraea* showed stronger downregulation, between *a* = −12.1 and *a* = −15.7, in four different experiments. Intraspecific variation was also low in the evergreen gymnosperms *Pinus taeda* and *Picea abies*, but higher in the deciduous angiosperms *Liquidamber styraciflua*, *Fagus sylvatica* and *Quercus rubor*. In the latter, stomatal downregulation was *a* = −5.7 and *a* = −7.0 in two studies, and *a* = −23.1 in another (Table S1).

3.6 | The effect of variation in stomatal sensitivity to CO_2 on tree flux exchange

The multi-species Swiss FACE experiment in a mature, natural forest provided an opportunity to test the direct consequences of the divergence in stomatal sensitivity to CO_2 between evergreen gymnosperms and deciduous angiosperms on tree water and carbon exchanges. Among the six studied species, transpiration reduction was evident among the different angiosperm species, to variable extent, but not in the gymnosperm species *Picea abies* (Figure S2). Measurements of photosynthesis on the same trees showed a similar enhancement of 2.5–3.0 µmol m^{-2} s^{-1} 100 ppm^{-1} across the species, including the *Picea*. Consequently, the ratio between photosynthesis and transpiration rates, that is, the water-use efficiency, was an order of magnitude higher in *Picea* versus the neighbouring angiosperm species (24.3–60.7 vs. 2.5–6.5 mmol CO_2 mol H_2O^{-1}). Reduced transpiration and enhanced photosynthesis were not limited to forest trees under temperate climate. In a greenhouse experiment on lemon saplings with three CO_2 levels, stomatal downregulation of *a* = −19.4 was related to −0.23 mmol m^{-2} s^{-1} 100 ppm^{-1} reduction in transpiration (Figure S2). As a result, soil water content increased with CO_2 by 1.03% 100 ppm^{-1}. Despite the stomatal downregulation, photosynthesis still increased by 0.96 µmol m^{-2} s^{-1} 100 ppm^{-1}, as seen in forest trees, albeit to lesser extent.

4 | DISCUSSION

CO_2-induced stomatal downregulation was evident in 52 of the 57 tree species tested, and to significantly larger extent in angiosperms compared to gymnosperms. The effect of leaf habit was not consistent, although stomatal downregulation of the only deciduous gymnosperm (*Larix laricinia*) was similar with those of deciduous angiosperms and not with evergreen gymnosperms. Among angiosperms, evergreens were more sensitive than deciduous species (Figure 2d). The difference between deciduous and evergreen angiosperms essentially reflects their contrasting distributions; while all 18 deciduous angiosperms were temperate and boreal species, only two of 31 evergreen angiosperms were temperate species, the others coming from low- and mid-latitude biomes. The unequal, contrasting distributions of functional groups across biomes prevent a complete interpretation of the observed biome differences. Nevertheless, the clear decreasing trend Mediterranean > subtropical > semi-arid > tropical (Figure 2b) suggested for the existence of an underlying mechanism. The fact that evergreen gymnosperms and deciduous angiosperms, which coexist in temperate and boreal forests, were so different indicated a fundamental difference in stomatal sensitivity to CO_2 between conifers and broadleaves.

4.1 | Robustness of the results considering the methodological limitations

Is it safe to deduce about a species' sensitivity to CO_2 from two-level studies? Technical and financial considerations limited most of the experiments in our dataset to just two levels of CO_2 concentrations. Here, we took advantage of data coming from seven species with more than two studies available (Table S1). Our analyses showed that, for most of the species, the slope term (*a*) was rather conserved across three or four independent species. Another approach to testing the robustness of the results takes advantage of studies with more than two CO_2 levels. For example, using just two of the three CO_2 levels measured for *Pinus koraiensis* (Zhou & Han, 2005) would yield a slope term (*a*) of −10.0 or −6.5 (for the lower and upper two levels, respectively). These values are close to the three-level slope of −7.9, supporting the assumption that two-level slope terms represent well a species' sensitivity to CO_2 across a relevant range. Similar results were obtained for another conifer species and for three broadleaf species. Yet for four tropical evergreen broadleaf species measured in the greenhouse (Reekie & Bazzaz, 1989), trends were different. Specifically in that experiment, stomatal responses to an increase between 350 and 525 ppm were different from those between 525 and 700 ppm. It is therefore important to acknowledge that our understanding about trees' responses to the increasing levels of atmospheric CO_2 is as limited as the experiment conducted.

4.2 | Conifers are less sensitive to CO_2 than broadleaves

Gymnosperms classify into the 'fast' plant economics (unlike most angiosperms, which classify into 'slow'; Reich, 2014) and hence should have high rates of resource acquisition. Per the 'fast–slow' plant economics, the lower stomatal sensitivity of gymnosperms (compared to angiosperms) could be interpreted as means to maintain a high resource flux rate. Considering our hypotheses regarding processes underlying the differences in $g_s(CO_2)$ among conifers and broadleaves, our analyses supported each of the three hypotheses. We brought evidence that stomatal sensitivity to CO_2 scales with the average level of stomatal conductance, that is, that higher baseline g_s offers a wider range for stomatal closure (H1); the decrease

in leaf thickness, since the thinner the leaf, the larger the water savings (H2); and the decrease in atmospheric CO_2 level at the time of taxa diversification, an evolutionary driver of high stomatal sensitivity (H3). Considering (H1), why is g_s lower in gymnosperms versus angiosperms? A hypothesis of higher vascularization in the latter finds support at a coarse scale, but not at finer scales (Rockwell & Holbrook, 2017). In (H2), a hidden assumption in H2 is that leaf thickness is analogous to the water-bearing tissue, which in fact only partly represents the whole leaf. This assumption was confirmed in a study comparing 34 woody angiosperm species. Leaf thickness (assessed by the leaf volume to area ratio) variation was strongly and positively related with mesophyll thickness for both deciduous and evergreen species, and also with the thickness of epidermis, vascular and sclerenchyma tissues for evergreen species (de la Riva, Olmo, Poorter, Ubera, & Villar, 2016). Our observations call for focused experiments to test each of the three outlined hypotheses. It could well be that mechanisms are complementary, and disentangling each effect would be challenging. For example, g_s and leaf thickness seem to diverge simultaneously among groups and species (Figure 3c). A future study could focus on the $g_s(CO_2)$ response of low-g_s broadleaves and high-g_s conifers (H1). A different approach can use synthetic structures for physical experiments, for example, using a wind tunnel, to test the leaf thickness effect on water loss potential (H2).

4.3 | Consequences for forests in a high-CO_2 future

Are water savings expected in broadleaf forests but not in conifer forests? Caution must be taken when upscaling tree-scale observations in space and time. Still, the divergence between the soil moisture response to CO_2 enrichment application in broadleaves versus conifers, even in trees growing at the same forest (Leuzinger & Körner, 2007, Leuzinger & Bader, 2012, respectively), cannot be overlooked. Our results show that such differences are not case-specific, but rather reflect a general trend of significantly stronger CO_2-induced stomatal downregulation in broadleaves versus conifers. Therefore, and depending on the turnover rates of feedbacks with soil and atmosphere (Cech, Pepin, & Körner, 2003; Leuzinger & Körner, 2007; Reekie & Bazzaz, 1989; Uddling, Teclaw, Pregitzer, & Ellsworth, 2009), effects on regional water budget and the hydrological cycle can potentially diverge between forest types. Notably, broadleaves and conifers are not equally distributed across Earth's forests: the share of conifers typically increases with latitude. Therefore, water savings might be more plausible in tropical, subtropical and temperate broadleaf ecosystems, and less so in temperate and boreal conifer ecosystems. Tree water use should decrease also in semi-arid forests (Figure S2), potentially having a beneficial influence in these water-limited ecosystems. In our dataset, we note the disproportional representation of temperate tree species, most of which are deciduous angiosperms (Table S1). Data on stomatal sensitivity to CO_2 in tropical tree species are mostly from experiments conducted >20 years ago (Berryman et al., 1994; Eamus et al., 1993; Goodfellow et al., 1997; Leuzinger & Bader, 2012; Oberbauer, Strain, & Fetcher, 1985), but see Ref. (Lima, Jarvis, & Rhizopoulou,

2003; Lovelock, Virgo, Popp, & Winter, 1999). New studies are needed to close the knowledge gap on stomatal sensitivity to CO_2 in tropical trees and forests.

Finally, to improve predictions of future forest biogeochemistry, our results should feed into CO_2-parameterized vegetation models, which are currently lacking the notion of group-based divergence. For example, for *Picea abies*, where CO_2-induced stomatal downregulation was close to zero (Figure 1) and hence there was no CO_2 effect on soil moisture, dynamic global vegetation models predicted 9%–18% reduction in transpiration (Leuzinger & Bader, 2012). Such predictions did not consider the low CO_2 sensitivity of the conifers in comparison with broadleaves and could hence lead to reduced accuracy of predictions. Our results indicate that functional divergence between needle-leaf and broadleaf forests should intensify as atmospheric [CO_2] continues to rise.

CONFLICT OF INTEREST

The authors declare no competing financial interests and no conflict of interest.

AUTHORS' CONTRIBUTIONS

Data collection was performed by both authors. T.K. performed the data analysis and wrote the manuscript.

DATA ACCESSIBILITY

All data used in the paper are available in Table S1, which has also been deposited in the Dryad digital repository: Provisional DOI: https://doi.org/10.5061/dryad.p6v01kd, (Klein & Ramon, 2019).

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SUPPORTING INFORMATION

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