Climate forcing due to optimization of maximal leaf conductance in subtropical vegetation under rising CO2

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Plant physiological adaptation to the global rise in atmospheric CO2 concentration (CO2) is identified as a crucial climatic forcing. To optimize functioning under rising CO2, plants reduce the diffusive stomatal conductance of their leaves (gs) dynamically by closing stomata and structurally by growing leaves with altered stomatal densities and pore sizes. The structural adaptations reduce maximal stomatal conductance (gsmax) and constrain the dynamic responses of gs. Here, we develop and validate models that simulate structural stomatal adaptations based on diffusion of CO2 and water vapor through stomata, photosynthesis, and optimization of carbon gain under the constraint of a plant physiological cost of water loss. We propose that the ongoing optimization of gsmax is eventually limited by species-specific limits to phenotypic plasticity. Our model reproduces observed structural stomatal adaptations and predicts that adaptation will continue beyond double CO2. Owing to their distinct stomatal dimensions, angiosperms reach their phenotypic response limits on average at 740 ppm and conifers on average at 1,250 ppm CO2. Further, our simulations predict that doubling today’s CO2 will decrease the annual transpiration flux of subtropical vegetation in Florida by =60 W m−2. We conclude that plant adaptation to rising CO2 is altering the freshwater cycle and climate and will continue to do so throughout this century.

climate change | physiological forcing | plant evolution

Plants respond to the complex of environmental signals they perceive by plastic changes in their phenotype to increase individual fitness (1). The most apparent environmental change that induces phenotypic adaptations in plants is the global increase in atmospheric CO2 concentration (CO2) (2). In response to this rise in CO2, plants reduce the diffusive stomatal conductance of their leaves [gs (mol m−2 s−1)] to increase drought resistance (3) and reduce physiological costs associated with water transport (4). Plants can reduce gs by dynamically closing their stomata within minutes (5, 6), and structurally within the lifespan of an individual by growing leaves with altered stomatal density [D (number of stomata m−2)] and pore size at maximal stomatal opening [gsmax (m2)] (7, 8). Structural adaptations thereby reduce maximal stomatal conductance [gsmax (mol m−2 s−1)], which critically reduces actual gs, especially when stomata are fully open during times with ample light and water (9).

Reduction of gs via structural adaptation of gsmax has the potential to reduce transpiration fluxes and, thus, cause land surface warming in addition to changes in the global hydrological cycle with rising CO2 (10). This climatic effect is termed the physiological forcing of CO2, which acts beside and independent of its radiative forcing. Despite advances to quantify this physiological forcing with global climate models (11, 12), these models rely on semiempirical relations to simulate gs from environmental variables (13, 14). Alternative models have been proposed on the mechanism that stomatal adaptations optimize carbon gain under the constraint of a cost of water loss (15, 16). Because of their mechanistic representation of stomatal responses, optimization models are potentially suitable to simulate canopy gas exchange under changing CO2. However, optimization models implicitly assume that plants will continue to adapt gs optimally to future rises in CO2. Whether this assumption holds for the current rate of CO2 increase is unknown, but structural stomatal responses might be constrained by limits to phenotypic plasticity (17, 18) and diffusion through stomatal pores (19).

To quantify physiological forcing of CO2 on past and future climate, two challenges must therefore be addressed. First we test if the observed structural adaptation of gsmax to rising CO2 can be explained by optimization of carbon gain under the constraint of a cost of water loss and second we predict at what level of CO2 this structural adaptation ceases due to limits to phenotypic plasticity.

Recent advances in stomatal modeling provide possibilities to tackle the first challenge, because the hypothesis that plants adapt gsmax structurally to rising CO2 to optimize carbon gain with water loss can be solved mathematically (15, 16). However, limited experimental data are available for model validation because experiments are generally too short to measure structural stomatal adaptation in forests that take decades or longer to fully adapt to elevated CO2 (20). A unique dataset provides measurements of structural adaptation of gsmax to the CO2 rise of the past century in eight C3 canopy species from natural subtropical vegetation in Florida (see Table 1 for species names) (8). Because these species are representative for vegetation in subtropical climates, these observations are crucial to validate models of stomatal adaptations for this climate zone.

The second challenge is more difficult to overcome because ideally species-specific limits to structural adaptation are observed in natural vegetation under rising CO2. However, no historic analog of the current high rate of CO2 increase can be found in the 400-million-year history of vascular plants (21, 22). The fossil record shows that CO2 has been driving genetic adaptation that allowed plants to develop ranges of phenotypic plasticity to optimize functioning under changing CO2 (22, 23). Despite these shifts in phenotypic plasticity at geologic timescales, structural adaptation of gsmax was always constrained by interdependence of D and eactmax in the form of a power law relationship (Fig. L4). Although D and eactmax are not the only variables to determine gsmax the constraint on their combined values does control the range of gsmax. Which is calculated as (23):


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Additionally, adaptations to rising \( g_{\text{smax}} \) independently, \( A_{s\text{low}} \) would eventually surpass \( A_{s\text{low}} \), and fall beyond the range of historic observations. We therefore suggest that structural response limits are determined by consistent species-specific strategies to reduce \( g_{\text{smax}} \) via adaptation of \( D \) and \( a_{\text{max}} \) along the linear relation between \( g_{\text{smax}} \) and \( A_{s\text{low}} \), until \( A_{s\text{low}} \) is reached.

With a mechanistic model of stomatal adaptation and an empirical method to estimate response limits at hand, we can now quantify the physiological forcing of past and future \( CO_2 \) in a subtropical climate at a decadal to centennial timescale. We first model how stomatal optimization reduces \( g_{\text{smax}} \) with rising \( CO_2 \) and validate these results against observations of eight C3 canopy species (8) that responded structurally to the \( CO_2 \) rise of the past century. Because we suggest that these adaptations are constrained by limits to phenotypic plasticity, we derive the upper limits for each species in terms of \( CO_2 \). Finally, we use the stomatal optimization model with structural response limits to calculate physiological forcing of \( CO_2 \) rising from preindustrial (280 ppm) through present (385 ppm), and up to double present levels (770 ppm).

**Results**

Our simulations of stomatal optimization are consistent with observations that report a 17–53% decrease in \( g_{\text{smax}} \) from preindustrial to present \( CO_2 \) (Fig. 2). Our model simulates \( g_{\text{smax}} \) for all species within the variability of observed \( g_{\text{smax}} \) (Fig. 2, Inset) as a consequence of adaptations to the complex of environmental factors determining \( D \) and \( a_{\text{max}} \), including \( CO_2 \) (Fig. S1) (24). Although not all variability in observed \( g_{\text{smax}} \) can be explained from adaptation to \( CO_2 \), the consistent decreases of \( g_{\text{smax}} \) observed at decadal to centennial timescales are accurately reproduced by our model. These results indicate that structural adaptations of \( g_{\text{smax}} \) to \( CO_2 \) rising from preindustrial to present levels can be explained from optimization of carbon gain under the constraint of a cost of water loss.

Furthermore, our simulations show that \( g_{\text{smax}} \) continues to decrease with \( CO_2 \) rising beyond present values (Fig. 2). Interpreting these model results, we find this ongoing decrease in

### Table 1. Species-specific limits of structural stomatal adaptations to rising \( CO_2 \)

<table>
<thead>
<tr>
<th>Species</th>
<th>( g_{\text{smax}} ) (mol m(^{-2}) s(^{-1}))</th>
<th>( CO_\text{low} ) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiosperm average</td>
<td>0.76</td>
<td>740</td>
</tr>
<tr>
<td>Acer rubrum (Ar)</td>
<td>0.69</td>
<td>830</td>
</tr>
<tr>
<td>ilex cassine (Ic)</td>
<td>0.46</td>
<td>770</td>
</tr>
<tr>
<td>Myrica cerifera (Mc)</td>
<td>0.73</td>
<td>670</td>
</tr>
<tr>
<td>Quercus laurifolia (Q)</td>
<td>0.95</td>
<td>635</td>
</tr>
<tr>
<td>Quercus nigra (Qn)</td>
<td>0.97</td>
<td>775</td>
</tr>
<tr>
<td>Conifer average</td>
<td>0.31</td>
<td>1,250</td>
</tr>
<tr>
<td>Pinus elliottii (Pe)</td>
<td>0.19</td>
<td>1,465</td>
</tr>
<tr>
<td>Pinus taeda (Pt)</td>
<td>0.19</td>
<td>1,060</td>
</tr>
<tr>
<td>Taxodium distichum (Td)</td>
<td>0.29</td>
<td>2,120</td>
</tr>
</tbody>
</table>

Species-specific limits of the structural stomatal adaptation to rising \( CO_2 \), denoted by the lower limit on \( g_{\text{smax}} \) (defined as \( g_{\text{low}} \)) and \( CO_2 \) when mean \( g_{\text{smax}} \) reaches \( g_{\text{low}} \) (defined as \( CO_\text{low} \)).

\[
g_{\text{smax}} = \frac{d_a}{v} \cdot \frac{D \cdot a_{\text{max}}}{l + \frac{1}{2} \sqrt{a_{\text{max}} / \pi}} \quad [1]
\]

where \( d_a \) (m\(^2\) s\(^{-1}\)) is the diffusivity of water vapor and \( v \) (mol m\(^{-3}\) s\(^{-1}\)) is the molar volume of air. The \( a_{\text{max}} \) is approximated from pore length \( L \) (m) on the premise that species studied here have ellipse-shaped apertures at \( a_{\text{max}} \) with width \( W = L/2 \). Pore depth \( l \) (m) is calculated from a species-specific relation with guard cell width and pore length (8) (Table S1). Note that \( g_s \) and \( g_{\text{smax}} \) are expressed as conductance to water vapor (mol m\(^{-2}\) s\(^{-1}\)). Additionally, \( D \) and \( a_{\text{max}} \) together express the percentage of leaf surface area allocated to stomatal pores as: \( A_{s\text{low}} = 100D \cdot a_{\text{max}} \). Fig. 1A shows how combined values of \( D \) and \( a_{\text{max}} \) relate to values of equal \( g_{\text{smax}} \) and equal \( A_{s\text{low}} \), which is distributed lognormally (Fig. 1B).

The lognormal distribution of \( A_{s\text{low}} \) allows for the estimation of species-specific limits to structural adaptation of \( g_{\text{smax}} \), because \( A_{s\text{low}} \) is bounded on the lower side by a generic value of 0.6% independent of \( g_{\text{smax}} \) defined as \( A_{s\text{low}} \) (Fig. 1B). Although the species independent power law relationship between \( D \) and \( a_{\text{max}} \) is bound by \( A_{s\text{low}} \), each species uses a specific strategy to reduce \( g_{\text{smax}} \) linearly with \( A_{s\text{low}} \) (Fig. 1C).

*Fig. 1.* An overview of relationships among stomatal density (\( D \)), pore size at maximal stomatal opening (\( a_{\text{max}} \)), and the resulting maximal stomatal conductance (\( g_{\text{smax}} \)) and leaf surface area allocated to stomatal pores at \( a_{\text{max}} \) (\( A_{s\text{low}} \)). (A) Power law relationship between \( D \) and \( a_{\text{max}} \) are plotted together with lines of equal \( g_{\text{smax}} \) (solid lines) and \( A_{s\text{low}} \) (dashed lines). See Eq. 1 and Table S1 for calculations of \( g_{\text{smax}} \). Note that logarithmic axes are used. (B) Cumulative probability of \( A_{s\text{low}} \), for woody angiosperm and conifer species fitted to a lognormal distribution. The value of 0.6 indicates the estimated lower bound (5% probability) on \( A_{s\text{low}} \), defined here as \( A_{s\text{low}} \). Note that a logarithmic x axis is used. (C) Species-specific strategies to adapt \( g_{\text{smax}} \) linearly with \( A_{s\text{low}} \). The dashed line denotes \( A_{s\text{low}} \). Lines of linear least squares regressions are indicated per species and used to determine the intersect with \( A_{s\text{low}} \) to predict the lowest attainable \( g_{\text{smax}} \) for each species, defined as \( g_{\text{low}} \). The \( r^2 \) values are: 0.97 (Ar), 0.96 (Ic), 0.86 (Mc), 0.96 (Ql), 0.91 (Qn), 0.85 (Pe), 0.98 (Pt), and 0.94 (Td) with \( P < 0.001 \) for all. Data FB09 are from ref. 23, others from ref. 8. Species names and their abbreviations are defined in the legend.
plants are likely to reach the limits of their phenotypic plasticity (17, 18, 25). We therefore predict structural response limits on the premise that the species-specific adaptation strategies observed remain unchanged at elevated CO₂ and will eventually be limited by Aₚₗₗ,low at the lowest attainable gₘₐₓ, defined here as gₘₐₓ (Fig. 1C). With our simulations of structural adaptation, we predict that values of gₘₐₓ will be reached in a CO₂ range between 635 and 1,465 ppm (Table 1). Consistent with observations of stomatal adaptations at evolutionary timescales (23), the angiosperms in our dataset (Ar, Ic, Mc, Ql, and Qn) have notably lower response limits than conifers (Pe, Pt, and Td) (740 and 1,250 ppm CO₂ on average, respectively). This difference might be related to the distinct leaf vascular designs of angiosperms and conifers, which are intrinsically linked to the gas exchange capacity of their leaves (4). Angiosperms evolved toward densely veined leaves, which require highly conductive leaf surfaces with many small stomata to maximize gas exchange under low CO₂ (26). Contrasting, conifers have less conductive leaf surfaces with fewer and larger stomata, matching the lower water transport capacity of the simpler leaf vascular design suited for higher CO₂ (27).

Structural stomatal adaptations potentially alter photosynthesis and canopy gas exchange because gₘₐₓ crucially constrains gₛ, especially when assimilation rates reach their daily maximum and stomata are fully open. To determine how photosynthesis and leaf gas exchange is altered by structural stomatal adaptation, we perform three model ensemble simulations: one with dynamic adaptation (GfixMod), one with structural and dynamic adaptation (GoptMod), and one with constant preindustrial gₘₐₓ (GlimMod), each of which consists of eight species members. The differences in simulated gₛ between GfixMod, and GoptMod and GlimMod ensemble averages that structural adaptation of gₘₐₓ constrains daily average gₛ (Fig. 3A). From preindustrial to present CO₂, daily average gₛ decreases by 20% in the GlimMod and GoptMod ensembles and by 5% in the GfixMod ensemble. From present to double CO₂, gₛ decreases by 40% in the GlimMod and GoptMod ensembles and by 10% in the GfixMod ensemble. Because transpiration (E) is controlled by gₛ and humidity deficit in the lower atmosphere, E decreases in line with gₘₐₓ at increasing CO₂ with 1.0 mmol·m⁻²·s⁻¹ from preindustrial to present, and 1.8 mmol·m⁻²·s⁻¹ from present to double CO₂ in the GlimMod and GoptMod ensembles (Fig. 3B). The GfixMod ensemble shows considerably less change in E, with 0.15 and 0.35 mmol·m⁻²·s⁻¹ from preindustrial through present to double CO₂, because only dynamic adaptation reduces gₛ, whereas gₘₐₓ remains at its preindustrial value in this model ensemble.

Contrasting the large differences in transpiration among the three ensemble runs, it is clear that they all show a similar increase in assimilation (A) from 9 μmol·m⁻²·s⁻¹ at preindustrial CO₂ to 15 μmol·m⁻²·s⁻¹ at double CO₂ (Fig. 3C). The similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C). This similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C). This similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C). This similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C). This similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C). This similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C). This similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C). This similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C). This similarity in A increase indicates that gₘₐₓ does not strongly control A, but rather that A is controlled by CO₂ via changes in leaf interior CO₂ concentrations (Cᵢ) (Fig. 3C).
compared with GoptMod ensemble at double
backs and the contribution of transpiration to total surface latent
reduced canopy transpiration and shift the fractional contribu-
gional and continental scale could potentially compensate for
simulations based on optimization of carbon gain under the con-
around or above double present
end of this century (29) and response limits are generally reached
of their phenotypic plasticity. Because
plants will continue to adapt structurally until they reach the limits
 adaptation only (GfxMod), with structural and dynamic adaptation (Gopt-
Mod), and with CO₂ response limits included (GlimMod). Error bars for in-
dividual species denote SDs in daily average transpiration for preindustrial
double CO₂ error bars for mean values denote SDs between species
averages.

**Discussion**

We confirm that structural adaptations of \( \text{g}_{\text{max}} \) exert strong control on dynamic responses of \( g \) and thereby significantly reduce the annual transpiration flux from natural subtropical vegetation in Florida under rising CO₂. Our hypothesis is supported by model simulations based on optimization of carbon gain under the con-
straint of a plant physiological cost of water loss that reproduce the observed adaptation of \( \text{g}_{\text{max}} \) (which decreased with 17–55% from preindustrial to present CO₂) (8). We further expect that plants will continue to adapt structurally until they reach the limits of their phenotypic plasticity. Because CO₂ is likely doubled by the end of this century (29) and response limits are generally reached around or above double present CO₂ levels, structural stomatal adaptation in subtropical vegetation will continue to amplify the climatic forcing of CO₂ throughout this century.

Our simulations with the stomatal optimization model predict that a doubling of present CO₂ will decrease the annual transpiration flux from subtropical vegetation in Florida by \( \approx 60 \text{ W m}^{-2} \). This decrease is considerable because the current annual evapotranspiration flux in Florida is \( \approx 120 \text{ W m}^{-2} \) and transpi-
ration constitutes \( \approx 50\% \) of this total (12, 30). Feedbacks at re-
regional and continental scale could potentially compensate for reduced canopy transpiration and shift the fractional contribu-
tion away from transpiration (31). Accounting for these feed-
backs and the contribution of transpiration to total surface latent
heat flux, a comparable decrease in latent heat flux of 30 W m⁻²
has been simulated over subtropical forests with the Hadley Centre global climate model (11, 32), which uses a semiempirical stomatal response model (13). The finding that stomatal adap-
tations are reducing canopy transpiration is supported by in-
dependent empirical data from river runoff that suggest reduced continental scale evapotranspiration over the past century (33). We therefore conclude that plant adaptation to CO₂ is altering the hydrological cycle and climate and will continue to do so under further rising CO₂.

Despite this evidence for the climatic effects of stomatal adaptations, changes in transpiration could be compensated if forests respond to rising CO₂ by growing taller and denser and, thus, increase leaf area index (LAI) (34). However, in dense subtropical forests, self-shading and down-regulation of photo-
synthetic capacity often limits this effect of CO₂ fertilization (35), so only forest-floor species are likely to benefit from rising CO₂ and these have little impact on canopy transpiration (36). Moreover, increased photosynthesis might also increase turnover rates, leading to a more dynamic forest with unchanged biomass and LAI (37). Simulations with a global vegetation model, which takes these considerations into account, indicate that in sub-
tropical forests LAI increases by a maximum of 10% after a doubling of CO₂ (38). This marginal increase in LAI increases canopy transpiration by \( \approx 5\% \), which is not sufficient to com-
pensate for reduced transpiration at the leaf level. Decreased transpiration is therefore a robust response to increasing CO₂ in subtropical forests.

To estimate physiological forcing due to future CO₂ increase, it is essential to validate response limits to structural adaptation. We based estimates of response limits on the hypothesis that plant species adapt \( \text{g}_{\text{max}} \) by altering \( D \) and \( \text{g}_{\text{max}} \) until they reach a generic value of \( \text{A}_{\text{low}} \). Although the physiologic relevance of \( \text{A}_{\text{low}} \) is not yet fully understood, it might represent a tradeoff between leaf interior CO₂ transport and the structural costs asso-
icated with the required leaf water transport system (4, 39). Because angiosperms and conifers have different leaf hydraulic systems (27), it could be argued that they also have different limits on \( \text{A}_{\text{low}} \) and that a generic \( \text{A}_{\text{low}} \) overestimates phenotypic plasticity for either growth type (17, 40). However, our analysis does not show significant differences in the lower ranges of \( \text{A}_{\text{low}} \) between angiosperms and conifers (Fig. S2). Therefore, we cannot reject the hypothesis that \( \text{A}_{\text{low}} \) is a generic lower limit of \( \text{A}_{\text{low}} \) and, thus, the use of equal \( \text{A}_{\text{low}} \) for angiosperms and conifers is appropriate. Response limits based on \( \text{A}_{\text{low}} \) might therefore represent upper limits of ambient CO₂ to which the current water transport system of each species is optimized. Our prediction indicates that response limits are lower for angiosperms than for conifers (on average 740 and 1,250 ppm CO₂, respectively), roughly reflecting the ambient CO₂ under which these lineages evolved (41, 42).

Comparative differences in stomatal adaptation between angiosperms and conifers have been noted in free-air carbon enrichment (FACE) and greenhouse experiments under elevated CO₂ (28, 43). These studies indicate that angiosperms respond with a higher sensitivity in \( g \) to elevated CO₂ than conifers. Our results suggest that differences in CO₂ response could result from the plant physiological cost of water loss, represented by the Lagrangian multiplier (λ) (Table S2) in the optimization procedure (15). According to the optimization hypothesis, an-
giosperm species with a low λ can resort to high values of \( \text{g}_{\text{max}} \) to function under low CO₂, whereas conifer species with a high λ cannot. Conversely, a rise in CO₂ reverses this adaptation and, therefore, shows an (initial) stronger response in angiosperms than conifers. However, because conifers are expected to have higher response limits than angiosperms, they might continue to optimize \( \text{g}_{\text{max}} \) at CO₂ levels when angiosperms have reached their limit of phenotypic plasticity.

Because CO₂ is rising at exceptional rates, plants face the challenge of increasing individual fitness with plastic responses in their phenotype. Although modern plants have adapted their physiology to the historically low CO₂ by increasing the diffusive conduction of their leaves over the past million years, the cur-
rent rise in CO₂ allows a reversal of this adaptation (23). Reducing maximum leaf conductance under rising CO₂ makes individual plants more productive and drought resistant but also has the climatic consequence of reduced transpiration and associated changes in surface energy balance and the hydrological cycle (10, 33). With CO₂ continuing to increase, it is crucial to estimate the global magnitude of this climatic forcing via plant physiological responses and the two-way coupling between vegetation and climate (44, 45). Furthermore, the ongoing rise in CO₂ might give competitive advantage to plant lineages that evolved under high CO₂ and, thereby, allow a shift of existing vegetation composition favoring plant lineages tied to an earlier time (25).

Model Equations

A biochemical model of photosynthesis (46) is used to simulate assimilation of CO₂ [A (mol·m⁻²·s⁻¹)]:

\[
A = \left(1 - \frac{1}{C_i}\right) \cdot \min(W_e, W_j) - R_d
\]

with

\[
W_e = V_{\text{max}} \frac{C_i}{C_i + K_c \left(1 + \frac{C_o}{K_c}\right)}, \quad W_j = 2 \frac{f_c}{C_i + \frac{1}{2} \Gamma}
\]

in which \(\Gamma\) (mol·mol⁻¹) is the CO₂ compensation point in absence of dark respiration \(R_d\) (mol·m⁻²·s⁻¹), \(C_i\) (mol·mol⁻¹) is the intercellular CO₂ concentration, \(W_e\) and \(W_j\) (mol·m⁻²·s⁻¹) are the Rusbisco and RuBP limited rates of carboxylation, \(V_{\text{max}}\) (mol·m⁻²·s⁻¹) is the maximum carboxylation capacity, \(K_c\) (mol·mol⁻¹) and \(K_o\) (mol·mol⁻¹) are the Michaelis-Menten constants for carboxylation and oxygenation and \(p_o\) (mol·mol⁻¹) is the partial pressure of oxygen. The rate of electron transport \(J\) (mol·m⁻²·s⁻¹) depends on the photon flux density \(Q\) (mol·m⁻²·s⁻¹), the rate and maximum rate of electron transport (15) and temperature response of photosynthesis parameters (47). Furthermore, \(V_{\text{max}}\) and \(J_{\text{max}}\) exhibit down-regulation in response to rising CO₂ (22) (see SI Text for details on parameter values).

Structural stomatal adaptations to changes in atmospheric CO₂ concentrations [CO₂ (mol·mol⁻¹)] are simulated from optimization of carbon gain under the constraint of a plant physiological cost of water loss (15). The underlying assumption of this approach is that plants cannot transpire more water than they can transport from the soil, through their roots and stem up to their leaves (48). As maximum transpiration generally occurs during maximum photosynthesis, this model calculates an optimal \(g_{\text{opt}}\) [defined as \(g_{\text{opt}}\) (mol·m⁻²·s⁻¹)] according to daily maximum photosynthesis and water availability at this time:

\[
g_{\text{opt}} = \min \left\{ \frac{q(K + \Gamma)[CO_2(q - R_d) - (q\Gamma + KR_d)]}{(CO_2 + K - 2aw_d)aw_d}, \right. \left. \frac{a}{CO_2 + K} \right\}
\]

in which:

\[
q = \begin{cases} V_{\text{max}} & \text{if } W_e \leq W_j \\ \frac{1}{2} J & \text{if } W_e > W_j \end{cases}, \quad K = \begin{cases} K_c \left(1 + \frac{\gamma}{2}\right) & \text{if } W_e \leq W_j \\ \frac{1}{2} \Gamma & \text{if } W_e > W_j \end{cases}
\]

and the Lagrangian multiplier \(\lambda\) (mol·mol⁻¹) represents a species specific empirical constant for the cost of water loss (Table S2), \(w_d\) (mol·mol⁻¹) is the water vapor deficit calculated from relative and saturated atmospheric humidity \([w_{\text{rel}}\) (mol·mol⁻¹)] and \(w_{\text{sat}}\) (mol·mol⁻¹)] as \(w_d = w_{\text{sat}}(1 - w_{\text{rel}})\) and \(a\) (mol·mol⁻¹) is the ratio between diffusivity of water vapor and CO₂ vapor \([d_w\) and \(d_c\) (m²·s⁻¹)]. Saturation value of water vapor and diffusivities of CO₂ and water vapor are calculated depending on ambient temperature (15, 49).

We obtain \(g_{\text{opt}}\) for every 5 ppm CO₂ interval from 280 to 2,000 ppm from maximum \(g_{\text{opt}}\) by prescribing an average diurnal cycle of environmental boundary conditions for the season when leaves are formed (March, April, and May in Florida). Meteorological data are obtained from the AmeriFlux database (50) (Fig. S1). For each species, \(\lambda\) is calibrated on the highest CO₂ quartile of species-specific \(g_{\text{opt}}\) observations.

Dynamic stomatal responses are simulated with a stomatal response model (51) superimposed on the model of structural adaptation. This model simulates dynamic adaption of \(g_s\) to environmental boundary conditions from changes in osmotic gradients in guard cells as a function of water availability and photosynthesis. Simulated actual \(g_s\) is the product of \(g_{\text{opt}}\) and the closure related to guard cell turgor \(f_i\) (mol·mol⁻¹):

\[
g_s = g_{\text{opt}} f_i, \quad \text{with } f_i = \frac{\alpha - \gamma}{\alpha + K_c}
\]

in which \(\gamma\) (mol·mol⁻¹) is the hydroscopic compensation point, \(K_c\) (mol·mol⁻¹) is the Michaelis constant for the guard cell advantage \([\alpha\) (mol·mol⁻¹)], which is calculated as a function of guard cell turgor related to water availability and photosynthesis (51).

To solve the model for leaf level gas exchange, we first obtain values for \(g_{\text{opt}}\) at each CO₂ level and then force the dynamic and structural adaptation models with a diurnal cycle of annual average environmental boundary conditions (Fig. S1).

We upscale the leaf level simulations to canopy scale by considering photosynthesis at different heights in the canopy and the feedback between transpiration and moisture in the lower atmosphere. Differences in light conditions within the canopy are simulated from light interception using a simple exponential light decay scheme (Beer’s law) over 5 layers of equal LAI (52):

\[
Q(L_c) = Q(0)e^{-kL_c},
\]

where \(Q(L_c)\) (mol·m⁻²·s⁻¹) is the photosynthetically active radiation calculated from cumulative LAI \(L_c\) above the considered location in the canopy, photosynthetically active radiation at the canopy top \(Q(0)\) and the light extinction coefficient \(k\) (mol·m⁻²·s⁻¹). Feedback between transpiration and moisture in the lower atmosphere is included considering moisture redistribution in the planetary boundary layer (53).

To solve the model for canopy scale gas exchange over 1 y, the humidity of the upper atmosphere is iteratively calculated by forcing the model with an annual cycle of environmental boundary conditions (Fig. S1). Then, \(g_s\) and \(E\) are calculated for every CO₂ level in each layer of the canopy.

Because \(A\) depends on the total leaf conductance \(g_s\) (mol·m⁻²·s⁻¹) but, in turn, controls \(g_s\), \(C_i\) is expressed as a function of CO₂, \(A\) and \(g_s\):

\[
C_i = CO_2 - \frac{A\lambda}{g_s}, \quad \text{with } g_s^{\text{neg}} = g_s^{\text{neg}} + g_s^{\text{neg}} + g_s^{\text{neg}}.
\]

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Supporting Information

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**SI Text**

The biochemical model of photosynthesis (1) requires three species-specific photosynthesis parameters (at 25 °C) to be known: maximum carboxylation capacity \( V_{\text{cmax}} \) (mol m\(^{-2}\) s\(^{-1}\)), maximum rate of electron transport \( J_{\text{max}} \) (mol m\(^{-2}\) s\(^{-1}\)) and mitochondrial respiration rate \( R_{D25} \) (mol m\(^{-2}\) s\(^{-1}\)) (Table S2).

For *Pinus taeda* (Pr) and *Taxodium distichum* (Td), we derived these values from published \( A/C_i \) curves (2) and the empirical relation among \( V_{\text{cmax25}}, J_{\text{max25}}, \) and \( R_{D25} \) (3, 4):

\[
J_{\text{max25}} = (29.1 + 1.64 \cdot 10^6 \cdot V_{\text{cmax25}}) \cdot 10^{-6} \quad \text{[S1]}
\]

and

\[
R_{D25} = 0.015 \cdot V_{\text{cmax25}}. \quad \text{[S2]}
\]

For the other species (*Acer rubrum* (Ar), *Ilex cassine* (Ic), *Myrica cerifera* (Mc), *Quercus laurifolia* (Ql), *Quercus nigra* (Qn), *Pinus elliottii* (Pe), and *Pinus taeda* (Pt)), we derived \( V_{\text{cmax25}} \) and \( J_{\text{max25}} \) from foliar nitrogen content (5) and \( R_{D25} \) from Eq. S2:

\[
V_{\text{cmax25}} = 6.25 \cdot V_c M_A N_b P_R \cdot 10^{-6}, \quad \text{[S3]}
\]

where 6.25 is the ratio of weight of Rubisco to the weight of nitrogen in Rubisco (g g\(^{-1}\)), \( V_c \) is the specific activity of Rubisco at 25 °C [20.7 (μmol g\(^{-1}\) s\(^{-1}\))], \( M_A \) is the leaf mass (g m\(^{-2}\)), \( N_b \) is leaf nitrogen content per leaf dry mass (g g\(^{-1}\)) and \( P_R \) (-) is the fraction of nitrogen allocated to Rubisco, estimated at 0.15, and:

\[
J_{\text{max25}} = 8.06 \cdot \frac{J_m M_A N_b P_R}{10^{-6}}, \quad \text{[S4]}
\]

where 8.06 is the minimal nitrogen investment in cytochrome bioenergetics [μmol of cytochrome (g of N)\(^{-1}\)]. The potential rate of photosynthetic electron transport per unit cytochrome \( (J_{\text{MC}}) \) is estimated at 156 μmol electrons (μmol of cytochrome s\(^{-1}\)) at 25 °C and PB (g of N in cytochrome) is the fraction of N allocated to RuBP estimated at 0.035.

Down-regulation of the photosynthesis parameters \( V_{\text{cmax25}} \) and \( J_{\text{max25}} \) in response to rising \( CO_2 \) (6, 7) is simulated with an exponential decay function:

\[
V_{\text{cmax25}}(CO_2) = V_{\text{cmax25}}(385) \cdot e^{-\kappa (CO_2 - 385)} \quad \text{[S5]}
\]

and

\[
J_{\text{max25}}(CO_2) = J_{\text{max25}}(385) \cdot e^{-\kappa (CO_2 - 385)}, \quad \text{[S6]}
\]

where \( V_{\text{cmax25}}(385) \) and \( J_{\text{max25}}(385) \) represent the photosynthesis parameters \( V_{\text{cmax25}} \) and \( J_{\text{max25}} \) at their present day values (Table S2) and \( \kappa \) is a decay constant for the \( CO_2 \) response of \( V_{\text{cmax25}} \) and \( J_{\text{max25}} \). A value of 2×10\(^{-4}\) ppm\(^{-1}\) is chosen for \( \kappa \) to match estimated down-regulation of photosynthesis parameters at geological timescales (7). Furthermore, species specific values of leaf area index \( (LAI (-)) \) are derived from literature (Table S2) (8, 9).

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Environmental boundary conditions used to force stomatal adaptation models. Annual cycles of climatic boundary conditions of photosynthetic active radiation [$Q$ (mmol·m$^{-2}$·s$^{-1}$)] (A), ambient air temperature [$T$ (°C)] (B), and relative humidity [$w_{rel}$ (%)] (C) measured over a pine flatwoods ecosystem near Gainesville, FL during the year 2003 (10, 11). (D) Average diurnal cycles for $Q$, $T$, and $w_{rel}$ during leaf development (March, April, and May) are prescribed to the optimization model to determine $g_{s,max}$. Annual average diurnal cycles of these boundary conditions are prescribed to calculate gas exchange at the leaf level. A complete annual cycle of these boundary conditions is prescribed to calculate changes in annual canopy transpiration.

Empirical cumulative probability of $A_{b}$ for woody broadleaf and woody needle leaf species. Data are from Franks and Beerling (12). Red circles and green crosses denote data points; dotted red and green lines denote the fit of the empirical distribution together with their 95% confidence levels. Dashed red and green lines denote the lower 5% limit of $A_{b}$. On average, stomata occupy less space on leaves of woody broadleaf species than on leaves (needles) of woody needle leaf species. However, the 5% lower limit of $A_{b}$ (defined as $A_{b,low}$) for both distributions cannot be distinguished. Note that a logarithmic $x$ axis is used.
Table S1. Species specific relations between pore length and guard cell width

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean $C_w$, μm</th>
<th>$\sigma$, μm</th>
<th>$n$</th>
<th>Linear regression</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acer rubrum</td>
<td>6.79</td>
<td>0.94</td>
<td>36</td>
<td>$C_w = 0.36 \cdot L + 2.90$</td>
<td>0.49*</td>
</tr>
<tr>
<td>Ilex cassine</td>
<td>10.26</td>
<td>1.33</td>
<td>27</td>
<td>$C_w = 0.28 \cdot L + 6.19$</td>
<td>0.57*</td>
</tr>
<tr>
<td>Myrica cerifera</td>
<td>7.84</td>
<td>1.10</td>
<td>25</td>
<td>$C_w = 0.41 \cdot L + 5.37$</td>
<td>0.62*</td>
</tr>
<tr>
<td>Pinus elliottii</td>
<td>16.24</td>
<td>2.22</td>
<td>28</td>
<td>$C_w = 0.27 \cdot L + 6.66$</td>
<td>0.62*</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>11.51</td>
<td>1.22</td>
<td>33</td>
<td>$C_w = 11.5 \mu m$</td>
<td>—</td>
</tr>
<tr>
<td>Quercus laurifolia</td>
<td>6.72</td>
<td>0.77</td>
<td>22</td>
<td>$C_w = 0.27 \cdot L + 4.57$</td>
<td>0.49*</td>
</tr>
<tr>
<td>Quercus nigra</td>
<td>7.29</td>
<td>1.21</td>
<td>27</td>
<td>$C_w = 0.26 \cdot L + 3.55$</td>
<td>0.56*</td>
</tr>
<tr>
<td>Taxodium distichum</td>
<td>9.79</td>
<td>1.57</td>
<td>20</td>
<td>$C_w = 0.55 \cdot L + 1.52$</td>
<td>0.72*</td>
</tr>
</tbody>
</table>

Species specific relations between pore length ($L$) and guard cell width ($C_w$) are used to derive pore depth ($l$), based on the assumption that $l$ is equal to $C_w$ (1). The SD ($\sigma$) and number of measurements ($n$) are indicated, alongside the linear regressions and $r^2$ values. Species specific regressions between $C_w$ and $L$ are highly significant ($P < 0.0001$, indicated by *) with exception of $P$. taeda. We therefore derive $l$ from these species specific regressions, except for $P$. taeda for which a constant value is applied. The average slope of these regressions is used to calculate lines of equal $g_{\text{max}}$ in Fig. 1A.

Table S2. Species-specific model parameters

<table>
<thead>
<tr>
<th>Species</th>
<th>$\lambda$</th>
<th>LAI</th>
<th>$V_{\text{cmax}25}$</th>
<th>$J_{\text{max}25}$</th>
<th>$R_{\text{d25}}$</th>
<th>Derived from</th>
<th>$M_a$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acer rubrum</td>
<td>72</td>
<td>5.5</td>
<td>75.0</td>
<td>94</td>
<td>1.1</td>
<td>Foliar N</td>
<td>—</td>
<td>13</td>
</tr>
<tr>
<td>Ilex cassine</td>
<td>134</td>
<td>5.5</td>
<td>55.5</td>
<td>79.2</td>
<td>0.8</td>
<td>Foliar N</td>
<td>127 (15)</td>
<td>14</td>
</tr>
<tr>
<td>Myrica cerifera</td>
<td>99</td>
<td>5.5</td>
<td>62.5</td>
<td>89.1</td>
<td>0.9</td>
<td>Foliar N</td>
<td>101 (35)</td>
<td>14</td>
</tr>
<tr>
<td>Pinus elliottii</td>
<td>244</td>
<td>2</td>
<td>60.9</td>
<td>86.9</td>
<td>0.9</td>
<td>Foliar N</td>
<td>—</td>
<td>14</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>87</td>
<td>2</td>
<td>47.0</td>
<td>77.1</td>
<td>0.7</td>
<td>$A/C_i$ curves</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Quercus laurifolia</td>
<td>62</td>
<td>5.5</td>
<td>54.0</td>
<td>77.0</td>
<td>0.8</td>
<td>Foliar N</td>
<td>102 (34)</td>
<td>15</td>
</tr>
<tr>
<td>Quercus nigra</td>
<td>58</td>
<td>5.5</td>
<td>64.8</td>
<td>92.4</td>
<td>1.0</td>
<td>Foliar N</td>
<td>96 (10)</td>
<td>15</td>
</tr>
<tr>
<td>Taxodium distichum</td>
<td>55</td>
<td>3</td>
<td>30.0</td>
<td>49.2</td>
<td>0.5</td>
<td>$A/C_i$ curves</td>
<td>—</td>
<td>2</td>
</tr>
</tbody>
</table>

Species specific model parameters. Lagrangian multiplier ($\lambda$ (μmol.mol$^{-1}$)), leaf area index (LAI (-)) and photosynthesis parameters $V_{\text{cmax}25}$, $J_{\text{max}25}$ and $R_{\text{d25}}$ (μmol·m$^{-2}$·s$^{-1}$) and how photosynthesis parameters are derived. If photosynthesis parameters are based on foliar nitrogen (N) concentrations on a leaf mass base, measurements of leaf mass with area ($M_a$ (g·m$^{-2}$)) and their SDs are indicated. LAI values for conifers (8, 9) are doubled in the model to account for their amphistomatic leaves.