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Uniform regulation of stomatal closure across temperate tree species to sustain nocturnal turgor and growth

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Water loss and carbon gain are balanced by stomatal control[1], a trade-off that has allowed trees to survive and thrive under fluctuating environmental conditions[2–4]. During periods of lower water availability, stomatal closure prevents excess water loss[5]. Various strategies of stomatal control have been found among tree species[6,7], but the trigger for this behaviour remains elusive. We found a uniform pre-dawn water potential threshold (−1.2 MPa) for stomatal closure across species, which coincided with stem-growth cessation. Meanwhile, midday water potentials at stomatal closure were more variable across species and stomatal control did not follow species-specific thresholds of hydraulic failure, a commonly adopted theory in plant biology[8–10], and often used in predictive water-use modelling[11,12]. This indicates that nocturnal rehydration, rather than daytime hydraulic safety is an optimization priority for stomatal closure in trees[13]. We suggest that these processes are critical for forecasting the global carbon cycle dynamics.

Plants must balance water loss through stomatal pores on the leaf surface with carbon uptake via photosynthesis. In the short term—daily or seasonally—plants can reduce water loss to the atmosphere by reducing stomatal conductance (g_s) to water vapour. While stomatal closure decreases transpiration during periods of low water availability, it also constrains carbon uptake[1]. Understanding the environmental and physiological thresholds for stomatal closure in mature trees is crucial, as forests are estimated to absorb around a fourth of the annual anthropogenic carbon emissions[14]. However, different tree species have been reported to reduce g_s at varying environmental thresholds, creating uncertainty in predictions of global water and carbon cycles[6]. Explaining g_s responses to low water availability is of particular importance, as global observations indicate significant variability in g_s and photosynthesis responses to environmental variables[3,7,15].

Stomatal closure occurs when adjacent guard cells lose turgor either through passive diffusion of water or via active metabolic pathways[16]. This process responds to a decrease in the internal water status of tissues, typically quantified with leaf water potential measurements (Ψ_{leaf})[5]. As Ψ_{leaf} decreases, g_s also decreases. A common explanation for species-specific g_s responses to midday Ψ_{leaf} is that trees optimize carbon uptake against the risk of lethal hydraulic conductance loss in the xylem which occurs at low Ψ_{leaf} (refs. 2,9,17), known as the 'safety-efficiency trade-off'. Earth system models use this principle[12], and recent studies supported this theorem in juvenile trees[8]. These models assume that species-specific stomatal sensitivity to reduced water availability is guided by the species-specific point of loss of plant hydraulic conductivity[4]. However, recent observations in mature trees challenge this trade-off and suggest that maintaining turgor within

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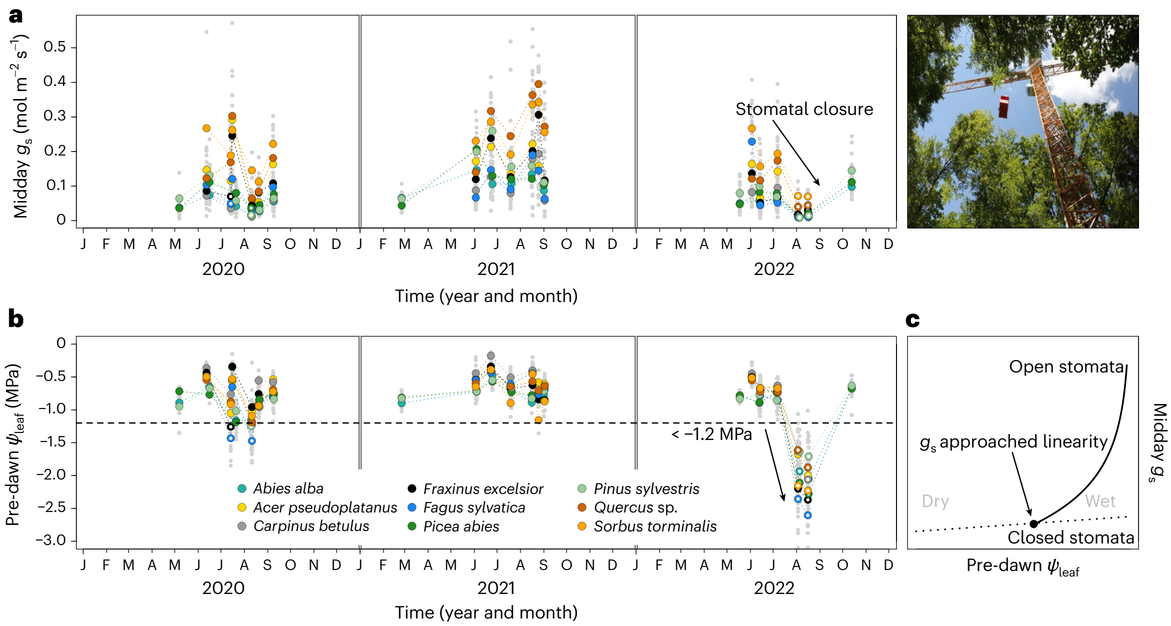

Fig. 1 | Time series of stomatal conductance (g_s) and pre-dawn leaf water potentials (Ψ_{leaf}) collected using a canopy crane at the Swiss Canopy Crane II (SCCII) site. a, Mean species-specific midday g_s measured on leaves from the top of the canopy, shown with large, coloured circles. Open circles indicate measurement dates where Ψ_{leaf} was below −1.2 MPa. Grey dots represent raw measurements. **b**, Pre-dawn (04:00–06:00 CET) Ψ_{leaf}, with coloured dots indicating the species mean. In 2022 an exceptional drought caused all Ψ_{leaf} to drop below −1.2 MPa (shown with open circles). **c**, Theoretical example of how the point of stomatal closure (P_{st}) was defined. P_{st} was assumed when the negative exponential behaviour of g_s changed to a linear decrease (Supplementary Methods). Midday Ψ_{leaf} values are presented in Supplementary Fig. 3. J through D, months of January through December.

growing tissues during night-time is the optimization priority for g_s across temperate tree species[13,18]. Tree growth occurs mainly at night[19] and depends on the nocturnal water status. Delayed stem rehydration after a day of transpiration due to low water availability in the soil can hamper cambial turgor recovery[20]. Conversely, very negative Ψ_{leaf} values, which cause lethal embolisms[10], typically occur around midday. To test whether trees optimize g_s primarily to avoid daytime embolisms or are more conservative and induce stomatal closure already around the point of night-time cambial turgor loss, we can compare the uniformity of pre-dawn and midday Ψ_{leaf} thresholds for stomatal closure across species. Yet, empirical evidence for such a test is lacking as long-term in situ measurements on tall trees are rare, limiting our general understanding of species-specific g_s responses to diel Ψ_{leaf} dynamics.

We collected unique empirical evidence to characterize the water status conditions under which mature tall trees (~20–35 m in height and 80–150 years of age; Supplementary Tables 1 and 2) of various species reduce g_s, considering diel Ψ_{leaf} dynamics and growth (Fig. 1). Over 3 years of intensive canopy and stem monitoring of 95 trees from 9 common temperate tree species, we made observations across a wide range of environmental conditions (Supplementary Fig. 1). This natural climatic variability allowed us to cover a unique hydration spectrum in the target species. The hydration status of the trees ranged from well hydrated, early in the season (pre-dawn Ψ_{leaf} −0.22 to −0.69 MPa; Fig. 1b and Supplementary Table 3), to pre-dawn Ψ_{leaf} values below −1.5 MPa (Supplementary Table 3), close to the expected turgor loss points of leaves for these species (Supplementary Table 4). We observed a wide range of g_s values, from fully open stomata to stomatal closure (Fig. 1a). Using these data, we tested whether stomatal closure is better explained by pre-dawn or midday Ψ_{leaf} conditions and if stomatal closure occurs under more uniform pre-dawn or midday Ψ_{leaf} conditions across species. We defined the point of stomatal closure (P_{st}) by analysing the relationship between stomatal conductance (g_s) and leaf water potential (Ψ_{leaf}). The P_{st} was identified as the Ψ_{leaf} point where g_s transitions from a negative exponential decrease to low values[21] that followed a slight linear decline[10] (Fig. 1c)[22].

We first examined how well pre-dawn and midday Ψ_{leaf} values can explain the in situ g_s measurements (Fig. 2). The pre-dawn Ψ_{leaf} showed a high explanatory power for the g_s response across species ($R^2 = 0.58$, $P < 0.01$), which was greater than that of midday Ψ_{leaf} ($R^2 = 0.41$, $P < 0.01$). As pre-dawn Ψ_{leaf} largely reflects soil water availability dynamics, the strong relationship between pre-dawn Ψ_{leaf} and g_s suggests that stomatal responses are influenced by insufficient tissue rehydration caused by drying soils, with stomata remaining closed unless the soil is adequately wet. Thus, pre-dawn Ψ_{leaf} appears to be a primary control mechanism for stomatal closure in temperate trees. Including vapour pressure deficit (VPD) in the analysis substantially increased the goodness-of-fit, particularly under wetter conditions with less-negative pre-dawn Ψ_{leaf} ($R^2 = 0.68$, $P < 0.01$ in Supplementary Table 5). This suggests a secondary control mechanism or an association between pre-dawn Ψ_{leaf} and the hidden variable controlling stomatal conductance (for example, turgor in the guard cells).

We quantified the pre-dawn and midday P_{st} thresholds across species. For these thresholds, we both determined the point where g_s response to Ψ_{leaf} approaches linearity (Fig. 1c) and the point where g_s crosses a fixed lower g_s threshold (Supplementary Fig. 4), to confirm the robustness of our P_{st} quantification. Importantly, the variance in the estimated point of stomatal closure (P_{st}) across different species was much smaller for pre-dawn than midday Ψ_{leaf} (s.d. of 0.2 versus 0.7 MPa, respectively; Fig. 2). This indicates not only that pre-dawn Ψ_{leaf} is a primary control for stomatal closure in temperate trees, but also that a uniform pre-dawn Ψ_{leaf} threshold for stomatal closure of approximately −1.2 MPa across species exists.

Our data shed light on several basic aspects of tree water relations and their control mechanisms. P_{st} occurred at substantially more negative Ψ_{leaf} when considering midday Ψ_{leaf} (−2.3 ± 0.7 MPa, Fig. 2b) compared to pre-dawn Ψ_{leaf} (1.2 ± 0.2 MPa, Fig. 2a), indicating that if the soil is sufficiently wet, trees allow more negative midday water potentials. Moreover, P_{st} coincided with the inflection point of the nonlinear relationship between pre-dawn and midday Ψ_{leaf} (ref. 23) (referred to as the hydroscape), indicating an important hydraulic threshold at which we

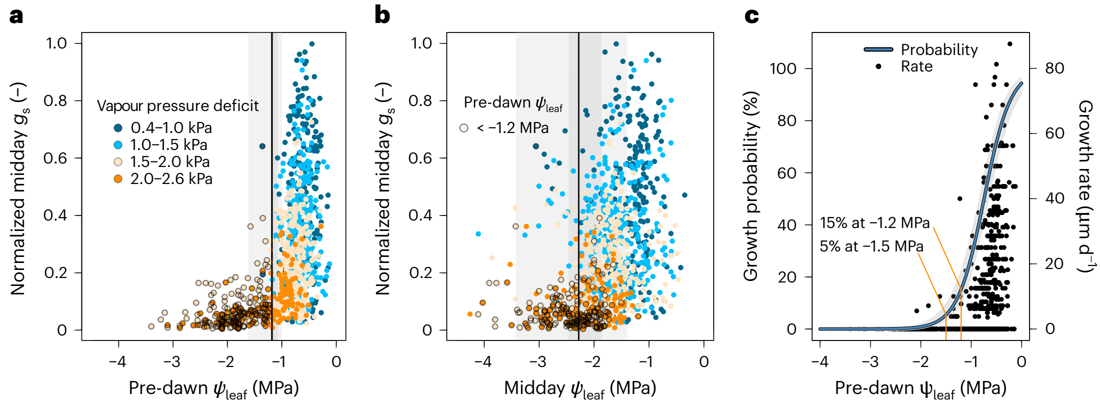

Fig. 2 | Relationship between pre-dawn and midday Ψ_{leaf} and normalized midday g_s or growth of mature temperate tree species. a, The relationship between normalized midday g_s and pre-dawn Ψ_{leaf} for all studied species at the SCCII site. The point of stomatal closure (P_{st}) was quantified for each species individually. The across-species mean P_{st} is indicated by a bold line, with the 50th and 95th percentile ranges shown as grey shading (Supplementary Fig. 4). **b**, Instantaneous response conditions between midday g_s and midday Ψ_{leaf}, presented as in **a**. The across-species mean, as well as the 50th and 95th percentile ranges for P_{st}, are depicted as for **a**. **c**, Growth probability and rate responses to pre-dawn Ψ_{leaf}, obtained from weekly band dendrometer readings. Zero growth rates versus non-zero growth rates were analysed to determine the growth probability using a mixed-effect model with a binomial distribution. Axes labelled (-) indicate unitless values, resulting from normalization to the maximum value. The grey shading around the line represents the 95% confidence interval.

defined the P_{st} (ref. 22) (Supplementary Fig. 5). Furthermore, although it is commonly assumed that P_{st} should occur at the point of turgor loss in the leaves (T_{lp}; refs. 23,24), for most species P_{st} approached less-negative values than the minimum T_{lp} for the species (Supplementary Figs. 6 and 7). Finally, P_{st} did not correspond to species-specific sensitivity to embolism formation (Supplementary Table 4 and Supplementary Figs. 6 and 7). We conclude that stomatal closure might only occur close to the T_{lp} under well-hydrated pre-dawn conditions (Supplementary Fig. 4), and the pre-dawn water status modulates commonly used g_s responses to midday Ψ_{leaf} and VPD[7], particularly when pre-dawn Ψ_{leaf} values are more negative (Supplementary Fig. 8).

To test whether stomatal control is optimized to facilitate growth by sustaining turgor within the cambium[18], we examined whether growth rates of the monitored trees at the Swiss Canopy Crane II (SCCII) site would halt at similar pre-dawn water status conditions as stomatal closure. Weekly manual readings of stem diameters, using band dendrometers, allowed us to relate tree-specific pre-dawn Ψ_{leaf} to daily growth rates driven by xylem and bark cell production and expansion[25]. Our findings showed that all species reduced stem growth rates to nearly zero after reaching −1.2 MPa pre-dawn Ψ_{leaf} (Fig. 2c), which corresponds to the water status at which midday stomatal closure occurs[20]. Additionally, the probability of growth across species rapidly decreased after −1.2 MPa, with only a 5% probability for any stem growth to occur after −1.5 MPa (Fig. 2c, $P < 0.01$). Indeed, growth cessation in herbaceous plants is often observed at less-negative water potentials[26] than those reported in this study. One possible explanation is that acclimation through osmotic adjustments within cambial tissues may enable growth at more negative water potentials[27], although this hypothesis warrants further investigation.

To analyse whether pre-dawn Ψ_{leaf} plays a critical role in regulating whole-canopy g_s, we compared the constraints imposed by pre-dawn and midday Ψ_{leaf} on whole-tree transpiration across multiple monitoring sites in Europe (Supplementary Table 5). We confirmed a strong relationship across species, showing that a more negative water status (that is, pre-dawn Ψ_{leaf} more negative than −1.2 MPa) significantly decreased sap flux density ($R^2 = 0.32$, $P < 0.0001$; Fig. 3a,b). In contrast, midday Ψ_{leaf} did not predict a consistent or significant reduction in daily maximum sap flux density ($R^2 = 0.01$, $P = 0.218$; Supplementary Fig. 9). Moreover, we again found a clear change in the hydroscape behaviour (or the relationship between pre-dawn and midday Ψ_{leaf}), where a less steep decrease in midday Ψ_{leaf} with pre-dawn Ψ_{leaf} was found below −1.2 MPa (Supplementary Fig. 10), suggesting a clear shift in the whole-tree hydraulic response beyond this threshold. The importance of pre-dawn Ψ_{leaf} was also observed across four common tree genera growing in Australia along a steep precipitation gradient (278–1,705 mm of annual precipitation; Fig. 3c,d and Supplementary Table 6). Although full halt of conductance ($g_s < 0.05$ mol m^{-2} s^{-1}) was not measured in these species, we nevertheless found again a shift in their stomatal behaviour and pre-dawn Ψ_{leaf} relationship at −1.2 MPa (ref. 22) (Supplementary Fig. 10). Despite expectations of g_s adjustment to drier conditions[28], we found that across species g_s was substantially reduced with decreasing pre-dawn Ψ_{leaf} ($R^2 = 0.28$, $P < 0.0001$), particularly when it approached −1.2 MPa.

Our data demonstrated that decreasing nocturnal water status of the entire tree plays a crucial role in inducing stomatal closure. Across natural forest sites, we found that pre-dawn Ψ_{leaf} is of primary importance for predicting g_s response, often forcing stomatal closure long before the midday water status reaches T_{lp} of leaves (Supplementary Fig. 7). Until now the −1.2 MPa pre-dawn Ψ_{leaf} threshold was solely reported in potted juvenile trees and shrubs[22,29], without considering this in the context of midday Ψ_{leaf} or whether it would be a similar threshold for mature trees. The combined results we report here clearly indicate that pre-dawn water status is critically important for stomatal control across tree species, age classes and ecosystems. Here, we do, however, need to note that we still do not fully understand the exact interplay between midday VPD and pre-dawn Ψ_{leaf} on g_s (compare Supplementary Figs. 11 and 12).

Our results highlight that stomatal closure behaviour is not explained by the variance in vulnerability to xylem embolisms across species[8,17]. Instead, trees were observed to be conservative with their water usage, allowing stomata to remain open until the T_{lp} only when well hydrated in the morning (pre-dawn $\Psi_{leaf} > -1.2$ MPa; Supplementary Table 4). This behaviour is probably not easily detected in small trees, which are exposed to reduced water availability[8] as a result of their smaller water-storage capacity, compared to tall trees, which makes the time between T_{lp} and the occurrence of xylem embolisms shorter. Larger trees probably benefit from greater storage capacity, which decreases the colinear behaviour between pre-dawn and midday water status[20]. The occurrence of stomatal closure when a mature tree stem cannot rehydrate over night can be physiologically explained, as negative pressures on the phloem hinder both sugar transport in the phloem and growth, and unconstrained water use until T_{lp} could cascade into ever-increasing water loss[13]. However, this does not diminish the importance of daytime conditions. Under well-hydrated pre-dawn

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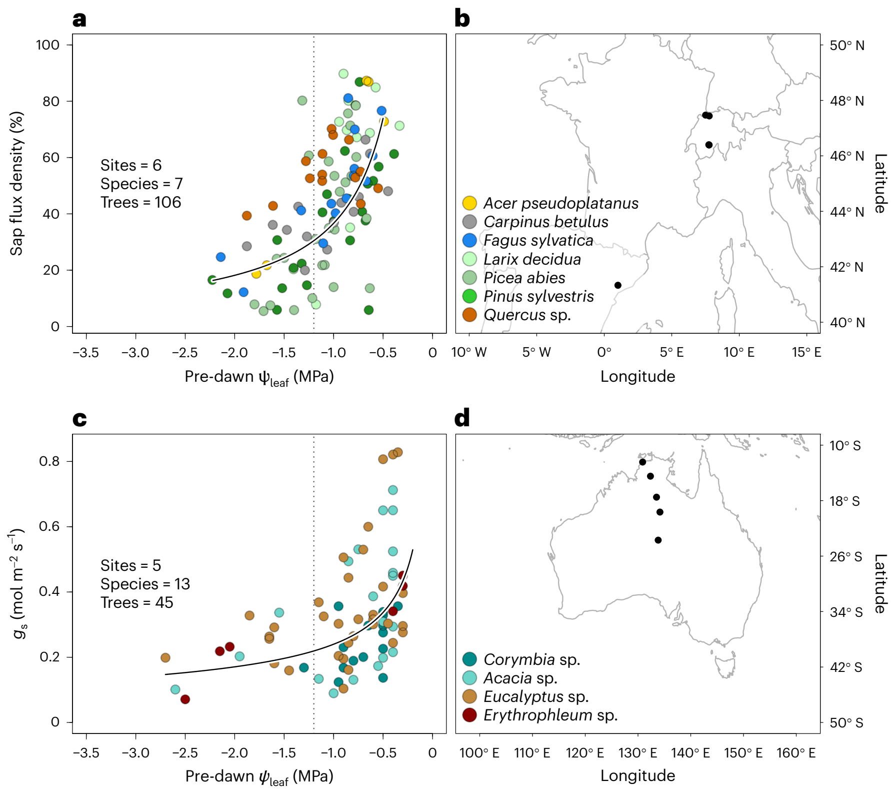

Fig. 3 | Sap flow and g_s response to pre-dawn Ψ_{leaf} across broad spatial scales. **a**, Pre-dawn Ψ_{leaf} measurements related to daily maximum sap flux density (expressed as a percentage of the maximum sap flux density of the tree). Species are distinguished by different colours. The line represents a significant log-transformed relationship, with species considered as a random effect to generate an across-species response curve. The grey dotted line is shown at −1.2 MPa for reference. **b**, Locations of the sites included in this analysis, as detailed in Supplementary Table 5. **c**,**d**, Relationship between pre-dawn Ψ_{leaf} and g_s for Australian tree species (**c**), distinguishable by colours as presented in the location map (**d**).

conditions, mature trees appear to optimize stomatal closure according to species-specific osmotic adjustments in the leaf tissue[23].

It is well-known that plant hormones such as abscisic acid (ABA) regulate stomatal closure[5,16]. However, the signalling pathways trees use to propagate hormonal response to a pre-dawn water status to control midday g_s response—as our data suggest—remain unclear. Leaf turgor has been identified as a key sensor within the signalling pathway[30], yet this does not explain why stomata close long before leaf turgor is lost (Supplementary Fig. 7). Declining turgor associated with decreasing water potential of the leaves could still be important[31]. Alternative signalling could come from surrounding tissues, such as the mesophyll or phloem cells[17], possibly through circadian processes that make these tissues more sensitive during night-time conditions. There is experimental evidence for high foliar ABA production in 8-year-old vines when the pre-dawn water status begins to decline[32]. However, the exact mechanisms by which tall trees perceive their water status in other tissues, such as the cambium of the stem, and at earlier times of day, remain unknown. The greater importance of pre-dawn water status reported here could explain why some studies on smaller plants emphasize the significance of root–soil connectivity for stomatal control[33], as this is the primary process affecting rehydration in plants with lower water-storage capacity.

Water potential thresholds at which mature tree species close stomata are theorized to optimize carbon assimilation versus water availability in the living tissues, affecting other physiological functions such as growth. Current investigations of these thresholds for different tree species often focus on the instantaneous, daytime cost of turgor loss in the leaf tissue[8,23]. However, our findings suggest that pre-dawn water status in mature trees is probably more important. This underscores the necessity for trees to conserve water during the day to avoid compromising turgor-driven growth in the following night. Moreover, the inhibition of tissue formation at relatively high water potentials, closer to our observed pre-dawn threshold of −1.2 MPa than to the threshold of hydraulic failure, supports the importance of carbon sink activity in modulating stomatal conductance[26]. This dynamic diel controlling mechanism is currently not considered in most g_s modelling approaches, which typically simulate an instantaneous midday stomatal response (but see ref. 13). Our observed pre-dawn thresholds should thus be integrated into these models to avoid overestimating carbon assimilation at the landscape scale and beyond, particularly during drought events.

Methods

Study sites for detailed monitoring of gas exchange and sap flow

The majority of measurements were conducted in a mature mixed temperate forest at the SCCII site in Hölstein, Switzerland (47.439° N, 7.776° E, 500 m above sea level). Since 2018, the site has included a 50-m-tall canopy crane with a 62.5-m jib (Supplementary Fig. 2), allowing access to the canopies of over 300 trees via a manned gondola. Ecophysiological measurements were collected during 35 diurnal campaigns from 2020 until 2022, for a total of 95 trees (Supplementary Table 1). The tallest trees within the range of the crane from each of the nine species were selected as target trees. Diurnal campaigns were typically conducted from May until October, where each campaign included pre-dawn sampling before sunrise (04:00–06:00 Central European Time (CET)) and midday sampling (12:00–14:00 CET). In addition to the SCCII site, we included multiple European sites which have data on concurrent pre-dawn and midday Ψ_{leaf} combined with high-resolution sap flow measurements (Supplementary Table 6).

Finally, we included a unique study along a North Australian climate gradient which collected g_s and both midday and pre-dawn Ψ_{leaf} for the genera *Eucalyptus*, *Corymbia* and *Acacia* (Supplementary Methods).

Ecophysiological measurements and meteorological data

At the SCCII site, point measurements of g_s were made from branches in the upper canopy of the target trees. We used a LI-6800 Portable Photosynthesis System (LI-COR Biosciences), where for broadleaved trees, we selected healthy, sun-exposed leaves, whereas for conifers, we chose healthy sun-exposed second-year ramets to ensure fully developed leaves throughout the growing season. In Australia, point measurements of g_s were recorded using a Li-Cor 1600 Steady-State-Porometer (LI-COR).

Sap flow data collected from European forest sites (Supplementary Table 3) used thermal dissipation sap flow sensors, including either the SFS2-M sensors (UP GmbH) or self-made sensors (for data processing see Supplementary Methods). All sensors were installed at a height of 1.5 m on the northeast side of the main tree bole, where dead bark was removed without damaging the phloem tissue. Raw measurements were obtained from either Hölstein or the corresponding authors of the published data (Supplementary Table 3), providing ΔT time series spanning periods where concurrent pre-dawn and midday Ψ_{leaf} measurements were performed.

At all sites, measurements of Ψ_{leaf} were performed by using a Scholander-type pressure chamber (PMS Instrument Company). All measurements were conducted immediately after sampling of sun-exposed apical branches with multiple healthy leaves attached to each one. In June and August of 2023, we sampled additional branches to generate pressure–volume curves with which we established tree-specific leaf turgor loss points (T_{lp}).

At the SCCII site, band dendrometers (D1 tree girth band, Meter GmbH) were mounted at breast height (1.3 m above the ground) on the stems of all target trees (Supplementary Table 1). Before mounting, the stem surface was cleaned of other vegetation and uneven parts of the outer bark. Approximately weekly readings were performed manually throughout the monitoring years to record the diameter at breast height (DBH in cm). Moreover, to confirm the accuracy of the band dendrometers (ZN11-T-WP type, Natkon), for some of the target trees point dendrometers were installed which automatically recorded radial changes in a higher precision and a temporal resolution of 10 min.

On-site air temperature (T_a in °C), relative humidity (%), solar irradiance (W m^{-2}) and precipitation (mm) were monitored using a weather station placed in a forest gap at the SCCII site (Davis Vantage Pro 2, Scientific Sales). Additional measurements of solar irradiance (W m^{-2}) and precipitation (mm) were collected from the top of the crane using the same climate station, where precipitation patterns were averaged over all sensors.

Data treatment and statistical analyses

The relationship between Ψ_{leaf} and g_s was tested using linear mixed-effect modelling. This process involved model selection, model assumption testing, and finally, post hoc tests and model application. Absolute g_s values vary between species and can introduce issues when performing analyses that include multiple species. For this reason, we normalized our g_s data to the maximum species-specific g_s values. We performed a log transformation on both the g_s measurements and VPD (measured by the LI-6800) to better describe the nonlinear behaviour and avoid violating normality assumptions for the residuals. Tree number was included as a random effect, while species was added as a fixed effect.

To determine the point of stomatal closure (P_{st}) due to Ψ_{leaf}, we used the linear mixed-effect model to predict the linear part of the data after stomatal closure (at more negative Ψ_{leaf} values), where VPD conditions are set to high values (VPD = 2.4 kPa; Supplementary Fig. 5). When the data points approach the 95% confidence interval of this projection under high VPD conditions, we define this as the P_{st} as the behaviour becomes statistically indistinguishable from the approached linear decrease (schematically shown in Fig. 1c). To generalize the behaviour of all data, we used a generalized additive mixed-effect model (GAMM) function. The intercept between the 95% confidence interval and the GAMM model was used to represent the point when stomatal behaviour reaches an inflection point, transitioning from active closure to approaching g_s reduction due to loss of conductivity to soil water reservoirs. Moreover, we tested the robustness of the P_{st} by using the fitted GAMM to quantify the Ψ_{leaf} point at which g_s was <0.05 mol m^{-2} s^{-1}, a common stomatal closure threshold used in literature[21] (Supplementary Fig. 4). We modelled the stomatal response to pre-dawn Ψ_{leaf} for both sap flow-derived stomatal conductance and g_s measurements from Australia using a linear mixed-effects model with log-transformed dependent and independent variables, treating species and site as random effects.

Growth rates were calculated by determining the difference in DBH between each monitoring session and dividing this by the number of days between measurements. We applied the zero-growth concept to the DBH data before calculating the radius growth rates, to ensure that no negative growth will be included in the analyses (due to drought induced shrinkage). For the analyses we only considered June, July and August, to avoid the inclusion of growth halt due to winter dormancy of the cambium. A generalized linear mixed-effect model with a binomial distribution was applied to assess the probability of growth occurrence across species, using species and tree nested in species as random effects. See Supplementary Fig. 13 for the results from the daily point dendrometer measurements. More detailed information on the statistical methods is provided in the Supplementary Methods.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Data are available in the main text and Supplementary Information. The gas exchange, sap flow, leaf water potential and growth data used in this study are available via Zenodo at https://doi.org/10.5281/zenodo.14852038 (ref. 34). Source data are provided with this paper.

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Author contributions

R.L.P. and A.K. conceived the study and wrote the first draft of the manuscript. M.A. conceived the experimental design to perform pre-dawn and midday measurements and organized the first 2 years of data collection, including the band dendrometer readings. R.L.P., M.A., G.H., C.Z. and T.Z. collected the data at the SCCII site. Other data included in the manuscript were generated by A.K., S.K.A., L.A.C. and R.P. R.L.P. performed the data analysis and prepared the figures. All authors discussed the manuscript and supported the writing process.

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Competing interests

The authors declare no competing interests.

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☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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Software and code

Policy information about availability of computer code

Data collection	The stomatal conductance measurements were obtained with the program provided on the LI-6800 Portable Photosynthesis System (LI-COR Biosciences GmbH, Bad Homburg, Germany). In Australia, stomatal conductance measurements were obtained by using the software on the LI-COR 1600 Steady-State-Porometer (LiCor Inc., Lincoln, Nebraska, USA). The needle area was then extracted using a dedicated digital image analysis tool (github.com/dabasler/LeafAreaExtraction). At all sites, measurements of Ψleaf were performed by using a Scholander-type pressure chamber (PMS Instrument Company, Albany, Oregon, USA).
Data analysis	All data analyses were performed within the R software environment (Version: 4.2.2, R Core Team 2022). Openly available R packages were used for the analyses, including "datacleanr" (V: 1.0.3), "nlme" (V: 3.1-165), "lme4" (V: 1.1-35.5), "emmeans" (V: 1.10.3), "mgcv" (V: 1.9-1), "plantecophys" (V: 1.4-6), and "TREX" (V: 1.0.2, Location: https://github.com/the-Hull/TREX).

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All data are available in the main text, supplementary materials, and supplementary data files supplied with the initial submission. The gas exchange, sap flow, leaf water potential, and growth data used in this study are available in the Zenodo repository under the accession code https://doi.org/10.5281/zenodo.14852038.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	This information was not collected in this study, as it was not relevant to the main research question focused on tree physiology.
Reporting on race, ethnicity, or other socially relevant groupings	This information was not collected in this study, as it was not relevant to the main research question focused on tree physiology.
Population characteristics	This information was not collected in this study, as it was not relevant to the main research question focused on tree physiology.
Recruitment	No recruitment was needed for this study, as it did not involve human subjects.
Ethics oversight	No approval was needed for this study, as it did not involve human subjects.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We collected unique empirical evidence to characterize the water status conditions under which mature tall trees (approximately 20-35 m in height) of various species reduce stomatal conductance (gs), considering diel leaf water potential (Ψleaf) dynamics and growth. Over three years of intensive canopy and stem monitoring of 95 trees from 9 common temperate tree species, we made observations across a wide range of environmental conditions. Using these data, we tested whether stomatal closure occurs under more uniform pre-dawn or midday Ψleaf conditions.
Research sample	For this study, we measured leaf-level stomatal conductance (gs) from sun-exposed branches in the upper canopy of trees. Concurrently, we measured leaf water potential (Ψleaf) from canopy branches. Moreover, (bi)weekly observations of stem growth at breast height were collected (using manual band dendrometers). We included broadleaved species such as Fagus sylvatica L., Acer pseudoplatanus L., Fraxinus excelsior L., Carpinus betulus L., and Sorbus torminalis Crantz, as well as conifers Picea abies Karst., Abies alba Mill., and Pinus sylvestris L. Additionally, Quercus trees, which were hybrids of Quercus petraea Liebl. and Quercus robur L. to varying degrees, were treated as a single species for this study.
Sampling strategy	We used a 50 m tall canopy crane at the Hölstein research site in Switzerland, with a 62.5 m long jib, to monitor gs and leaf water potentials (Ψleaf) in the crowns of over 95 mature trees from 9 common European species (>1000 measurements). During 35 sampling dates from 2020 to 2022, we measured concurrent midday (12:00 – 14:00 CET) gs and Ψleaf from 95 individual trees, as well as pre-dawn (04:00 – 06:00 CET) Ψleaf to establish tree hydration status. To validate the found patterns, data from existing studies was compiled with concurrent pre-dawn and midday Ψleaf combined with high-resolution sap flow measurements in Europe. Moreover, a unique dataset along a North Australian climate gradient was made available, featuring concurrent measurements of midday (14:00 – 15:00) gs and both midday and pre-dawn Ψleaf.
Data collection	For broadleaved trees, we selected healthy, sun-exposed leaves, whereas for conifers, we chose healthy sun-exposed second-year ramets to ensure fully developed leaves throughout the growing season. For each tree, we selected 2 to 3 small apical branches, 5-10

	cm long, with multiple healthy sun-exposed leaves or needles attached to it. Data was collected by: Richard L. Peters, Matthias Arend, Cedric Zahnd, Günter Hoch, Tobias Zhorzel, and Ansgar Kahmen. Data from published sources was provided by: Stefan Arndt, Lucas Cernusak, and Rafael Poyatos.
Timing and spatial scale	Diurnal campaigns were typically conducted from May until October for three years (2020-2022), when leaves were fully developed. Each campaign (35 in total) included pre-dawn sampling before sunrise (04:00 – 06:00 CET) and midday sampling (12:00 – 14:00 CET).
Data exclusions	Environmental monitoring data and sap flow data were inspected for outliers using the "datacleanr" package. Erroneous data points, such as drastic single outlier values, abrupt jumps, and random noise generated by sensor failure, were identified and removed. For the stomatal conductance (gs) measurements, we normalized our data by dividing each gs value by the maximum gs value recorded for the species. This maximum value was excluded from the analyses to prevent data inflation towards a value of 1. Additionally, negative gs values were excluded from the analysis, as these values result from sensor inaccuracies. We applied the zero-growth concept to the band dendrometer data before calculating growth rates to ensure that no negative growth, caused by drought-induced shrinkage, was included in the analyses. For the analyses, we only considered data from June, July, and August to avoid the inclusion of growth halt due to winter dormancy of the cambium.
Reproducibility	We tested whether pre-dawn Ψleaf is more constraining to whole-tree transpiration across multiple monitoring sites in Europe and Australia. However, the data consist solely of natural observations.
Randomization	The tallest trees within the crane's range from each of the nine species were selected as target trees, representing the primary contributors to forest stand transpiration. A similar sampling strategy was applied in the other included studies.
Blinding	Blinding was not relevant to our study because we focused on obtaining natural observations rather than applying an experimental design.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Diurnal campaigns were only planned on days without rain. The field conditions were ambient temperature from May until October.
Location	Measurements were conducted at the Swiss Canopy Crane II (SCCII) site in Hölstein, Switzerland (47.439 °N, 7.776 °E, 500 m a.s.l.). Moreover, measurements were considered from published sources: the Swiss Lötschental valley, Hofstetten (Switzerland), the Tillar valley within the Poblet nature reserve (Prades Mountains, northeast Spain), and a North Australian climate gradient from Darwin to Alice springs.
Access & import/export	All research infrastructure and data collection were carried out in accordance with local governmental authorities.
Disturbance	The crane was constructed in a natural forest, with all activities designed to avoid threatening biodiversity at the site.

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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☒	☐	Dual use research of concern
☐	☒	Plants

Methods

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☒	☐	ChIP-seq
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Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

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☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
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Plants

Seed stocks	No plants were removed from their natural locations, and all observations were conducted in the field.
Novel plant genotypes	No novel plant genotypes were produced.
Authentication	No authentication procedure was required.