Supplementary data

Title: Increasing temperature and vapor pressure deficit lead to hydraulic damages in the absence of soil drought

Authors: Leonie C. Schönbeck[1,2,3], Philipp Schuler[4,5], Marco M. Lehmann[4], Eugénie Mas[1,2], Laura Mekarni[1,2], Alexandria L. Pivovaroff[6], Pascal Turberg[1,2], Charlotte Grossiord[1,2]

[1] Plant Ecology Research Laboratory PERL, School of Architecture, Civil and Environmental Engineering ENAC, EPFL, CH-1015 Lausanne, Switzerland

[2] Community Ecology Unit, Swiss Federal Institute for Forest, Snow and Landscape WSL, CH-1015 Lausanne, Switzerland

[3] Department of Botany & Plant Sciences, University of California, 2150 Batchelor Hall, Riverside, CA, 92521 USA

[4] Forest dynamics unit, Swiss Federal Institute for Forest, Snow and Landscape WSL, CH-8903 Birmensdorf, Switzerland

[5] Institute of Agricultural Sciences, ETH Zurich, Zurich Switzerland

[6] Biology Division, Glendale Community College, Glendale, CA, 91208, USA

The following supplementary data is available:

Table S1: Hydraulic characteristics of the three tree species *F. sylvatica, Q. pubescens, Q. ilex*
Table S2: Results of the ANOVA analysis for $\psi_{leaf,md}$, PLA, and PLC
Table S3: Results of the ANOVA analysis for leaf hydraulic traits
Figure S1: Distribution and climatic envelops of the three species *F. sylvatica, Q. pubescens, Q. ilex*
Figure S2: Diurnal pattern of temperature and VPD, and mean daily VPD and soil moisture throughout the experiment
Figure S3: Schematic overview of the experimental setup and pictures of the three species at the end of the experiment
Figure S4: g_s *vs.* VPD curves for all individuals
Figure S5: Stomatal sensitivity (*m*) to $g_{s,ref}$ ratio
Figure S6: Sugar concentrations and K_{max} values in the different treatments
Methods S1: Methods for curve fitting of the g_s *vs.* VPD response curves

Table S1. Average values of stomatal closure (P_{close}), minimum and maximum values of turgor loss point (ψ_{TLP}) and P50 found in the literature for the three studied species. K_{max} values come from direct measurements in this study.

Species	**P_{close}**	**ψ_{TLP} (MPa)**	**P50 (stem, MPa)**	**K_{max} (kg m^{-2} s^{-1} MPa^{-1})**	**Reference**
F. sylvatica	-2.50	-2.04 – -2.50	-3.15	0.014	(Aranda *et al.*, 2001; Choat *et al.*, 2012)
Q. pubescens	-2.75	-2.24 – -2.80	-3.3 – -4.81	0.013	(Choat *et al.*, 2012; Nardini *et al.*, 2012)
Q. ilex	-3.18	-2.84 – -3.15	-3.3 – -6.9	0.007	(Nardini *et al.*, 2012; Martin-StPaul *et al.*, 2014)

Table S2. Results from the ANOVA analysis for midday water potential (ψ_{md}, MPa), loss of conductive area (PLA, %), and conductivity (PLC, %). The interaction is shown when significant ($p<0.05$). Otherwise, only the results from the additive model are shown. Bold numbers indicate significant treatment effects.

		Ψ_{md}		*PLC*		*PLA*	
F. sylvatica	**df**	**F**	**p**	**F**	**p**	**F**	**p**
Temperature	**1**	**4.27**	**0.041**	4.07	0.053	**10.12**	**0.002**
VPD	**2**	**29.28**	**<0.001**	**21.29**	**<0.001**	**10.39**	**0.002**
Temp*VPD	**2**	**13.25**	**<0.001**				
Q. pubescens							
Temperature	**1**	**7.57**	**0.007**	2.47	0.128	**6.82**	**0.011**
VPD	**2**	**3.95**	**0.022**	**5.76**	**0.024**	**4.05**	**0.049**
Temp*VPD	**2**	**6.86**	**0.001**				
Q. ilex							
Temperature	**1**	0.60	0.443	0.40	0.534	0.17	0.685
VPD	**2**	**9.03**	**0.005**	0.16	0.690	1.27	0.265
Temp*VPD	**2**						

Table S3. Results from the ANOVA analysis for leaf water evaporation (E, mmol m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}), stomatal sensitivity to VPD (*m,* mmol m^{-2} s^{-1} kPa^{-1}), minimum conductance (g_{min}, mmol m^{-2} s^{-1}), and turgor loss point (ψ_{TLP}, MPa). The interaction is shown when significant (p<0.05). Otherwise, only the results from the additive model are shown. Bold numbers indicate significant treatment effects.

		E		***g_s***		***m***		***g_{min}***		***Ψ_{TLP}***		***Sugar***	
F. sylvatica	**df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
T	**1**	0.78	0.387	0.24	0.632	**5.08**	**0.033**	3.87	0.058	0.45	0.451	0.31	0.580
VPD	**2**	**8.05**	**0.008**	3.97	0.058	0.89	0.354	0.09	0.925	1.34	0.255	0.16	0.686
T*VPD	**2**							**5.25**	**0.029**				
Q. pubescens													
T	**1**	2.08	**0.005**	1.37	0.251	**8.31**	**0.005**	0.40	0.529	**5.34**	**0.023**	**5.66**	**0.020**
VPD	**2**	9.11	0.161	0.18	0.677	0.22	0.638	1.68	0.204	**7.36**	**0.008**	**7.09**	**0.010**
T*VPD	**2**									**7.75**	**0.006**		
Q. ilex													
T	**1**	4.04	0.055	2.83	0.105	0.72	0.407	**6.28**	**0.017**	**7.44**	**0.010**	0.33	0.566
VPD	**2**	3.81	0.061	0.84	0.369	1.27	0.271	0.74	0.396	**10.41**	**0.003**	0.12	0.727
T*VPD	**2**									**6.85**	**0.013**		

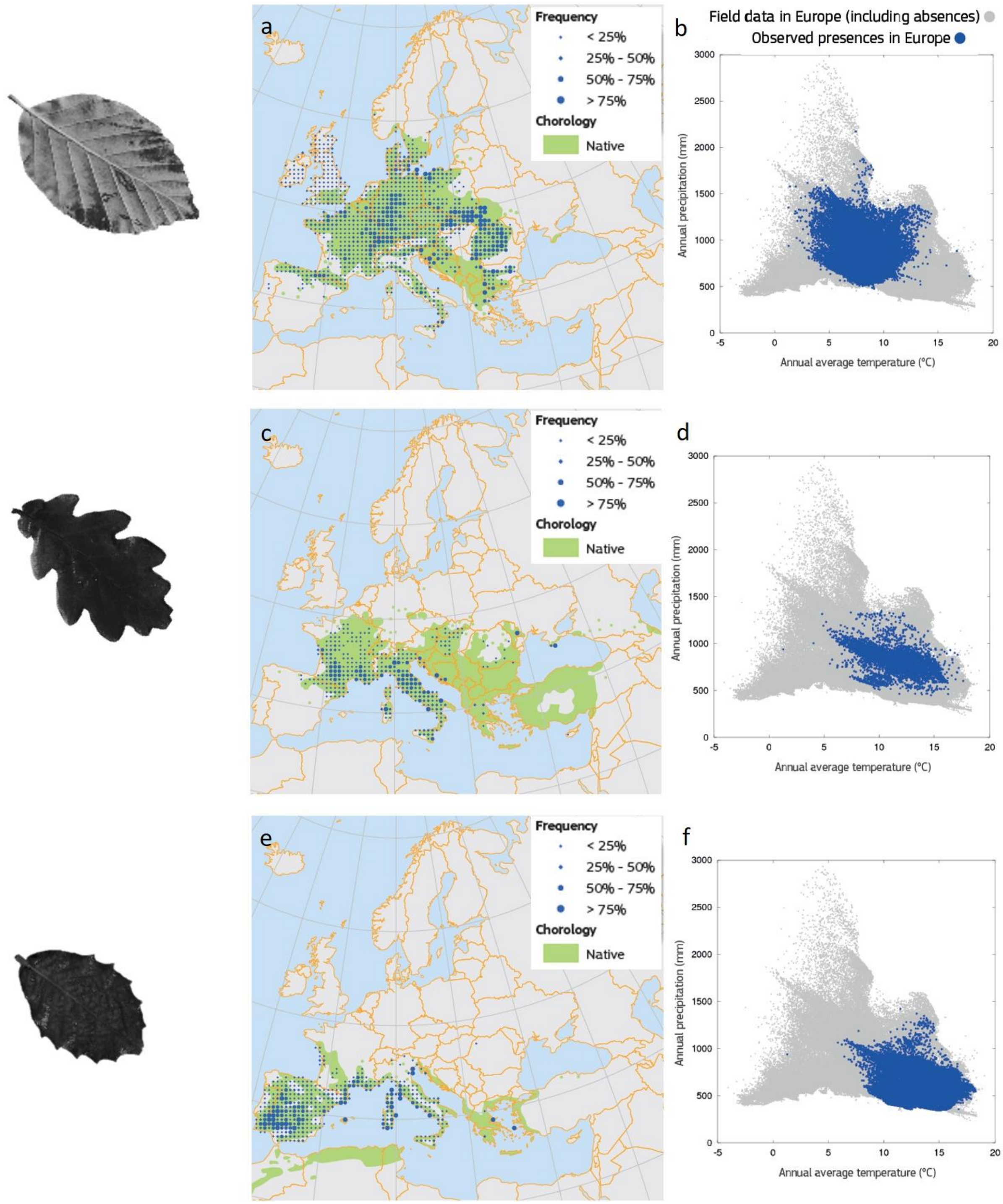

Figure S1. Distribution throughout Europe and the climatic envelopes of *Fagus sylvatica* L. (a, b), *Quercus pubescens* Wildd (c, d), and *Quercus ilex* L. (e, f) (Distribution maps from EUFORGEN, euforgen.org).

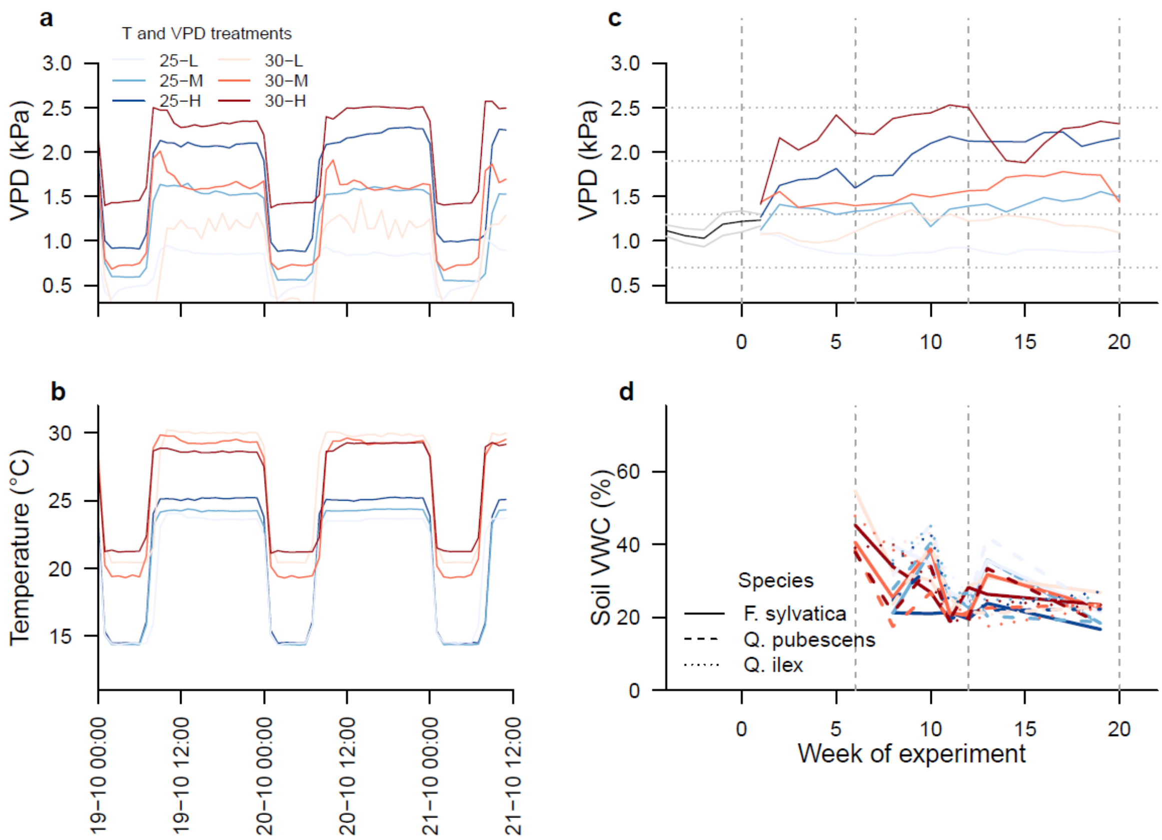

Figure S2. a & b) Example of diurnal patterns over the course of 3 days of VPD (a) and temperature (b) in the six climate chambers; **c)** Weekly means of daytime VPD throughout the experiment. The black line indicates the average VPD in all six chambers during the acclimation period, with the standard error indicated by the grey lines.; **d)** Soil volumetric water content, measured using a TDR 100 Soil Moisture Probe. Line types indicate the three species. In all panels, colors indicate the different T and VPD treatments in the six climate chambers.

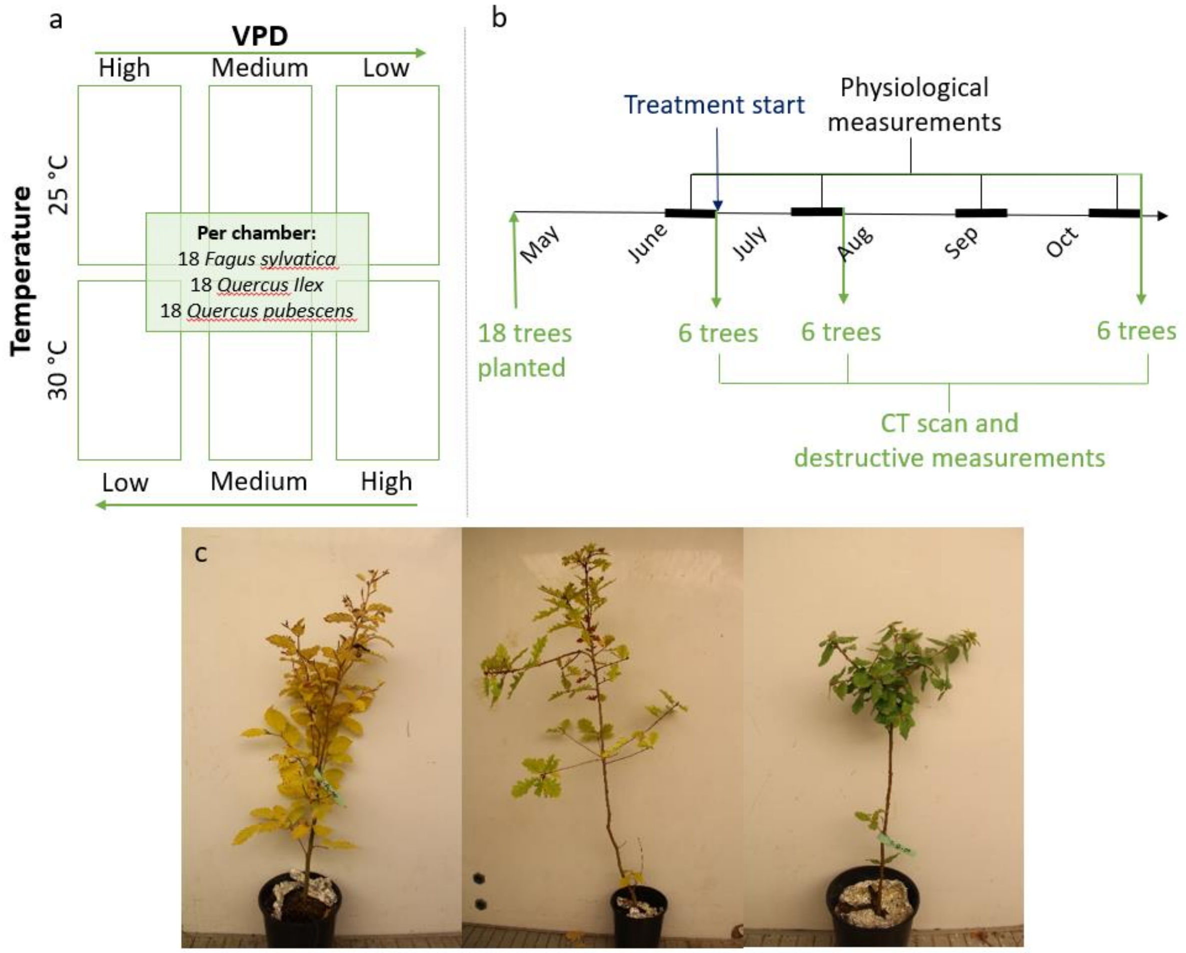

Figure S3. Schematic overview of the experimental setup. a) Climate settings of the six climate chambers, with three chambers set to daytime temperatures of 25°C and three chambers to 30°C. Within each temperature level, chambers were set to low (0.7-1.3 kPa), medium (1.3-1.9 kPa) or high (1.9-2.5 kPa) VPD. b) Timeline of the experiment. All trees were left for acclimation over five weeks at the start of the experiment. The week before the treatments started, physiological measurements were carried out, and six trees per species were harvested and scanned using µCT (campaign 1). After 5, 10, and 15 weeks of treatment, similar physiological measurements were carried out (campaigns 2-4). After 5 and 15 weeks, another six individuals per species were harvested and scanned using µCT. c) Pictures of an individual of each species *F. sylvatica* (l), *Q. pubescens* (m), and *Q. ilex* (r) before the final harvest.

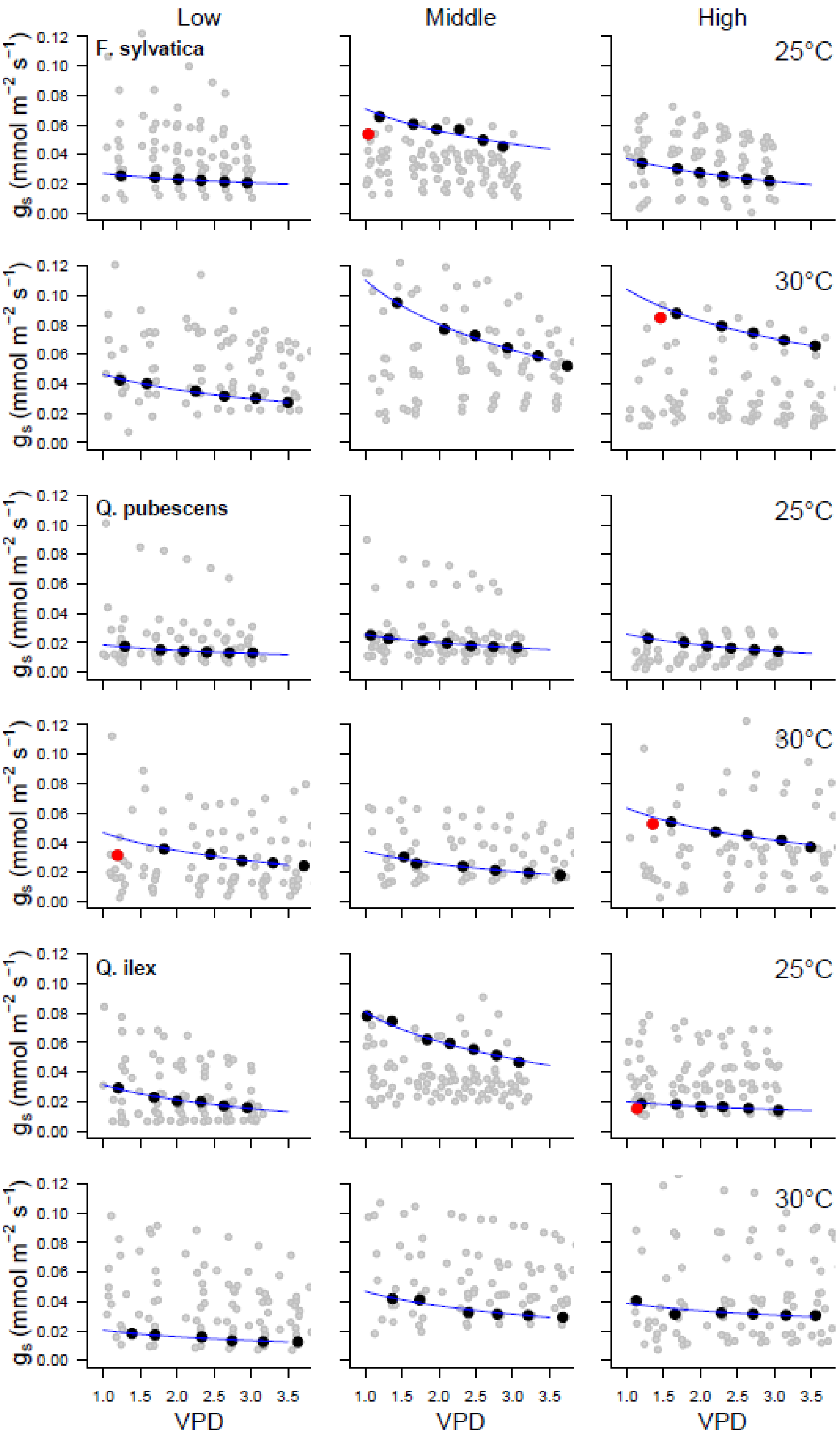

Figure S4. G_s *vs.* VPD curves for each species during all measurement campaigns in the six climate chambers: Low, middle, and high VPD at 25°C and 30°C. Blue lines indicate the fitted model of one curve (black dots) to highlight the method used (see Methods S1). Model fitting started at the VPD level where g_s was the highest, in some cases thereby eliminating the first point of a curve, indicated here by red dots.

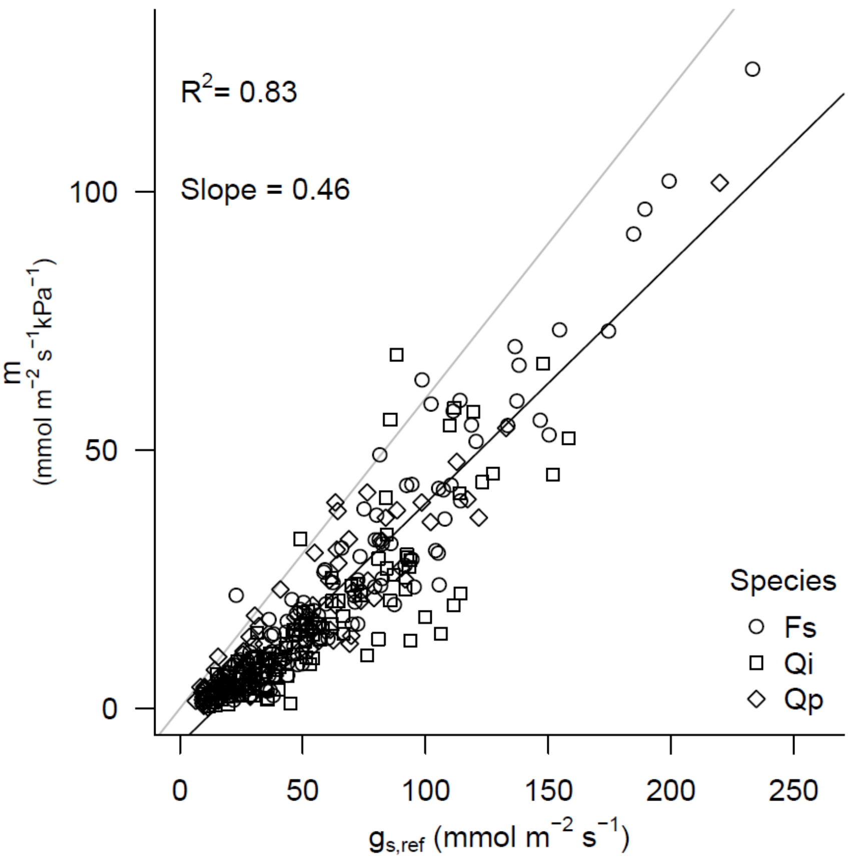

Figure S5. Stomatal sensitivity (*m*, the slope of the logarithmic curve of g_s to VPD, see Fig. S5) as a function of the reference stomatal conductance ($g_{s,ref}$). The universal ratio of 0.6 suggested by Oren et al. (1999) is indicated by a grey line. The black line indicates the ratio measured in this study (slope = 0.46, R^2 = 0.83).

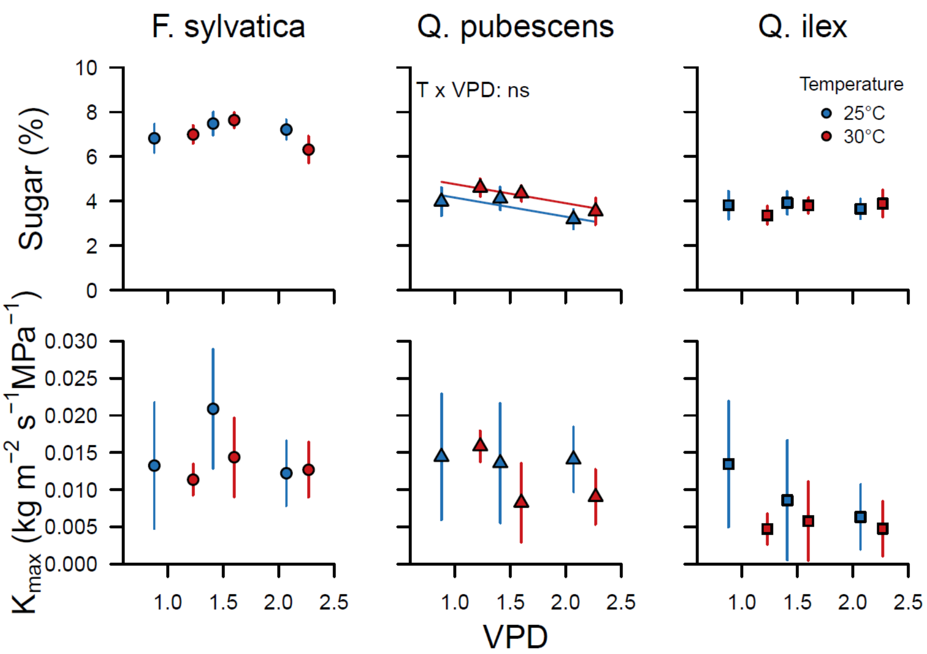

Figure S6. Sugar concentration in the leaves and maximum xylem hydraulic conductance (K_{max}) in *Fagus sylvatica, Quercus pubescens,* and *Quercus ilex* in the two temperature and three VPD treatments. Data are shown in relation to the average VPD in the chambers during the treatment period. Symbols indicate the mean ± SE of three measurement campaigns (n = 18). Colored lines – blue for 25°C and red for 30°C – indicate the VPD effects in the different temperature treatments in case of a T and VPD effect or interaction.

Methods S1

First, apparent outliers of g_s were cleaned with visual inspection and by removing g_s values below 0 and above 1.5 mol m^{-2} s^{-1} (Ely *et al.*, 2021). Different fitting curves were tested to calculate the sensitivity of g_s to VPD. The Oren model (Oren *et al.*, 1999) was used in the first instance, assuming a logarithmic decrease in g_s with increasing VPD, but many response curves seemed to follow different patterns. For example, we sometimes observed an initial increase of g_s with increasing VPD followed by a logarithmic decrease. Accordingly, polynomial (2^{nd} and 3^{rd} degree), logarithmic curves, and a logarithmic curve starting from the maximum measured g_s, independent of the VPD where it was measured, were tried, and the goodness of fit was compared. Fitting the logarithm from the maximum g_s resulted in the best fit that was comparable between all species, treatments, and campaigns.