

New Phytologist Supporting Information

Article title: Contrasting anatomical and biochemical controls on mesophyll conductance across plant functional types

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Table S1: Overview of leaf-level traits reported in this study.

Table S2: Statistics of the main results in this study with g_m standardised by two different temperature response functions.

Figure S1: Number of studies per year and measurement methods applied to determine g_m . Year refers to the year in which the study was published in issue, not published online. Studies published in issue after 2020 are not shown.

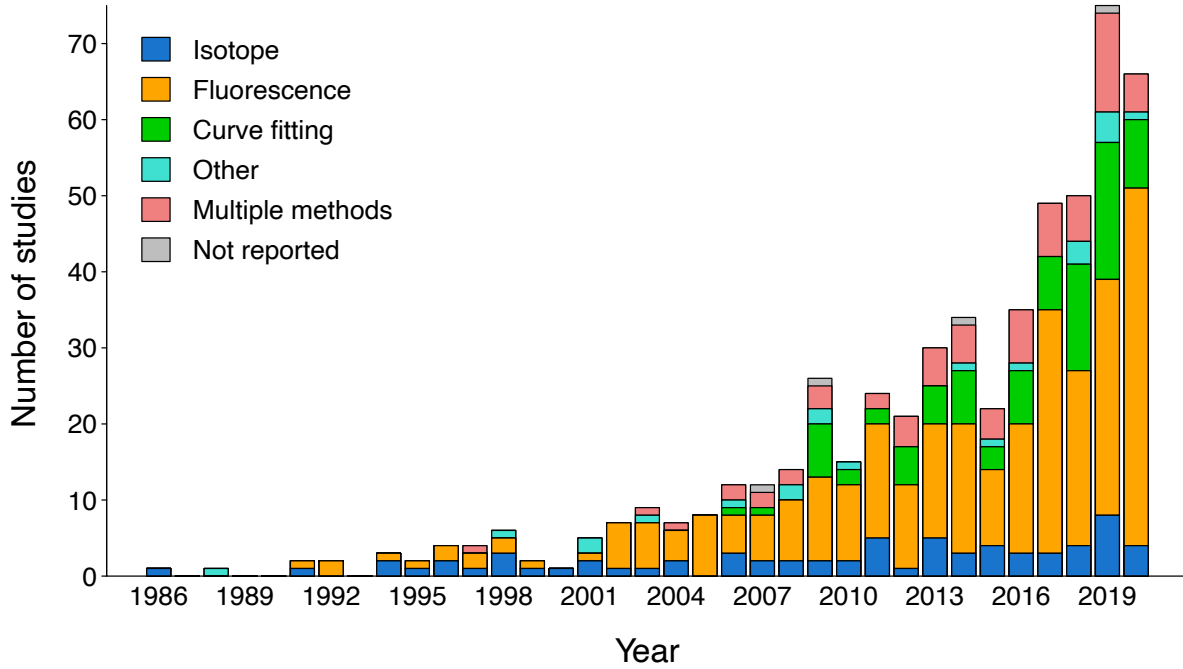


Figure S2: Violin plots showing mesophyll conductance standardised to 25 °C ($g_{m,25}$) for different plant functional types grouped according to measurement method (C: curve fitting, F: fluorescence, I: isotope, other: all other methods pooled). Dots represent group medians, black bars represent the interquartile range, and black lines represent 1.5 times the interquartile range. Shown are only groups with at least seven observations ($n \geq 7$). Letters above the panel indicate differences in the mean according to Dunn's test of multiple comparisons ($p < 0.05$).

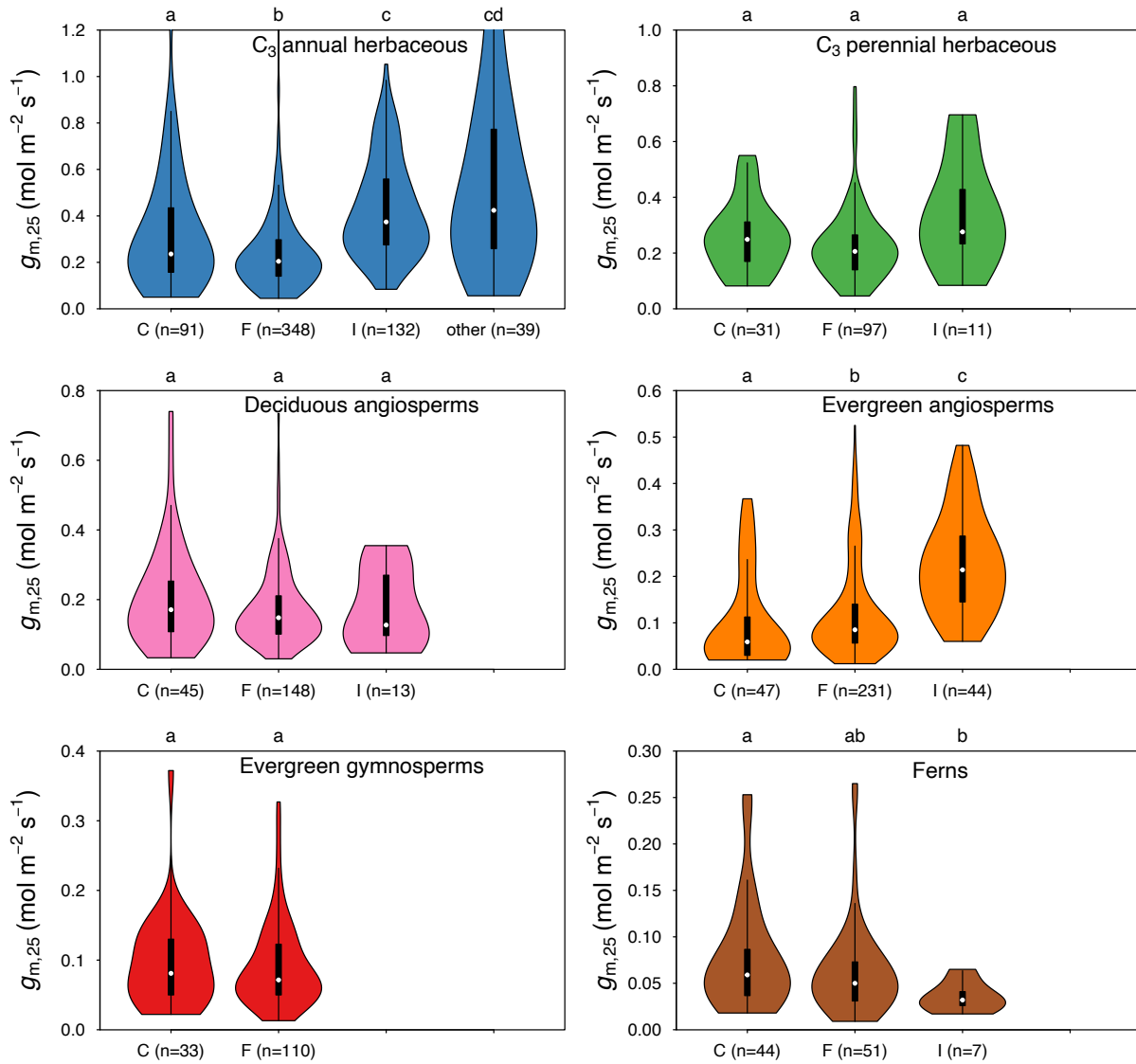


Figure S3: Violin plots showing mesophyll conductance standardised to 25 °C ($g_{m,25}$) for herbaceous crop species with at least five individual measurements. The white dots represent the species median, the black bars represent the interquartile range, the black lines represent 1.5 times the interquartile range, and the dashed grey line represents the median g_m across all measurements in herbaceous crops ($0.26 \text{ mol m}^{-2} \text{ s}^{-1}$). The last two violins show all measurements of annual and perennial crops, respectively, including species with less than five measurements.

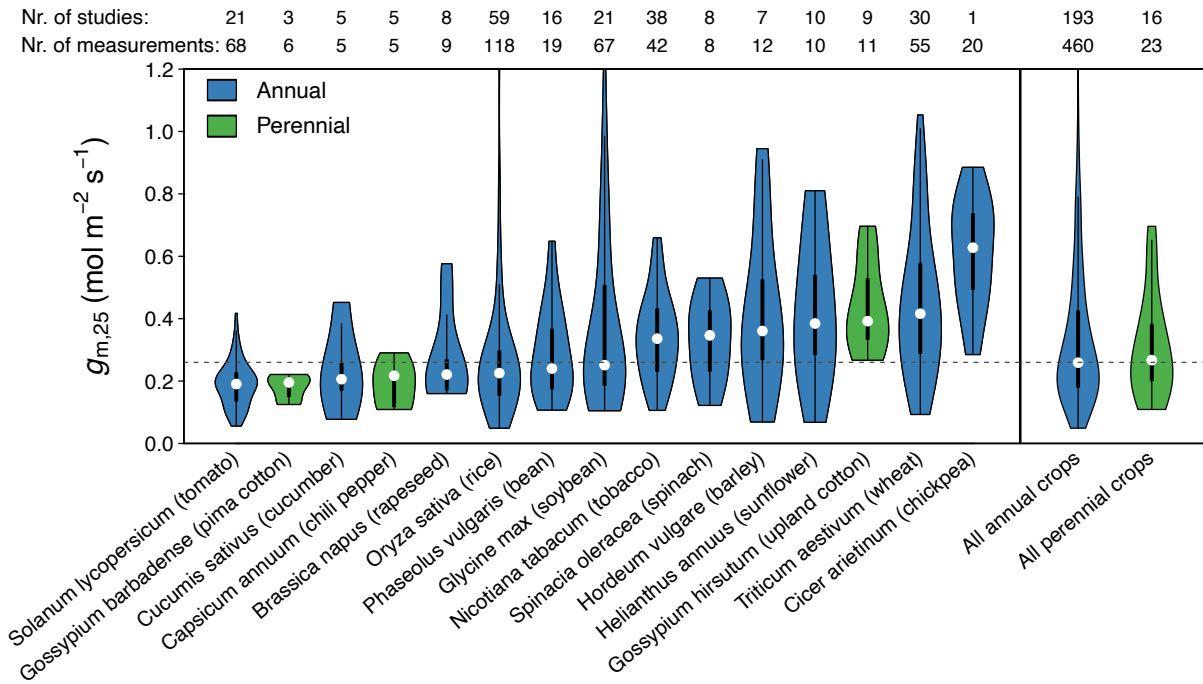


Figure S4: Relationships between mesophyll conductance standardised to 25 °C ($g_{m,25}$) and leaf structural traits: (a) leaf dry mass per area (LMA), (b) leaf density (D_{leaf}), (c) leaf thickness (T_{leaf}), (d) mesophyll thickness ($T_{\text{mesophyll}}$), (e) fraction of leaf mesophyll composed of intercellular air spaces (f_{ias}), (f) stomatal length (L_{stomata} ; abaxial and adaxial combined), (g) stomatal area (A_{stomata} ; abaxial and adaxial combined), (h) stomatal density abaxial (SD_{abaxial}) (i) stomatal density adaxial (SD_{adaxial}). Lines represent robust linear regression fits, which are only drawn if $p < 0.05$.

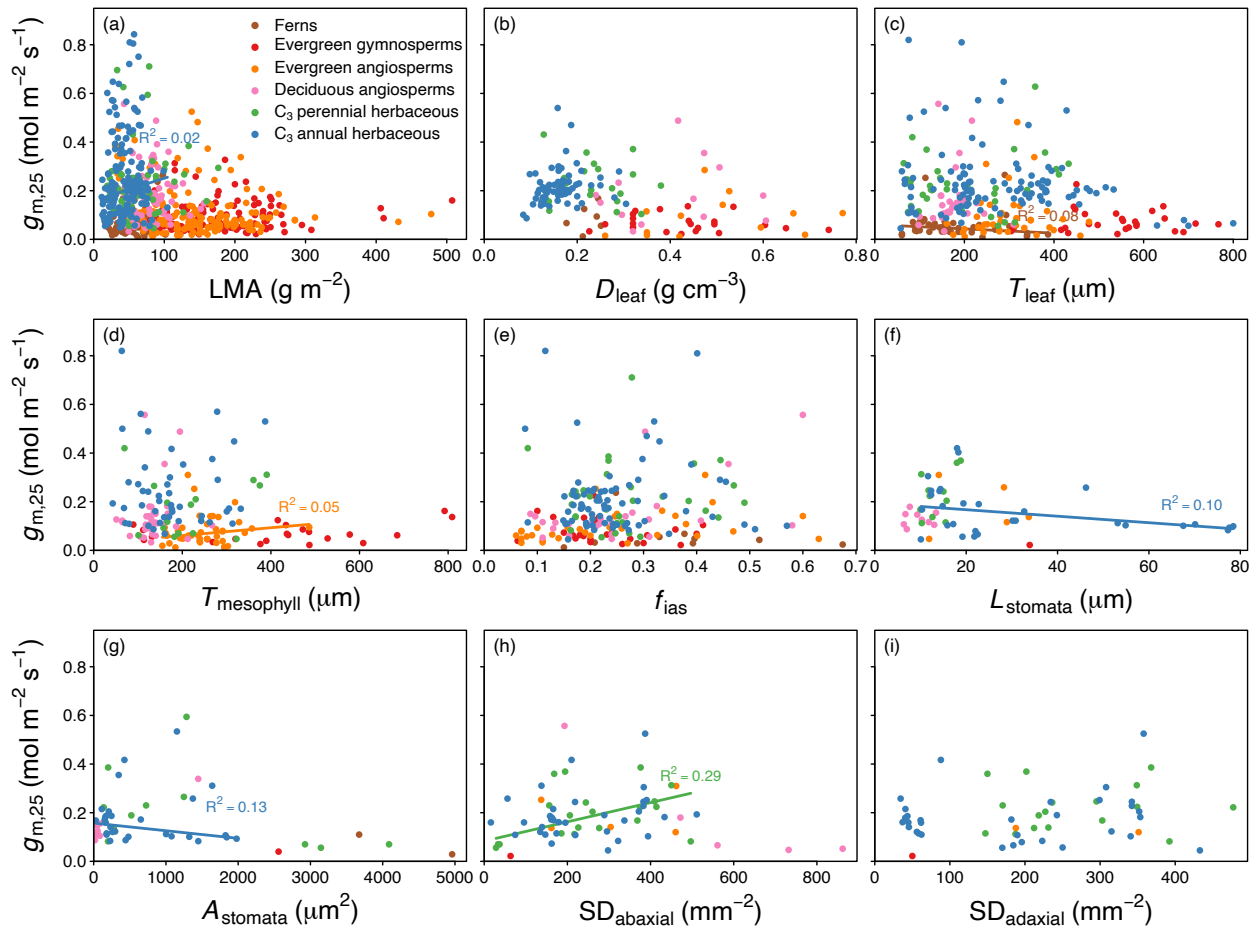


Figure S5: Relationships between mesophyll conductance standardised to 25 °C ($g_{m,25}$) and leaf anatomical traits: (a) surface area of mesophyll cells exposed to the intercellular airspaces per unit leaf area (S_m), (b) ratio of chloroplast surface area exposed to the intercellular airspaces per unit leaf area (S_c) to S_m , (c) cytosol thickness (T_{cytosol}), (d) chloroplast thickness ($T_{\text{chloroplast}}$), (e) chloroplast length ($L_{\text{chloroplast}}$), (f) chloroplast surface area ($S_{\text{chloroplast}}$). Lines represent robust linear regression fits, which are only drawn if $p < 0.05$.

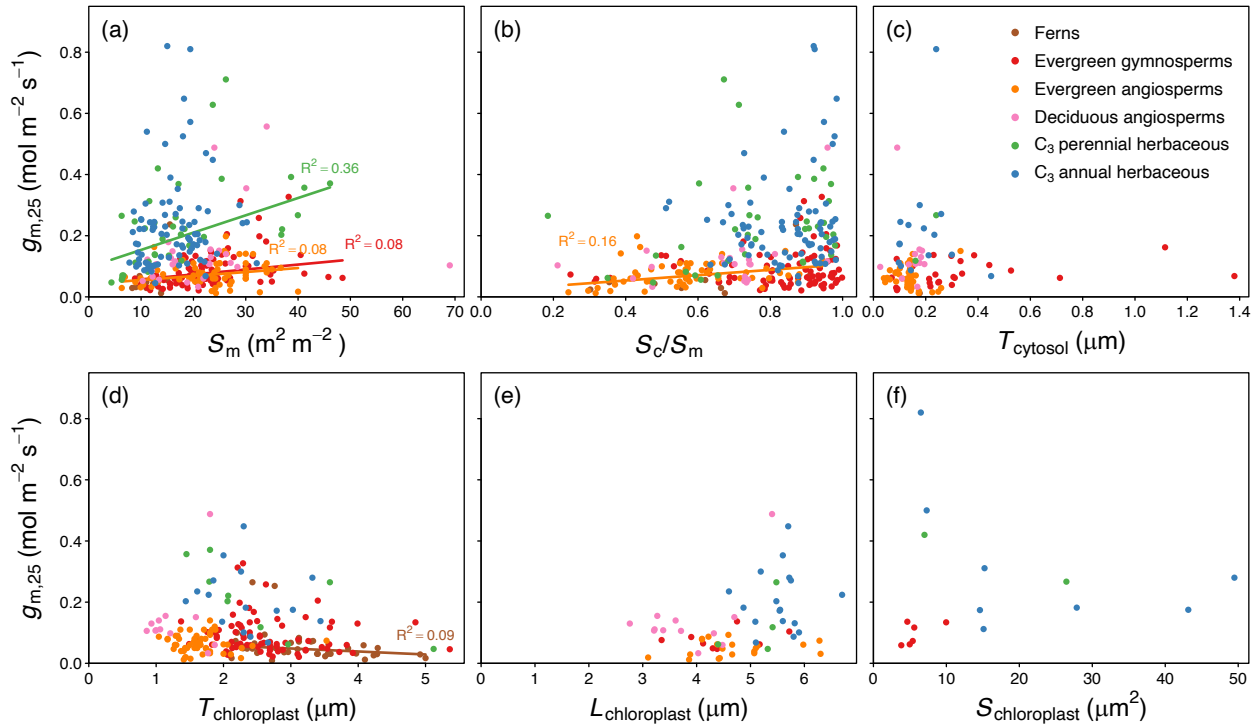


Table S1: Overview of leaf-level traits reported in this study. The number of available data as well as the statistics refer to the dataset after filtering as described in the Methods section.

Trait group	Trait symbol	Definition	Unit	Number of measurements (% of all measurements)	Number of studies (% of all studies)	Min	1 st Q	Median	Mean	3 rd Q	Max
Leaf physiology	g_m	Mesophyll conductance to CO ₂ at measurement temperature standardised to 100 kPa	mol m ⁻² s ⁻¹	1337 (100)	476 (100)	0.009	0.089	0.172	0.228	0.305	1.97
	$g_{m,25}$	Mesophyll conductance to CO ₂ standardised to 100 kPa and 25 °C	mol m ⁻² s ⁻¹	1337 (100)	476 (100)	0.009	0.087	0.164	0.215	0.281	1.50
	A_n	Net photosynthesis rate	μmol m ⁻² s ⁻¹	1130 (84.5)	394 (82.8)	1.5	9.0	14.7	16.0	22.4	48.3
	$g_{s,c}$	Stomatal conductance to CO ₂	mol m ⁻² s ⁻¹	763 (57.1)	250 (52.5)	0.007	0.103	0.174	0.194	0.263	0.955
	$V_{cmax,Cc}$	Maximum carboxylation rate on C _c -basis	μmol m ⁻² s ⁻¹	590 (44.1)	220 (47.5)	17.0	63.8	115.4	125.4	164.0	397.0
	C_i	Intercellular CO ₂ concentration	μmol mol ⁻¹	501 (37.5)	210 (44.1)	131.0	239.7	260.7	261.5	289.6	365.6
	C_c	Chloroplast CO ₂ concentration	μmol mol ⁻¹	417 (31.2)	173 (36.3)	48.5	128.0	167.9	166.5	200.0	304.0
	L_m	Photosynthetic limitation by g_m	%	46 (3.4)	26 (5.5)	1.1	7.7	18.3	20.4	29.0	55.0
Leaf structure	LMA	Leaf mass per area	g m ⁻²	627 (46.9)	180 (37.8)	11.4	45.1	77.2	100.8	144.5	507.6
	T_{leaf}	Leaf thickness	μm	281 (21.0)	80 (16.8)	58	159	231	268	349	800
	f_{ias}	Fraction of intercellular airspaces	-	198 (14.8)	66 (13.9)	0.06	0.175	0.231	0.253	0.305	0.675
	$T_{mesophyll}$	Mesophyll thickness	μm	155 (11.6)	63 (13.2)	43	132	190	226	279	809
	D_{leaf}	Leaf density	g cm ⁻³	146 (10.9)	26 (5.5)	0.08	0.16	0.21	0.27	0.33	0.77

	$SD_{abaxial}$	Stomatal density (abaxial)	mm^{-2}	65 (4.9)	26 (5.5)	16	162	219	272	383	862
	$L_{stomata}$	Stomatal length	μm	55 (4.1)	19 (4.0)	6.4	11.9	15.7	23.7	28.6	78.5
	$SD_{adaxial}$	Stomatal density (adaxial)	mm^{-2}	52 (3.9)	15 (3.2)	34	82	215	213	322	477
	$A_{stomata}$	Stomatal area	μm^2	51 (3.8)	15 (3.2)	13	167	346	929	1348	4961
Leaf anatomy	S_c	Chloroplast surface area exposed to the intercellular airspaces per unit leaf area	$m^2 m^{-2}$	339 (25.4)	68 (14.3)	1.2	8.7	13.0	13.7	18.0	36.0
	S_m	Mesophyll surface area exposed to the intercellular airspaces per unit leaf area	$m^2 m^{-2}$	305 (22.8)	66 (13.9)	4.3	13.2	19.3	20.1	24.4	69.0
	T_{cw}	Cell wall thickness	μm	298 (22.3)	52 (10.9)	0.095	0.183	0.307	0.351	0.449	1.220
	$T_{chloroplast}$	Chloroplast thickness	μm	207 (15.5)	26 (5.5)	0.86	1.74	2.26	2.38	2.88	5.36
	$T_{cytosol}$	Cytosol thickness	μm	110 (8.2)	22 (4.6)	0.026	0.129	0.143	0.193	0.215	1.380
	$L_{chloroplast}$	Chloroplast length	μm	61 (4.6)	19 (4.0)	2.76	4.12	4.70	4.76	5.48	6.70
	$S_{chloroplast}$	Chloroplast surface area	μm^2	16 (1.2)	7 (1.5)	3.82	5.55	8.64	15.44	18.04	49.48
Leaf biochemistry	Leaf N	Leaf nitrogen content	$g m^{-2}$	313 (23.4)	116 (24.4)	0.59	1.38	1.87	2.02	2.31	6.08
	Rubisco	Leaf Rubisco content	$g m^{-2}$	77 (5.8)	41 (8.6)	0.24	1.51	1.85	2.38	3.11	10.78
	Leaf K	Leaf potassium content	$g m^{-2}$	26 (1.9)	19 (4.0)	0.28	0.73	1.06	1.09	1.37	2.22

Table S2: Model and regression results with two different temperature standardisations of g_m : Bernacchi et al. (2002) as in the main manuscript and Walker et al. (2013). Values in brackets represent 95% confidence intervals (n.s. = non-significant).

Plant Functional Type		Bernacchi et al. (2002)			Walker et al. (2013)		
Median g_m (Figure 2a)	Ferns		0.050			0.050	
	Evergreen gymnosperms		0.075			0.075	
	Evergreen angiosperms		0.095			0.102	
	Deciduous angiosperms		0.149			0.157	
	C ₃ perennial herbaceous		0.210			0.220	
	C ₃ annual herbaceous		0.240			0.283	
		Intercept	Slope	Adj. R ²	Intercept	Slope	Adj. R ²
$g_m - S_c$ relationship (Figure 3a)	All PFTs	0.052 [0.029, 0.074]	0.0040 [0.0024, 0.0056]	0.12	0.054 [0.029, 0.080]	0.0046 [0.0027, 0.0065]	0.11
	Ferns	-	-	n.s.	0.024 [0.007, 0.041]	0.0040 [0.0009, 0.0070]	0.18
	Evergreen gymnosperms	0.011 [-0.027, 0.049]	0.0038 [0.0014, 0.0063]	0.23	0.013 [-0.026, 0.052]	0.0038 [0.0013, 0.0062]	0.21
	Evergreen angiosperms	0.007 [-0.010, 0.024]	0.0049 [0.0034, 0.0063]	0.47	0.009 [-0.008, 0.026]	0.0047 [0.0033, 0.0062]	0.46
	Deciduous angiosperms	0.036 [-0.018, 0.089]	0.0049 [0.0017, 0.0082]	0.22	-	-	n.s.
	C ₃ perennial herbaceous	0.124 [0.075, 0.172]	0.0067 [0.0039, 0.0095]	0.30	0.128 [0.082, 0.175]	0.0096 [0.0064, 0.0127]	0.43
	C ₃ annual herbaceous	-	-	n.s.	-	-	n.s.
		a	b	Adj. R ²	a	b	Adj. R ²
$g_m - T_{cw}$ relationship (Figure 3b)	All PFTs	0.031 [0.022, 0.039]	-0.989 [-1.148, -0.829]	0.33	0.029 [0.021, 0.038]	-1.081 [-1.249, -0.914]	0.32
	Ferns	0.019 [0.011, 0.027]	-0.784 [-1.082, -0.486]	0.14	0.020 [0.012, 0.028]	-0.752 [-1.033, -0.471]	0.12

	Evergreen gymnosperms	0.047 [0.033, 0.062]	-0.752 [-1.063, -0.442]	0.21	0.048 [0.033, 0.063]	-0.762 [-1.085, -0.440]	0.21
	Evergreen angiosperms	0.025 [0.008, 0.041]	-0.952 [-1.566, 0.338]	0.10	0.027 [0.009, 0.046]	-0.858 [-1.468, -0.247]	0.06
	Deciduous angiosperms	-	-	n.s.	-	-	n.s.
	C ₃ perennial herbaceous	0.036 [-0.005, 0.078]	-1.02 [-1.634, -0.407]	0.20	0.043 [-0.005, 0.090]	-1.056 [-1.647, -0.465]	0.24
	C ₃ annual herbaceous	-	-	n.s.	-	-	n.s.
			R ²			R ²	
glm model (Table 1)	All PFTs		0.48			0.50	
	Ferns		0.57			0.59	
	Evergreen gymnosperms		0.46			0.44	
	Evergreen angiosperms		0.34			0.28	
	Deciduous angiosperms		0.55			0.64	
	C ₃ perennial herbaceous		0.46			0.55	
	C ₃ annual herbaceous		0.09			0.08	
<i>g_m</i> - leaf N relationship (Figure 4a)		Intercept	Slope	R ²	Intercept	Slope	R ²
	Deciduous angiosperms	0.031 [-0.051, 0.113]	0.080 [0.033, 0.127]	0.18	0.041 [-0.042, 0.125]	0.078 [0.031, 0.125]	0.16
	C ₃ perennial herbaceous	0.148 [0.046, 0.251]	0.030 [0.005, 0.055]	0.28	0.085 [0.012, 0.158]	0.051 [0.031, 0.071]	0.63
	C ₃ annual herbaceous	-0.109 [-0.224, 0.007]	0.214 [0.148, 0.280]	0.51	-0.077 [-0.183, 0.029]	0.207 [0.146, 0.267]	0.48
<i>g_m</i> - leaf K relationship (Figure 4b)		Intercept	Slope	Adj. R ²	Intercept	Slope	Adj. R ²
	C ₃ annual herbaceous	0.002 [-0.097, 0.102]	0.168 [0.048, 0.288]	0.44	0.046 [-0.035, 0.126]	0.123 [0.040, 0.206]	0.35

References

Bernacchi CJ, Portis AR, Nakano H, von Caemmerer S, Long SP. 2002. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. *Plant Physiology* **130**: 1992–1998.

Walker B, Ariza LS, Kaines S, Badger MR, Cousins AB. 2013. Temperature response of in vivo Rubisco kinetics and mesophyll conductance in *Arabidopsis thaliana*: comparisons to *Nicotiana tabacum*. *Plant, Cell & Environment* **36**: 2108–2119.