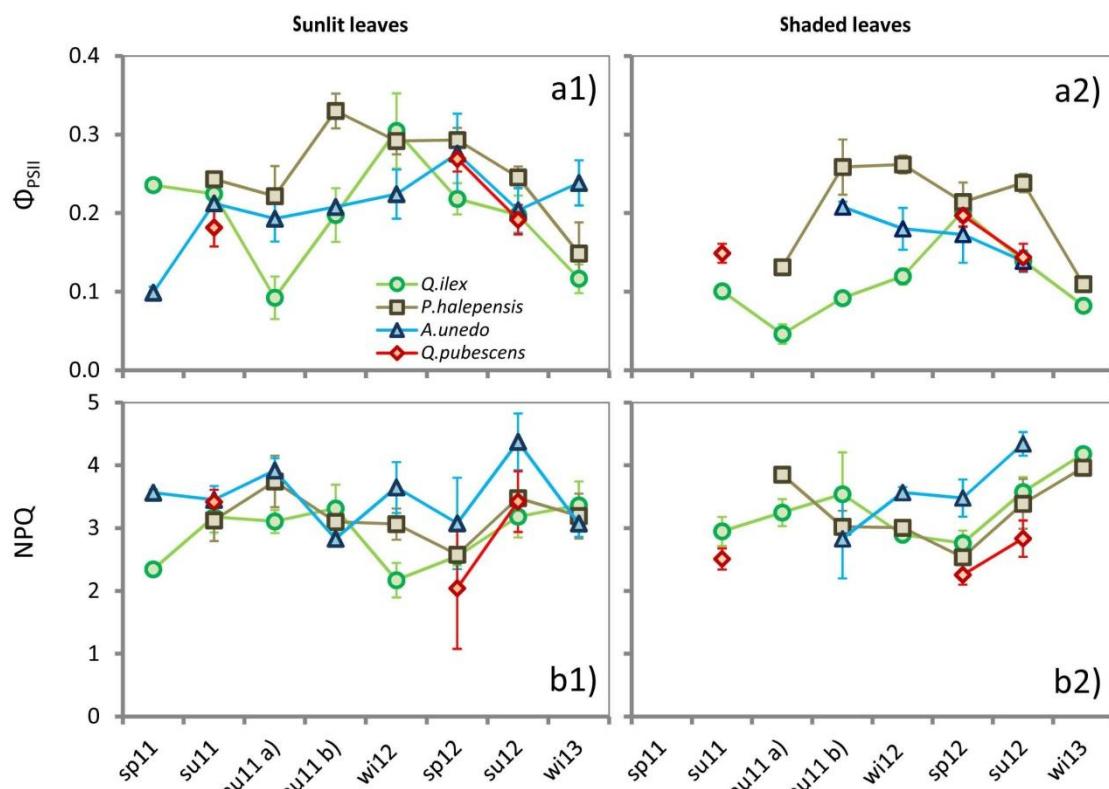


1 Supplementary Figures

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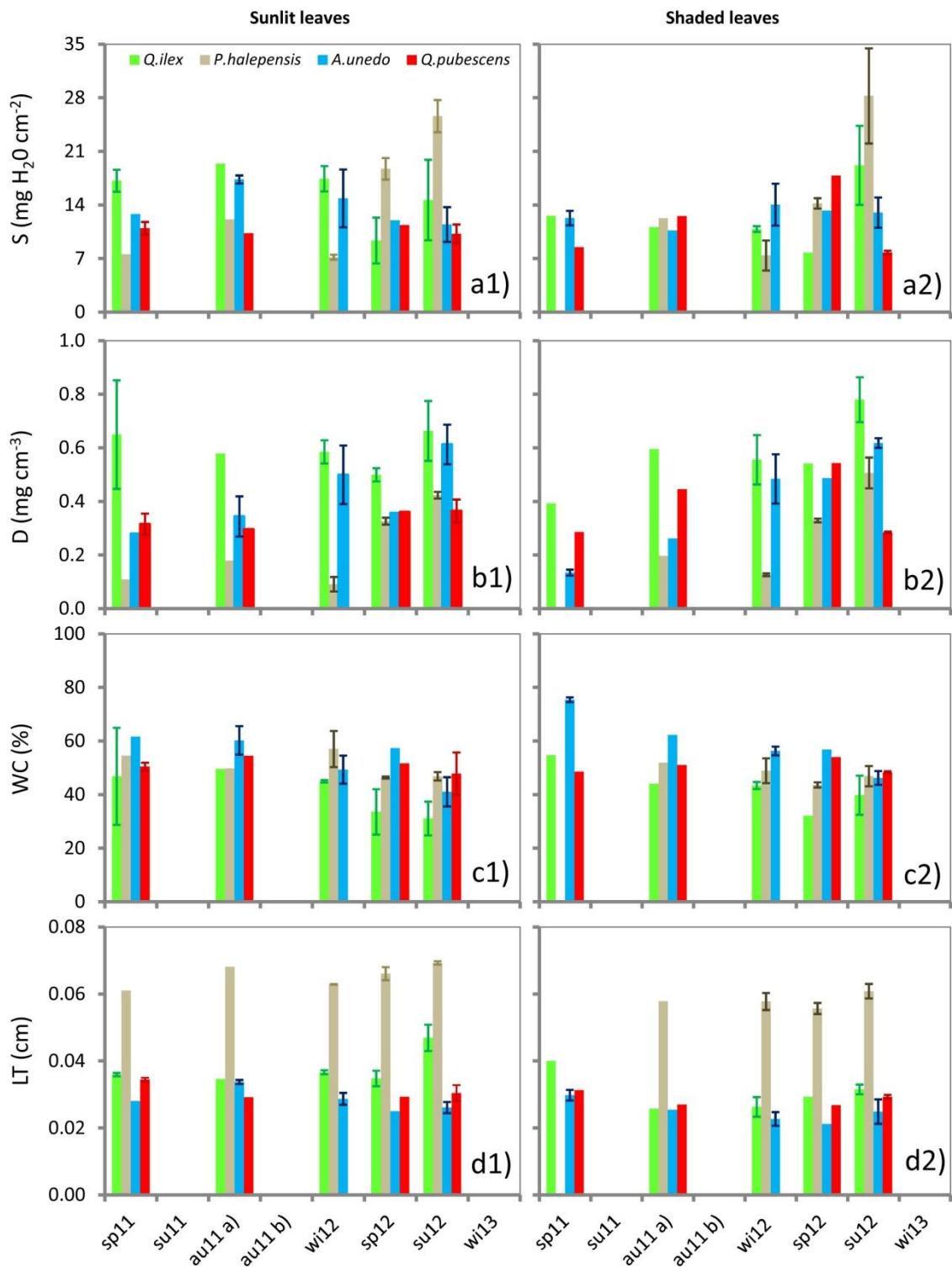


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Fig. S1. Line graphs depicting seasonal changes of a) effective quantum efficiency of PSII (Φ_{PSII}), and b) nonphotochemical quenching (NPQ) for *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in sunlit (1) and shaded (2) leaves. Missing data points were due to limitations of labour and equipment. Vertical bars indicate standard errors of the means ($n = 3-5$).

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This is a pre-copyedited, author-produced PDF of supplementary material of an article accepted for publication in *Tree physiology* following peer review. The version of record Sperlich, D., et al. "Seasonal variability of foliar photosynthetic and morphological traits and drought impacts in a Mediterranean mixed forest" in *Tree physiology*, Vol. 35 issue 5 (May 2015), p. 501-520 is available online at: DOI [10.1093/treephys/tpv017](https://doi.org/10.1093/treephys/tpv017)



9
10 **Fig. S2.** Bar charts depicting seasonal changes of a) succulence (S), b) leaf density (D), c) water content
11 (WC), and d) leaf thickness (LT) for *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in sunlit (1) and
12 shaded (2) leaves. Error bars indicate standard errors of the means ($n = 3-5$).
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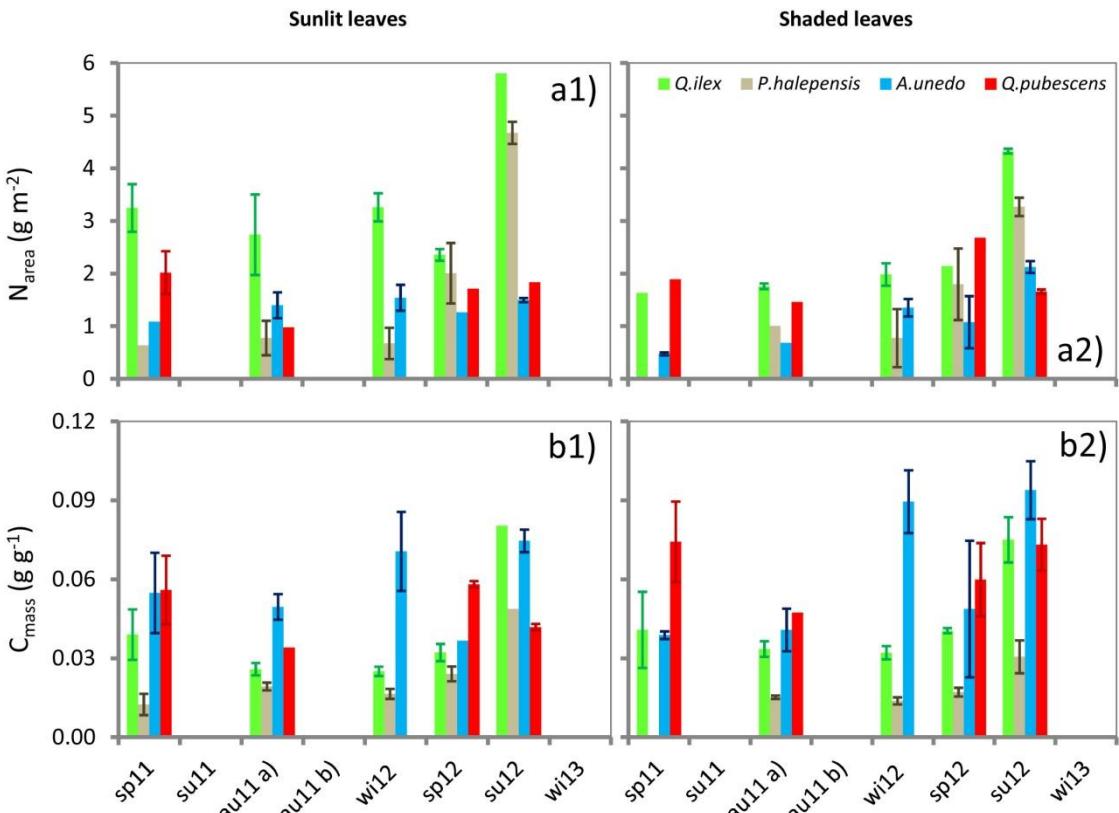


Fig. S3. Bar charts depicting seasonal changes of a) nitrogen per unit leaf area (N_{area}) and b) carbon per unit leaf mass (C_{mass}) for *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in sunlit (1) and shaded (2) leaves. Error bars indicate standard errors of the means ($n = 3-5$).

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Supplementary tables

Table S1. Regression equations and coefficients of determination (R^2) for A_{net}/g_s for sunlit and shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling campaigns.

Campaign	Leaf position	<i>Q. ilex</i>		<i>P. halepensis</i>		<i>A. unedo</i>		<i>Q. pubescens</i>		All species	
		Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
Total	sunlit	$y = 60.7x + 2.4$	0.85	$y = 35.5x + 2.7$	0.61	$y = 80.5x + 1.9$	0.79	$y = 110.5x + 0.5$	0.74	$y = 57.9x + 2.3$	0.69
	shaded	$y = 69.6x + 1.5$	0.72	$y = 37.9x + 2.8$	0.58	$y = 57.7x + 2.4$	0.71	$y = 104.9x + 1.3$	0.78	$y = 57.0x + 2.4$	0.55
Spring 2011	sunlit	$y = 42.7x + 4.1$	0.99			$y = 26.2x + 4.1$	0.72	$y = 31.5x + 3.6$	-0.50	$y = 42.7x + 3.5$	0.89
	shaded			$y = 10.0x + 4.7$	0.98	$y = 62.4x + 1.3$		$y = 42.7x + 4.5$	0.80	$y = 16.1x + 4.7$	-0.11
Summer 2011	sunlit	$y = 93.3x + 1.0$	0.73	$y = 96.3x + 0.5$	0.23	$y = 43.4x + 6.8$	0.79	$y = 92.0x - 0.3$	0.76	$y = 116.6x - 0.1$	0.87
	shaded	$y = 146.9x + 0.5$	0.29					$y = 53.6x + 2.6$	0.52	$y = 54.7x + 2.7$	0.51
Autumn 2011 ^a	sunlit	$y = 215.0x - 1.8$	0.91	$y = 56.0x - 0.6$	0.97	$y = 145.3x - 1.6$				$y = 62.9x + 0.4$	0.57
	shaded	$y = 120.7x - 1.3$	0.99							$y = 114.0x - 1.0$	0.96
Autumn 2011 ^b	sunlit	$y = 107.0x - 0.6$	0.7	$y = 31.1x + 2.6$	0.96					$y = 21.9x + 4.8$	0.14
	shaded	$y = 70.9x + 0.3$	0.95	$y = 32.4x + 1.9$	0.95	$y = 96.5x - 3.1$				$y = 38.4x + 2.1$	0.73
Winter 2012	sunlit	$y = 85.4x + 1.3$	0.97	$y = -23.3x + 11.3$	0.51	$y = 122.9x - 0.1$	0.89			$y = 49.2x + 2.9$	0.54
	shaded	$y = 73.8x + 1.8$	0.86	$y = 40.3x + 3.3$	0.99	$y = 52.1x + 2.8$	0.49			$y = 52.6x + 2.6$	0.73
Spring 2012	sunlit	$y = 84.3x - 0.5$	0.95	$y = 4.5x + 6.9$	-0.90	$y = 92.9x + 0.8$		$y = 66.9x + 4.0$		$y = 83.5x + 1.3$	0.94
	shaded	$y = 27.6x + 4.7$		$y = 47.6x + 2.1$	0.97	$y = 78.9x + 1.2$	0.73	$y = 150.7x - 2.0$		$y = 109.6x + 0.03$	0.66
Summer 2012	sunlit	$y = 116.0x - 0.1$	0.86	$y = 50.0x + 2.6$	0.91	$y = 85.9x + 2.0$	0.93	$y = 107.9x + 1.3$	0.67	$y = 85.2x + 1.7$	0.83
	shaded	$y = -49.2x + 9.1$	0.47	$y = 54.0x + 2.4$	0.94	$y = 81.2x + 1.5$	0.69	$y = 78.7x + 2.4$	0.93	$y = 63.2x + 2.6$	0.73
Winter 2013	sunlit	$y = 83.4x + 0.1$	0.96	$y = 5.5x + 6.3$	-0.90	$y = 67.3x + 3.4$	0.93			$y = 89.7x + 0.9$	0.63
	shaded										

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29**Table S2.** Regression equations and coefficients of determination (R^2) for A_{net}/g_m for sunlit and shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling campaigns.

Campaign	Leaf position	<i>Q. ilex</i>		<i>P. halepensis</i>		<i>A. unedo</i>		<i>Q. pubescens</i>		All species	
		Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
Total	sunlit	$y = 91.2x + 1.4$	0.77	$y = 113.5x + 1.7$	0.50	$y = 52.4x + 2.5$	0.81	$y = 56.8x + 1.3$	0.67	$y = 54.9x + 2.9$	0.65
	shaded	$y = 15.6x + 3.6$	0.26	$y = 2.2x + 107.9$	0.52	$y = 15.6x + 4.8$	0.17	$y = 16.1x + 5.3$	0.22	$y = 16.4x + 4.6$	0.24
Spring 2011	sunlit										
	shaded										
Summer 2011	sunlit	$y = 67.6x + 2.5$	0.81	$y = 62.7x + 1.0$	0.95	$y = 37.8x + 4.5$	0.96	$y = 121.4x - 0.1$	0.44	$y = 62.5x + 1.4$	0.85
	shaded	$y = 16.6x + 1.7$						$y = 24.7x + 4.2$	-0.20	$y = 6.7x + 4.4$	-0.23
Autumn 2011 ^a	sunlit	$y = 69.4x + 0.3$	0.99	$y = 235.3x + 0.1$	0.99	$y = 40.2x + 3.0$				$y = 57.8x + 1.3$	0.70
	shaded	$y = 91.1x - 0.3$	0.96							$y = 86.9x - 0.1$	0.93
Autumn 2011 ^b	sunlit	$y = 72.2x + 2.7$	0.66	$y = 217.1x + 0.3$	0.86					$y = 54.2x + 4.3$	0.44
	shaded	$y = 47.5x + 2.3$	0.61	$y = 287.1x - 1.4$	0.95					$y = 65.0x + 3.1$	0.28
Winter 2012	sunlit	$y = 133.0x - 0.2$	0.91	$y = 104.2x + 3.9$	0.92	$y = 89.8x + 0.6$	0.94			$y = 107.2x + 1.4$	0.72
	shaded	$y = 15.2x + 3.2$	0.30	$y = 206.2x - 1.0$	0.99	$y = 6.4x + 5.1$	-0.40			$y = 8.0x + 5.2$	0.03
Spring 2012	sunlit	$y = 207.0x - 3.2$		$y = 53.9x + 4.8$	0.29	$y = 52.2x + 4.6$		$y = -121.3x + 22.1$		$y = 50.6x + 4.6$	0.83
	shaded	$y = -305.3x + 24.8$		$y = 204.9x - 1.1$	0.99	$y = 12.3x + 5.6$	-0.70	$y = 86.7x - 6.8$		$y = 23.0x + 4.7$	0.45
Summer 2012	sunlit	$y = 44.3x + 3.0$	0.83	$y = -143.7x + 11.6$	0.98	$y = 41.6x + 2.7$	0.36	$y = 36.2x + 3.1$	0.52	$y = 36.7x + 3.4$	0.59
	shaded	$y = 3.7x + 5.8$	0.98	$y = 189.8x - 1.0$	0.66	$y = 28.3x + 2.8$	0.73	$y = 9.0x + 6.0$	-0.20	$y = 11.2x + 5.3$	0.17
Winter 2013	sunlit	$y = 138.9x + 0.8$	0.98	$y = 13.6x + 6.0$	-0.60	$y = 78.9x + 1.7$	-0.10			$y = 73.3x + 2.5$	0.77
	shaded										

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34**Table S3.** Regression equations and coefficients of determination (R^2) for $J_{\max}/V_{c,\max}$ for sunlit and shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling campaigns.

Campaign	Leaf position	<i>Q. ilex</i>		<i>P. halepensis</i>		<i>A. unedo</i>		<i>Q. pubescens</i>		All species	
		Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
Total	sunlit	$y = 0.92x + 23$	0.85	$y = 0.67x + 45$	0.62	$y = 0.94x + 28$	0.86	$y = 0.62x + 51$	0.38	$y = 0.80x + 35$	0.77
	shaded	$y = 0.89x + 27$	0.73	$y = 0.99x + 3$	0.79	$y = 0.93x + 30$	0.66	$y = 0.42x + 49$	0.23	$y = 0.78x + 33$	0.74
Spring 2011	sunlit										
	shaded										
Summer 2011	sunlit	$y = 0.80x + 42$	0.74	$y = 0.45x + 65$	0.01	$y = 0.72x + 51$	0.99	$y = -3.34x + 686$		$y = 0.39x + 83$	0.35
	shaded							$y = 0.15x + 54$	0.83	$y = 0.12x + 59$	0.47
Autumn 2011 ^a	sunlit	$y = 1.44x - 38$		$y = 0.64x + 59$	0.65	$y = 0.05x + 142$				$y = 0.99x + 7$	0.82
	shaded	$y = 9.03x - 175$								$y = 0.70x + 24$	0.67
Autumn 2011 ^b	sunlit	$y = 0.56x + 83$	0.74	$y = 0.43x + 92$	0.12	$y = 0.27x + 126$				$y = 0.42x + 97$	0.75
	shaded	$y = 0.91x + 22$	-0.03	$y = 1.63x - 70$	0.98	$y = 0.27x + 126$				$y = 1.01x + 20$	0.87
Winter 2012	sunlit	$y = 0.81x + 41$	0.97	$y = 0.55x + 77$	0.86	$y = 0.95x + 32$	0.95			$y = 0.76x + 50$	0.93
	shaded	$y = 0.65x + 46$	0.65	$y = 0.24x + 115$	-0.67	$y = 0.97x + 36$	0.56			$y = 0.67x + 53$	0.81
Spring 2012	sunlit	$y = 3.11x - 200$	0.89	$y = 0.92x + 2$	0.92	$y = 3.40x - 243$		$y = 0.72x + 51$	0.26	$y = 0.45x + 86$	0.22
	shaded	$y = 0.37x + 79$		$y = 1.12x - 17$	0.96	$y = 0.98x + 19$	0.88	$y = 0.98x + 18$	0.45	$y = 0.73x + 37$	0.66
Summer 2012	sunlit	$y = 0.91x + 16$	0.23	$y = 0.16x + 104$	-0.31	$y = 0.55x + 66$	0.79	$y = 0.66x + 42$	0.98	$y = 0.49x + 61$	0.51
	shaded	$y = 0.89x + 31$	0.93	$y = 0.57x + 51$	0.27	$y = 0.57x + 47$	0.59	$y = 0.46x + 45$	0.26	$y = 0.60x + 44$	0.73
Winter 2013	sunlit										
	shaded										

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38 **Table S4.** Regression equations and coefficients of determination (R^2) for $J_{\text{amb}}/A_{\text{net}}$ for sunlit and shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*,
39 and *Q. pubescens* in eight sampling campaigns.

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Campaign	Leaf position	<i>Q. ilex</i>		<i>P. halepensis</i>		<i>A. unedo</i>		<i>Q. pubescens</i>		All species	
		Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
Total	sunlit	$y = 5.46x + 59$	0.37	$y = 5.58x + 90$	0.38	$y = 5.40x + 56$	0.35	$y = 2.84x + 539$	0.26	$y = 4.57x + 74$	0.27
	shaded	$y = 6.54x + 23$	0.58	$y = 8.91x + 59$	0.76	$y = 4.83x + 46$	0.26	$y = 3.51x + 81$	0.35	$y = 5.2x + 45$	0.26
Spring 2011	sunlit					$y = 2.10x + 12$				$y = 5.67x - 8$	0.99
	shaded										
Summer 2011	sunlit	$y = -2.95x + 123$	-0.27	$y = 3.87x + 102$	0.70	$y = 18.80x - 92$	0.85	$y = 4.66x + 83$	0.12	$y = 1.19x + 95$	-0.01
	shaded	$y = 1.62x + 39$	0.95					$y = -8.23x + 114$	0.97	$y = 2.68x + 43$	-0.12
Autumn 2011 ^a	sunlit	$y = 12.45x + 25$	0.99	$y = 11.30x + 74$	0.89	$y = 6.23x + 49$				$y = 8.18x + 55$	0.39
	shaded	$y = 4.56x + 17$	0.98							$y = 2.64x + 29$	-0.42
Autumn 2011 ^b	sunlit	$y = 13.40x - 3.8$	0.81	$y = -6.60x + 193$	0.18	$y = 6.23x + 49$				$y = 3.04x + 97$	-0.17
	shaded	$y = 5.21x + 21$	0.61	$y = 13.17x + 45$	0.74	$y = -2.47x + 117$				$y = 11.87x + 16$	0.34
Winter 2012	sunlit	$y = 10.78x + 56$	0.78	$y = 16.50x + 5$	0.37	$y = 9.08x + 60$	0.98			$y = 10.60x + 55$	0.84
	shaded	$y = 3.08x + 39$	0.91	$y = 5.87x + 79$	0.67	$y = 6.97x + 44$	0.11			$y = 7.63x + 38$	0.24
Spring 2012	sunlit	$y = 1.84x + 80$	0.74	$y = 17.50x + 5$	0.87	$y = 4.01x + 71$		$y = 4.30x + 88$		$y = 1.55x + 106$	0.07
	shaded	$y = -32.90x + 302$		$y = 9.69x + 49$	0.91	$y = 7.70x + 24$	0.57	$y = -1.08x + 110$	-0.38	$y = 1.18x + 82$	-0.02
Summer 2012	sunlit	$y = 5.93x + 41$	0.99	$y = 6.66x + 77$	0.44	$y = 5.24x + 47$	0.58	$y = 3.66x + 65$	-0.15	$y = 3.30x + 70$	0.15
	shaded	$y = 0.75x + 60$	-0.49	$y = 4.90x + 82$	0.49	$y = 0.72x + 61$	-0.30	$y = 3.39x + 42$	-0.05	$y = 2.75x + 58$	0.0002
Winter 2013	sunlit	$y = 10.96x + 29$	0.77	$y = 102.70x - 568$	0.37	$y = -1.09x + 132$	-0.30			$y = 12.17x + 29$	0.60
	shaded										

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45**Table S5.** Regression equations and coefficients of determination (R^2) for g_m/g_s for sunlit and shaded leaves of *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens* in eight sampling campaigns.

Campaign	Leaf position	<i>Q. ilex</i>		<i>P. halepensis</i>		<i>A. unedo</i>		<i>Q. pubescens</i>		All species	
		Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
Total	sunlit	$y = 0.665x + 0.01$	0.53	$y = 0.078x + 0.03$	0.03	$y = 1.44x + 0.001$	0.69	$y = 1.146x + 0.01$	0.34	$y = 0.501x + 0.02$	0.18
	shaded	$y = 0.658x + 0.05$	0.01	$y = 0.107x + 0.03$	0.05	$y = 0.58x + 0.06$	-0.02	$y = 1.090x + 0.07$	0	$y = 0.199x + 0.07$	-0.01
Spring 2011	sunlit										
	shaded										
Summer 2011	sunlit	$y = 0.484x + 0.03$	0.3	$y = 0.096x + 0.018$	-0.3	$y = 1.065x + 0.07$	0.58	$y = 0.338x + 0.01$	0.08	$y = 1.48x - 0.02$	0.66
	shaded	$y = 8.650x - 0.11$						$y = 0.319x + 0.05$	-0.92	$y = -0.202x + 0.10$	-0.33
Autumn 2011 ^a	sunlit	$y = 0.309x + 0.01$	0.92	$y = 3.613x - 0.12$	0.96	$y = 0.280x + 0.03$				$y = 0.485x + 0.01$	0.05
	shaded	$y = 1.291x - 0.01$	0.93							$y = 1.284x - 0.01$	0.96
Autumn 2011 ^b	sunlit	$y = 0.442x + 0.05$	0.12	$y = 0.128x + 0.01$	0.7					$y = -0.069x + 0.05$	-0.18
	shaded	$y = 0.936x - 1.13$	0.38	$y = 0.107x + 0.01$	0.84	$y = 0.830x + 0.07$				$y = 0.099x + 0.03$	-0.04
Winter 2012	sunlit	$y = 1.450x - 0.01$	0.78	$y = 1.240x - 0.002$	0.8	$y = 0.636x + 0.01$	0.68			$y = 0.194x + 0.0359$	0.06
	shaded	$y = 1.800x + 0.06$	-0.15	$y = -0.459x + 0.11$	0.99	$y = -0.099x + 0.06$	-0.43			$y = -0.0296x + 0.10$	-0.11
Spring 2012	sunlit	$y = 3.480x - 0.09$		$y = 1.783x - 0.07$	-0.5	$y = 0.561x + 0.041$		$y = -0.552x + 0.15$		$y = 1.257x - 0.03$	0.5
	shaded	$y = -0.091x + 0.07$		$y = 1.861x - 0.01$	0.93			$y = 3.114x - 0.05$		$y = 1.520x - 0.002$	0.19
Summer 2012	sunlit	$y = 0.308x + 0.03$	0.45	$y = 0.936x + 0.07$	0.96	$y = 0.401x + 0.02$	0.17	$y = 1.700x + 0.02$	-0.29	$y = 1.085x + 0.03$	0.25
	shaded	$y = -14.12x + 0.93$	0.61	$y = 2.331x - 0.02$	0.39	$y = 0.272x + 0.03$	0.51	$y = 0.728x + 0.131$	-0.22	$y = 0.351x + 0.01$	-0.05
Winter 2013	sunlit	$y = 1.62x + 0.01$	0.94	$y = 0.291x + 0.06$	0.12	$y = 1.570x - 0.06$	0.28			$y = 0.669x + 0.01$	0.18
	shaded										

47 **Table S6.** Non-linear regression equations and coefficients of determination (R^2) for a)
48 g_m/LMA and b) $V_{c,\text{max}}/N_{\text{area}}$ in four seasonal campaigns and for sunlit and shaded leaf
49 positions for *Q. ilex*, *P. halepensis*, *A. unedo*, and *Q. pubescens*.

50 Seasonal changes of the relationship for all species and leaf positions between a) mesophyll conductance
51 (g_m) and leaf mass per area (LMA). We used a subset of morphological and photosynthetic data. Non-
52 linear regression lines of the form $y = \text{LMA}^{-b}$ were fitted to the data. The upper curve is for summer 2012
53 ($b = 0.800$), the middle curve is for spring 2012 ($b = 0.800$) and the lower two overlaying curves are for
54 autumn 2011^{a)} ($b = 1.533$) and winter 2012 ($b = 1.486$).

55

Campaign	g_m/LMA
Total	$y = x^{-1.016}$
Autumn 2011 ^{a)}	$y = x^{-1.533}$
Winter 2012	$y = x^{-1.486}$
Spring 2012	$y = x^{-0.953}$
Summer 2012	$y = x^{-0.800}$

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59 **Supplementary notes**

60 **Note S1** Calculation of maximum quantum yield of PSII and nonphotochemical quenching

61
$$\frac{F_v}{F_m} = \frac{(F_m - F_o)}{F_m}$$
 (1)

62 where F_o is the minimal fluorescence measured under darkness, and F_m is the maximal
63 fluorescence measured after a saturating light pulse. Both parameters were obtained on a dark-
64 adapted leaf with closed PSII reaction centres as described in the previous sections. The F_v/F_m
65 ratio describes the fraction of absorbed photons used in photochemistry under dark conditions
66 and serves as the primary stress indicator of the photosystems. Typical values range between
67 0.74 and 0.85. Ratios <0.80 are indicative of induced photoprotection (sustained energy
68 dissipation), and ratios <0.74 are indicative of chronic photoinhibition (Björkman & Demmig,
69 1987; Maxwell & Johnson, 2000; Verhoeven, 2014).

70 The nonphotochemical quenching (NPQ) is estimated by both dark- and light-adapted
71 fluorescence signals, F_m and F_m' , as:

72

73
$$NPQ = \frac{(F_m - F_m')}{F_m'}$$
 (3)

74 where F_m and F_m' are the maximal fluorescence of a dark-adapted and light-adapted leaf,
75 respectively.

76

77 **Note S2** Light experiments and estimation of day respiration

78 Light-response curves (A/PPFD) were generated at a C_a of 400 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ by
79 automatically applying changes in the photosynthetically active radiation with the LI-6400XT
80 light source. To obtain precise responses at the low range of the light gradient for estimating the
81 daily mitochondrial respiration by the Kok effect (Kok, 1948), we used the following PPFD
82 sequence: 2500 → 2000 → 1500 → 1000 → 800 → 600 → 500 → 400 → 300 → 200 → 150
83 → 125 → 100 → 75 → 50 → 40 → 30 → 20 → 10 → 5 → 0 ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The
84 minimum and maximum times between each light level for the generation of the A/PPFD
85 curves were set to 1 and 2 min, respectively. The gradient from high to low light during an
86 A/PPFD curve led to a drop in T_{Leaf} as the light decreased. The rapid changes in the light levels
87 prevented the correct adjustment of T_{Leaf} while guaranteeing stable air and water fluxes and
88 avoiding noisy measurements of C_i and g_s . We thus decided to maintain a stable Peltier-block
89 temperature (T_{block}) in the leaf cuvette. T_{block} was adjusted first so that T_{Leaf} was 25 °C at the
90 beginning of the A/PPFD curve and then kept stable throughout the experiment. T_{Leaf} had
91 dropped by approximately 1-3 °C by the completion of the A/PPFD curve. From this curve, we
92 estimated night and day respiration. The term R_d is sometimes used for dark respiration

93 (Farquhar *et al.*, 1980; Turnbull *et al.*, 2003) but also for day respiration (Yin *et al.*, 2011;
94 Flexas *et al.*, 2012). We will use R_d to represent mitochondrial respiration during the day or
95 under lighted conditions and R_n to represent mitochondrial respiration at night or under dark-
96 adapted conditions. We estimated R_n during the day after darkening the leaf for at least 30 min.
97 R_d was estimated from the light-response curves with the combined GE and CF measurements
98 proposed by Yin et al. (2009), named the CF method. This method amended the Kok method
99 (Kok, 1948) by substituting the A/PPFD relationship with $A/PPFD * \Phi_{PSII}/4$ (Yin *et al.*, 2009).

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