https://doi.org/10.1071/FP19242_AC
Supplementary Material: *Functional Plant Biology*, 2020, 47(7), 611–627

Supplementary Material

Dynamic responses of gas exchange and photochemistry to heat interference during drought in wheat and sorghum

Lingling Zhu[A,B], *Lucas A. Cernusak*[C] *and Xin Song*[A,D]

[A]Shenzhen Key Laboratory of Marine Biological Resources and Ecological Environment, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, 518060, China.

[B]Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen, 518060, China.

[C]College of Science and Engineering, James Cook University, Cairns, Australia.

[D]Corresponding author. Email: xinsong@szu.edu.cn

Methods S1 Calculation of Φ_{npq} and Φ_{no}

The calculation of non-photochemical quenching Φ_{npq} and Φ_{no} is based on Kramer *et al.* (2004).

$$NPQ = \frac{F_m - F'_m}{F'_m} \quad (1)$$

$$q_L = \frac{(F'_m - F_s)F'_o}{(F'_s - F'_o)F_s} \quad (2)$$

$$\phi_{no} = \frac{1}{NPQ+1+q_L(F_m/F_o-1)} \quad (3)$$

$$\phi_{npq} = 1 - \frac{(F'_m - F_s)}{F'_m} - \phi_{no} \quad (4)$$

Methods S2 Limitation calculations for wheat

The calculation of limitations followed Grassi and Magnani (Grassi and Magnani 2005). Basically, three limitations were partitioned with regard to the changes of g_s, $g_{m,app}$ and V_{cmax}, with each assigned as stomatal limitation (L_S), mesophyll limitation (L_M) and biochemical limitation (L_B), respectively. The three limitations are presented in percentage that are comparable with each other.

$$\frac{dA}{A} = L_S + L_M + L_B = l_s \cdot \frac{dg_s}{g_s} + l_m \cdot \frac{dg_{m,app}}{g_m} + l_b \cdot \frac{V_{cmax}}{V_{cmax}} \quad (5)$$

$$l_s = \frac{g_{tot}/g_s \cdot \partial A/\partial C_c}{g_{tot}+\partial A/\partial C_c} \quad (6)$$

$$l_m = \frac{g_{tot}/g_{m,app} \cdot \partial A/\partial C_c}{g_{tot}+\partial A/\partial C_c} \quad (7)$$

$$l_b = \frac{g_{tot}}{g_{tot}+\partial A/\partial C_c} \quad (8)$$

g_{tot} is the total conductance to CO_2 between the leaf surface and carboxylation sites ($1/g_{tot} = 1/g_s + 1/g_{m,app}$). The relative change in net photosynthesis was defined as the ratio of the actual value of Amax over the maximum value throughout the experiment for each species as shown below.

$$\frac{dA}{A} \approx \frac{A_{max}^{ref} - A_{max}}{A_{max}^{ref}} \quad (9)$$

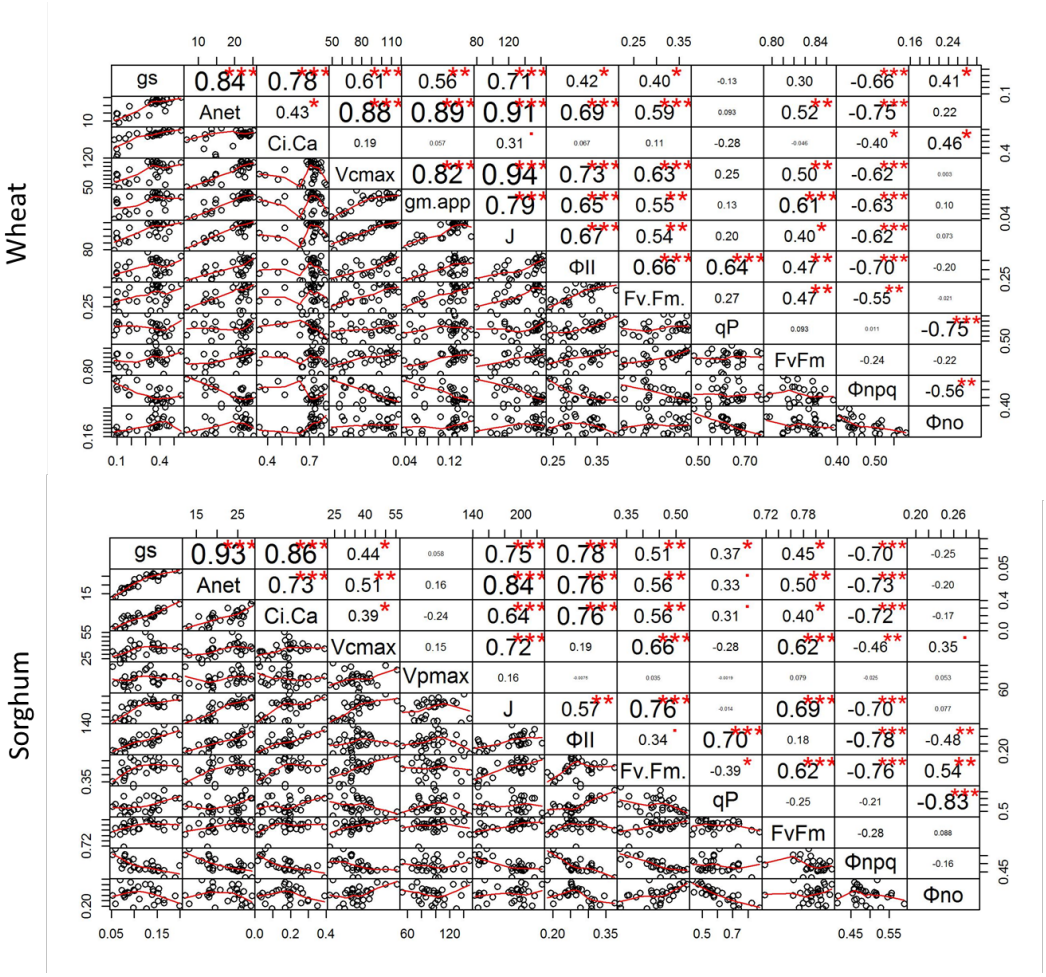
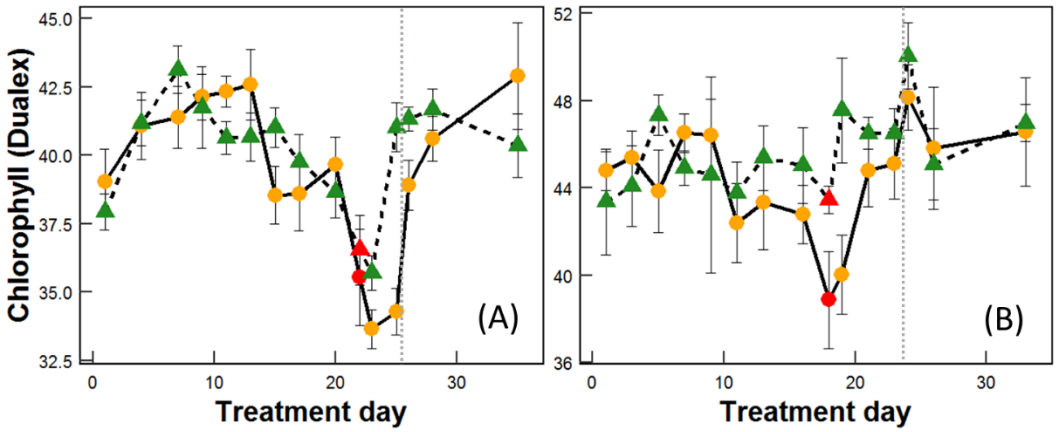


Figure S1. Correlation matrix of gas exchange and photochemical traits in responses to heat interference during prolonged drought. Note: Ci.Ca = C_i/C_a; Fv.Fm. =F_v'/F_m'; FvFm = F_v/F



825

826 **Figure S2.** Measured time series of chlorophyll content from Dualex reading in wheat (a) and
827 sorghum (b) in response to heat interference during drought (circles, DT) and well-watered
828 (triangles) conditions (WW). For the WW group of plants, green color represents control
829 temperature and red represents the second day of heat stress. For the DT group of plants, orange
830 color represents control temperature and red represents the second day of heat stress. Dashed
831 vertical gray line represents re-watering.

832

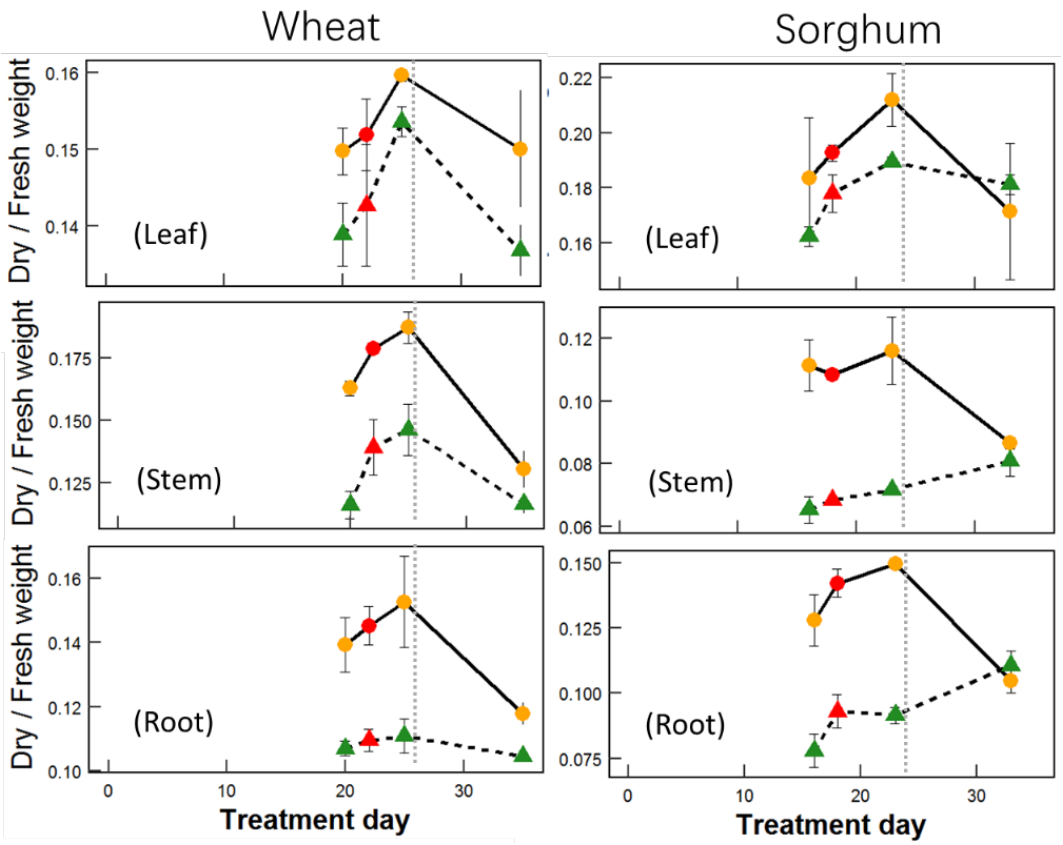


Figure S3. Changes of the ratio of dry to fresh weight of leaves, stems and roots in wheat and sorghum in response to heat interference during prolonged drought. Triangles represent the group of plants always under well-watered condition (WW plants). Green color represents WW plants under control temperature and red represents the second day of heat stress. Dots represent the group of plants gradually subject to drought stress then re-watering (DT plants). Orange color represents DT plants under control temperature and red represents the second day of heat stress. Dashed gray line represents re-watering.

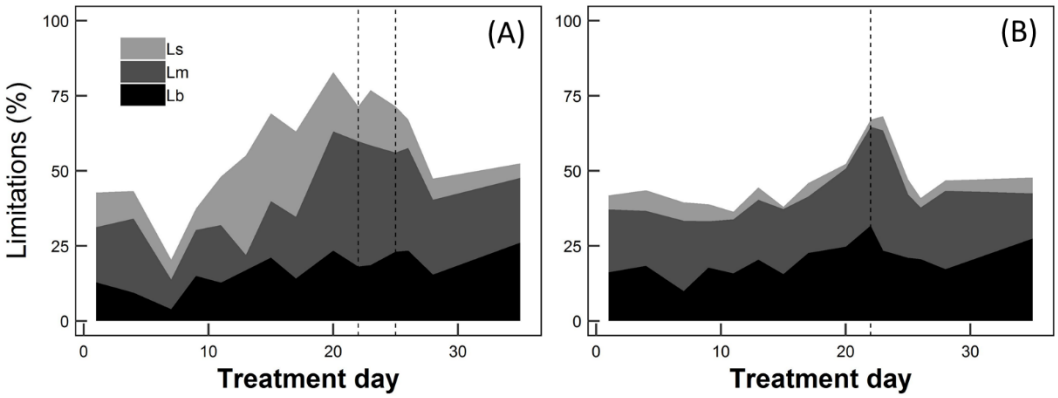


Figure S4. Quantitative limitation analysis of photosynthetic CO_2 assimilation in wheat leaves in response to heat interference during drought (A) and well-watered (B) conditions (WW). The shaded areas represent the percentage of stomatal (L_S), mesophyll (L_M), and biochemical (L_B) limitations. The first dashed vertical gray line represents the second day of heat stress; second represents re-watering.

Table S1. Measured time series data in wheat of stomatal conductance (g_s), net photosynthesis (A_{net}), intercelullar and ambient CO_2 concentration (C_i/C_a), maximum carboxylation rate (V_{cmax}), apparent mesophyll conductance ($g_{m,app}$), electron transport rate (J), quantum yield (Φ_{II}), quantum efficiency under light (F_v'/F_m'), quantum efficiency under dark (F_v/F_m), non-photochemical quenching through downregulatory processes (Φ_{npq}), and non-photochemical quenching through other energy losses (Φ_{no}).

Group	Day	g_s	A_{net}	C_i/C_a	V_{cmax}	$g_{m,app}$	J	Φ_{II}	F_v'/F_m'	qP	F_v/F_m	Φ_{npq}	Φ_{no}
DT	1	0.38±0.04	22.05±1.17	0.73±0.02	105.21±5.83	0.15±0.02	151.47±5.38	0.28±0.02	0.34±0.03	0.6±0.06	0.83±0.01	0.5±0.01	0.21±0.01
DT	4	0.32±0.04	21.71±0.51	0.68±0.03	112.25±8.07	0.13±0.01	157.58±4.98	0.39±0.02	0.33±0.05	0.77±0.06	0.84±0.01	0.44±0.01	0.17±0.02
DT	7	0.37±0.03	23.05±1.95	0.71±0.01	117.44±5.16	0.15±0.01	163.36±10.23	0.3±0.02	0.33±0.04	0.54±0.05	0.84±0.00	0.44±0.03	0.27±0.05
DT	9	0.35±0.03	23.89±1.29	0.69±0.01	105.45±7.15	0.15±0.01	152.88±9.51	0.36±0.04	0.36±0.04	0.68±0.08	0.84±0.01	0.44±0.02	0.2±0.02
DT	11	0.19±0.02	19.53±1.14	0.52±0.05	92.50±3.45	0.13±0.02	144.14±4.48	0.34±0.03	0.35±0.02	0.66±0.05	0.83±0.00	0.47±0.02	0.19±0.01
DT	13	0.1±0.01	14.85±0.95	0.34±0.04	82.87±6.51	0.22±0.00	129.7±8.42	0.28±0.01	0.28±0.01	0.61±0.03	0.82±0.01	0.52±0.01	0.21±0.02
DT	15	0.1±0.02	11.53±1.55	0.47±0.02	68.78±3.16	0.11±0.02	114.63±4.14	0.28±0.02	0.27±0.02	0.67±0.05	0.84±0.01	0.55±0.02	0.17±0.01
DT	17	0.1±0.01	14.66±1.39	0.38±0.08	79.60±6.50	0.1±0.01	114.63±8.19	0.32±0.03	0.33±0.03	0.69±0.06	0.84±0.01	0.5±0.03	0.18±0.01
DT	20	0.08±0.01	6.78±0.94	0.63±0.01	51.70±7.40	0.04±0.01	79.78±8.29	0.24±0.04	0.25±0.04	0.56±0.08	0.82±0.01	0.56±0.03	0.19±0.02
DT	22	0.18±0.01	11.86±0.84	0.71±0.02	59.88±9.94	0.06±0.00	102.37±8.56	0.28±0.03	0.28±0.03	0.6±0.05	0.8±0.01	0.46±0.03	0.26±0.03
DT	23	0.11±0.02	8.93±1.19	0.65±0.04	49.19±18.78	0.05±0.01	97.66±12.79	0.24±0.05	0.22±0.06	0.67±0.12	0.81±0.01	0.58±0.03	0.18±0.02
DT	25	0.16±0.02	12.06±0.26	0.64±0.04	63.12±3.62	0.07±0.01	105.66±4.63	0.32±0.05	0.32±0.04	0.77±0.11	0.81±0.01	0.51±0.03	0.17±0.02
DT	26	0.24±0.03	13.67±0.53	0.73±0.03	68.71±5.61	0.07±0.00	120.79±6.39	0.25±0.03	0.28±0.04	0.62±0.08	0.81±0.01	0.56±0.01	0.19±0.02

DT	28	0.34±0.03	21.36±0.55	0.71±0.01	101.72±2.58	0.12±0.00	160.73±6.03	0.36±0.02	0.23±0.04	0.69±0.01	0.81±0.04	0.44±0.00	0.2±0.02
DT	35	0.38±0.04	22.77±2.02	0.72±0.01	74.67±2.28	0.16±0.03	119.36±5.3	0.35±0.03	0.36±0.03	0.62±0.08	0.84±0.01	0.4±0.03	0.25±0.04
WW	1	0.38±0.05	23.47±1.35	0.73±0.01	112.42±5.23	0.13±0.01	154.29±5.92	0.36±0.03	0.35±0.03	0.67±0.09	0.83±0.00	0.43±0.01	0.21±0.04
WW	4	0.34±0.02	21.98±0.57	0.70±0.01	110.97±5.89	0.13±0.01	150.03±5.84	0.34±0.04	0.34±0.01	0.6±0.08	0.83±0.01	0.4±0.03	0.25±0.04
WW	7	0.37±0.03	22.52±1.05	0.72±0.01	109.29±4.87	0.13±0.01	154.43±4.93	0.36±0.03	0.37±0.04	0.64±0.06	0.86±0.00	0.43±0.01	0.2±0.03
WW	9	0.40±0.06	24.21±0.91	0.71±0.03	108.07±4.32	0.14±0.01	158.49±2.07	0.35±0.03	0.35±0.03	0.66±0.08	0.84±0.01	0.44±0.02	0.21±0.03
WW	11	0.48±0.04	24.26±0.74	0.76±0.01	105.94±7.49	0.13±0.01	158.52±8.63	0.33±0.03	0.35±0.04	0.62±0.07	0.84±0.01	0.43±0.01	0.24±0.02
WW	13	0.42±0.04	23.04±1.20	0.74±0.02	106.13±11.35	0.13±0.01	153.41±11.02	0.34±0.02	0.31±0.01	0.62±0.06	0.82±0.01	0.42±0.02	0.25±0.03
WW	15	0.55±0.01	25.2±0.81	0.78±0.01	109.59±5.49	0.13±0.01	162.87±5.47	0.37±0.03	0.37±0.03	0.75±0.04	0.84±0.01	0.46±0.02	0.16±0.01
WW	17	0.38±0.04	23.74±1.51	0.71±0.03	98.57±7.98	0.12±0.00	156.22±8.95	0.32±0.03	0.36±0.02	0.61±0.08	0.84±0.01	0.46±0.02	0.22±0.02
WW	20	0.46±0.02	21.65±0.91	0.78±0.01	89.91±3.32	0.12±0.02	132.49±0.84	0.29±0.03	0.3±0.03	0.56±0.07	0.85±0.01	0.5±0.03	0.21±0.03
WW	22	0.44±0.06	16.33±1.79	0.82±0.03	57.19±6.85	0.07±0.01	113.42±8.17	0.29±0.03	0.26±0.02	0.54±0.07	0.82±0.01	0.41±0.05	0.30±0.05
WW	23	0.33±0.04	13.60±1.65	0.80±0.02	61.83±9.69	0.06±0.01	123.37±10.16	0.24±0.04	0.29±0.03	0.5±0.07	0.82±0.01	0.52±0.04	0.24±0.02
WW	25	0.40±0.03	22.28±1.39	0.74±0.02	89.47±3.97	0.13±0.01	143.32±4.57	0.27±0.03	0.23±0.04	0.58±0.04	0.82±0.00	0.51±0.04	0.22±0.03
WW	26	0.45±0.01	23.91±0.6	0.75±0.01	97.41±5.36	0.14±0.01	161.39±5.8	0.34±0.03	0.28±0.02	0.69±0.1	0.82±0.01	0.45±0.02	0.21±0.04
WW	28	0.43±0.02	20.38±0.79	0.78±0.01	97.56±3.62	0.10±0.01	153.51±2.7	0.30±0.03	0.37±0.02	0.61±0.11	0.79±0.00	0.44±0.04	0.26±0.07
WW	35	0.37±0.01	21.04±0.89	0.74±0.01	81.01±5.16	0.13±0.01	130.3±5.44	0.27±0.02	0.28±0.03	0.48±0.06	0.83±0.00	0.43±0.02	0.3±0.04

Table S2. Measured time series data in sorghum of stomatal conductance (g_s), net photosynthesis (A_{net}), intercelullar and ambient CO_2 concentration (C_i/C_a), maximum carboxylation rate (V_{cmax}), maximum phosphoenolpyruvate carboxylase carboxylation (V_{pmax}), electron transport rate (J), quantum yield (Φ_{II}), quantum efficiency under light (F_v'/F_m'), quantum efficiency under dark (F_v/F_m), non-photochemical quenching through downregulatory processes (Φ_{npq}), and non-photochemical quenching through other energy losses (Φ_{no}).

Group	Day	g_s	A_{net}	C_i/C_a	V_{cmax}	V_{pmax}	J	Φ_{II}	F_v'/F_m'	qP	F_v/F_m	Φ_{npq}	Φ_{no}
DT	1	0.14±0.01	25.15±1.68	0.19±0.01	49.98±14.8	140.53±16.97	226.3±6.37	0.25±0.02	0.53±0.01	0.48±0.04	0.81±0	0.47±0.01	0.28±0.02
DT	3	0.09±0.01	18.94±0.47	0.17±0.02	45.86±8.16	73.59±19.8	200.44±2.85	0.25±0.02	0.52±0.03	0.49±0.05	0.81±0.01	0.46±0.03	0.29±0.03
DT	5	0.07±0.01	18.11±2.34	0.16±0.07	31.15±3.54	90.48±0.90	188.31±20.37	0.27±0.04	0.49±0.02	0.55±0.06	0.79±0.01	0.47±0.03	0.26±0.01
DT	7	0.11±0.02	24.08±3.14	0.20±0.06	41.93±3.63	112.52±16.61	198.64±14.28	0.31±0.03	0.46±0.03	0.68±0.06	0.78±0	0.46±0.04	0.23±0.02
DT	9	0.10±0.02	23.19±1.47	0.12±0.05	40.59±2.55	97.65±38.86	200.08±12.97	0.24±0.02	0.43±0.06	0.6±0.07	0.81±0.01	0.48±0	0.26±0.02
DT	11	0.05±0.01	12.24±1.71	0.03±0.00	33.07±4.93	136.39±33.94	142.77±13.95	0.21±0.04	0.35±0.05	0.58±0.09	0.76±0.02	0.56±0.06	0.24±0.01
DT	13	0.08±0.01	17.95±1.89	0.04±0.03	33.36±5.94	102.46±17.50	148.31±9.85	0.19±0.01	0.39±0.02	0.50±0.04	0.78±0.01	0.56±0.02	0.25±0.02
DT	16	0.06±0.01	13.25±2.42	0.06±0.02	29.19±0.94	80.25±17.34	157.51±9.91	0.20±0.03	0.33±0.04	0.61±0.06	0.79±0.02	0.59±0.02	0.21±0.01
DT	18	0.08±0.01	17.69±1.36	0.07±0.02	22.71±1.57	75.93±3.71	147.7±8.22	0.26±0.04	0.36±0.02	0.74±0.08	0.72±0.02	0.49±0.03	0.24±0.02
DT	19	0.07±0.01	16.28±1.67	0.01±0.01	24.25±5.28	148.1±14.83	154.94±1.43	0.18±0.04	0.42±0.03	0.44±0.08	0.76±0.02	0.52±0.03	0.3±0.03
DT	21	0.08±0.01	18.02±2.15	0.04±0.02	33.12±3.10	85.97±17.31	164.69±12.62	0.22±0.03	0.38±0.01	0.58±0.07	0.79±0.02	0.55±0.03	0.23±0.01
DT	23	0.10±0.01	19.51±3.03	0.17±0.08	27.4±4.02	97.66±7.44	146.54±19.49	0.28±0.03	0.33±0.05	0.88±0.1	0.77±0.01	0.53±0.03	0.19±0.01
DT	24	0.13±0.02	25.77±2.45	0.10±0.05	29.79±2.62	129.39±9.15	200.56±6.69	0.31±0.06	0.41±0.02	0.74±0.12	0.81±0.01	0.5±0.04	0.19±0.02

DT	26	0.14±0.02	26.03±2.06	0.19±0.03	36.00±2.04	111.7±4.38	204.01±9.82	0.33±0.02	0.45±0.01	0.74±0.04	0.80±0.01	0.46±0.01	0.20±0.01
DT	33	0.19±0.01	28.46±0.71	0.39±0.05	36.60±0.59	104.26±8.88	217.09±3.29	0.35±0.02	0.49±0.02	0.7±0.03	0.79±0	0.42±0.03	0.24±0.01
WW	1	0.12±0.02	23.61±2.62	0.17±0.02	55.63±5.63	125.06±24.87	205.7±7.34	0.25±0.04	0.51±0.01	0.5±0.08	0.83±0.01	0.47±0.02	0.28±0.03
WW	3	0.10±0.01	20.75±1.17	0.13±0.02	33.84±0.69	109.15±18.42	195.8±7.32	0.26±0.01	0.50±0.02	0.52±0.03	0.82±0.01	0.48±0.02	0.26±0.02
WW	5	0.09±0.00	18.71±1.8	0.20±0.07	36.65±8.57	53.13±13.37	184.64±9.06	0.24±0.01	0.44±0.01	0.44±0.09	0.79±0.01	0.51±0.01	0.25±0.01
WW	7	0.11±0.01	19.05±0.84	0.23±0.04	37.09±2.16	66.39±9.30	170.75±5.73	0.26±0.03	0.48±0.03	0.54±0.07	0.79±0.01	0.47±0.02	0.27±0.02
WW	9	0.14±0.02	24.80±2.79	0.22±0.05	42.93±2.09	87.4±15.16	203.06±13.98	0.28±0.03	0.5±0.03	0.57±0.07	0.80±0	0.46±0.02	0.26±0.03
WW	11	0.17±0.01	26.02±1.12	0.30±0.06	39.07±2.88	103.32±25.77	203.25±3.71	0.32±0.02	0.48±0.01	0.67±0.06	0.81±0.01	0.46±0.01	0.22±0.01
WW	13	0.14±0.02	25.24±1.80	0.20±0.05	37.82±3.98	103.55±33.4	197.5±6.86	0.24±0.03	0.46±0.02	0.52±0.06	0.82±0	0.52±0.02	0.24±0.02
WW	16	0.15±0.04	22.61±3.82	0.30±0.09	36.19±0.95	76.81±6.73	191.58±1.90	0.30±0.05	0.43±0.01	0.70±0.12	0.79±0.02	0.48±0.03	0.22±0.02
WW	18	0.08±0.01	15.55±1.85	0.19±0.09	25.79±2.33	69.74±17.11	149.66±14.13	0.24±0.03	0.43±0.02	0.57±0.09	0.81±0.01	0.52±0.02	0.24±0.03
WW	19	0.09±0.01	18.47±1.48	0.10±0.04	30.2±2.53	84.76±7.38	153.89±14.65	0.24±0.02	0.43±0.02	0.56±0.05	0.80±0.01	0.52±0.01	0.25±0.01
WW	21	0.15±0.03	24.70±2.12	0.26±0.08	33.76±5.14	81.03±10.66	195.38±7.42	0.27±0.02	0.52±0.03	0.52±0.03	0.80±0.01	0.46±0.03	0.26±0.01
WW	23	0.14±0.01	26.45±2.15	0.18±0.03	41.12±3.36	84.20±9.42	209.62±8.36	0.26±0.03	0.46±0.02	0.56±0.07	0.80±0.01	0.49±0.03	0.25±0.02
WW	24	0.20±0.02	28.12±0.96	0.35±0.07	38.67±2.32	94.84±3.57	214.15±7.20	0.31±0.04	0.44±0.02	0.70±0.07	0.82±0.01	0.49±0.03	0.20±0.01
WW	26	0.15±0.02	25.02±2.67	0.27±0.03	42.62±3.28	97.57±36.14	189.13±21.17	0.30±0.02	0.47±0.01	0.63±0.03	0.80±0.02	0.45±0.01	0.25±0.02
WW	33	0.17±0.02	26.83±1.68	0.26±0.05	34.03±0.21	99.45±6.57	188.54±5.47	0.37±0.03	0.45±0.01	0.82±0.03	0.78±0.02	0.41±0.01	0.21±0.02

Table S3. $\delta^{13}C$ data in leaves, stems and roots in wheat at four time points.

Group	Day	Organ	$\delta^{13}C$
DT	20	Leaf	-32.99±0.11
DT	22	Leaf	-33.04±0.11
DT	25	Leaf	-32.92±0.31
DT	35	Leaf	-31.11±0.14
DT	20	Root	-31.00±0.34
DT	22	Root	-30.67±0.20
DT	25	Root	-30.88±0.09
DT	35	Root	-31.04±0.12
DT	20	Stem	-32.19±0.05
DT	22	Stem	-31.90±0.20
DT	25	Stem	-32.01±0.20
DT	35	Stem	-32.48±0.10
WW	20	Leaf	-33.69±0.11
WW	22	Leaf	-34.12±0.17
WW	25	Leaf	-33.90±0.11
WW	35	Leaf	-33.74±0.10
WW	20	Root	-31.76±0.30
WW	22	Root	-32.59±0.21
WW	25	Root	-32.61±0.10
WW	35	Root	-32.97±0.10
WW	20	Stem	-33.88±0.11
WW	22	Stem	-33.87±0.23
WW	25	Stem	-33.74±0.19
WW	35	Stem	-34.01±0.11

Table S4. $\delta^{13}C$ data in leaves, stems and roots in sorghum at four time points.

Group	Day	Organ	$\delta^{13}C$
DT	14	Leaf	-14.85±0.14
DT	16	Leaf	-15.18±0.10
DT	21	Leaf	-15.49±0.11
DT	33	Leaf	-14.64±0.22
DT	14	Root	-14.79±0.13
DT	16	Root	-14.51±0.13
DT	21	Root	-14.97±0.23
DT	33	Root	-14.86±0.14
DT	14	Stem	-14.17±0.16
DT	16	Stem	-14.07±0.15
DT	21	Stem	-14.44±0.12
DT	33	Stem	-14.08±0.29
WW	14	Leaf	-14.85±0.12
WW	16	Leaf	-14.51±0.14
WW	21	Leaf	-14.66±0.14
WW	33	Leaf	-13.99±0.09
WW	14	Root	-14.94±0.34
WW	16	Root	-14.29±0.31
WW	21	Root	-14.95±0.48
WW	33	Root	-14.42±0.18
WW	14	Stem	-13.75±0.01
WW	16	Stem	-13.30±0.30
WW	21	Stem	-13.35±0.43
WW	33	Stem	-13.39±0.05

References:

Grassi, G, Magnani, F (2005) Stomatal, mesophyll conductance and biochemical limitations to

photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell & Environment* **28**, 834-849.

Kramer, DM, Johnson, G, Kiirats, O, Edwards, GE (2004) New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynthesis Research* **79**, 209.