

## Supplementary Material

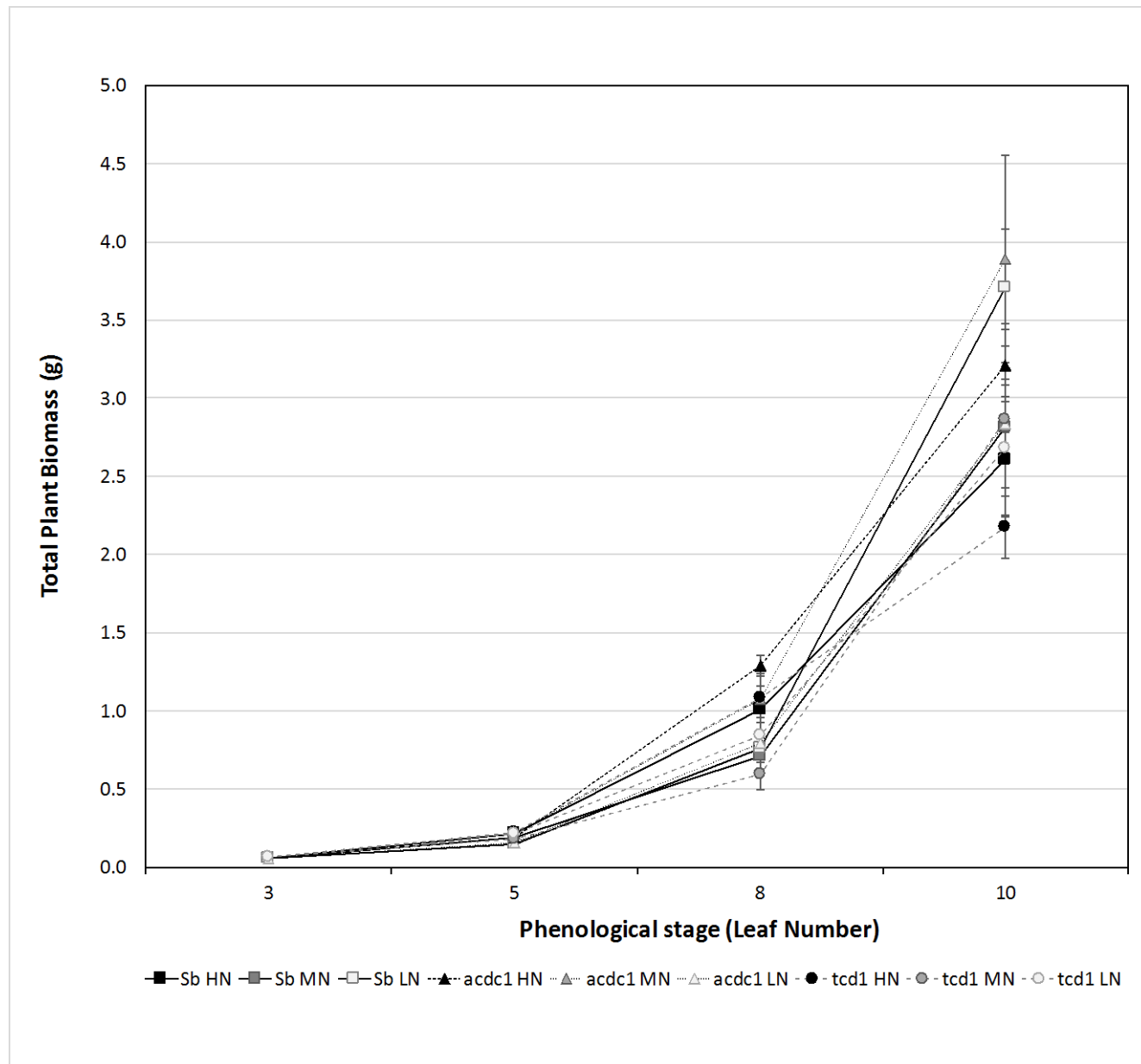
### **Counting the costs: nitrogen partitioning in *Sorghum* mutants**

*Cecilia K. Blomstedt*<sup>A</sup>, *Viviana C. Rosati*<sup>A</sup>, *Birger Lindberg Møller*<sup>B</sup> and *Ros Gleadow*<sup>A,C</sup>

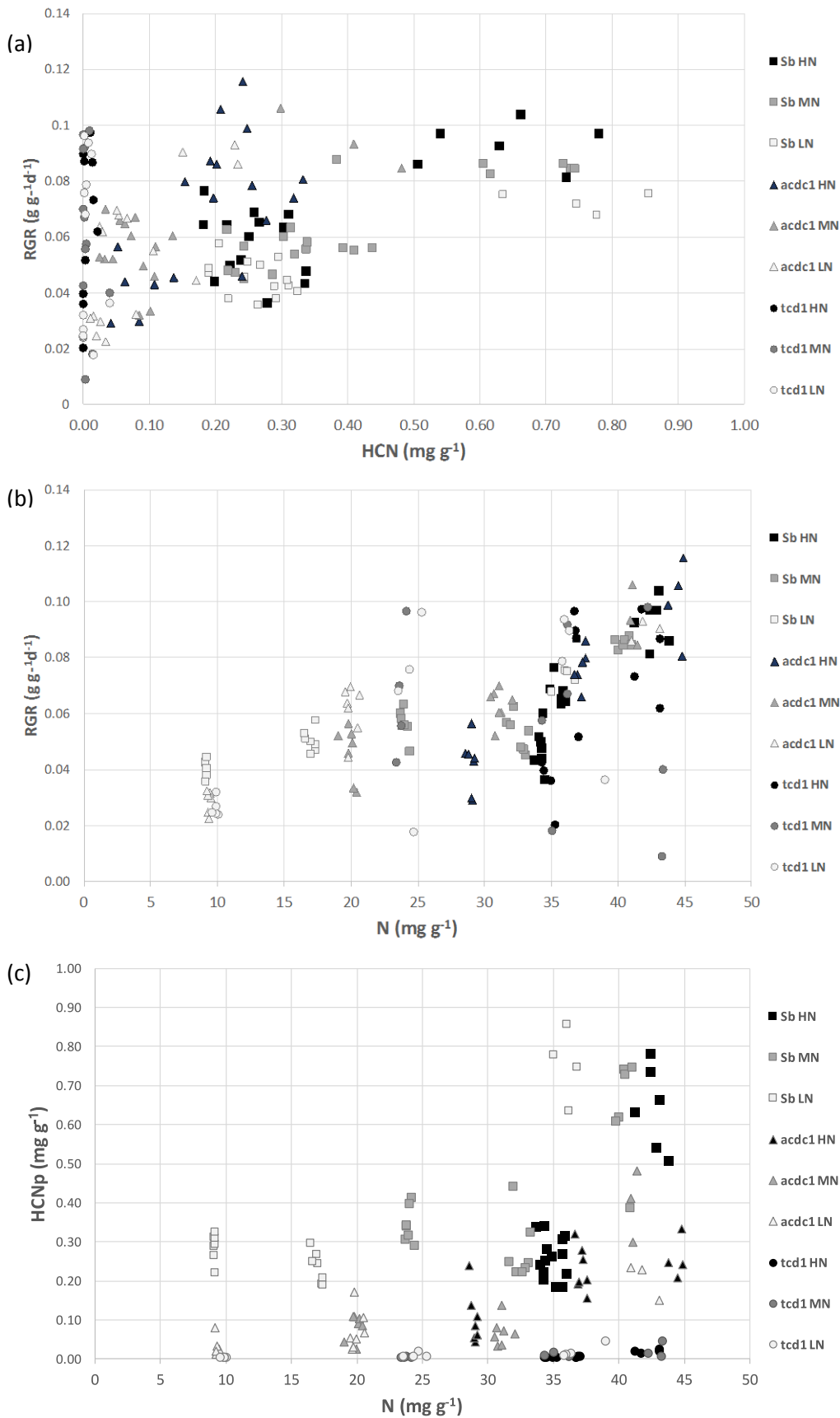
<sup>A</sup>School of Biological Sciences, Monash University, Clayton, Vic. 3800, Australia.

<sup>B</sup>Plant Biochemistry Laboratory and VILLUM Research Centre for Plant Plasticity,  
Department of Plant and Environmental Sciences, University of Copenhagen, 40  
Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark.

<sup>C</sup>Corresponding author. Email: [ros.gleadow@monash.edu](mailto:ros.gleadow@monash.edu)



**Fig. S1.** Total plant biomass (dry weight) of wild type *Sorghum bicolor* (Sb), totally cyanide deficient mutants *tcd1*, and mutants that are only cyanide deficient as adults (*adult cyanide deficient class 1, acdc1*) harvested at four different leaf stages and grown with Long-Ashton's nutrient solution modified to contain Low (LN, 2 mM), Medium (MN, 6 mM) and High (HN, 12 mM) Nitrogen.



**Fig. S2.** Relationship between (a) cyanide potential (HCNp) and overall Relative Growth Rate (RGR) (b) plant nitrogen (Total N) and overall Relative Growth Rate (RGR), and (c) nitrogen and HCNp in the three sorghum genotypes grown at three levels of nitrogen: Low (LN, 2mM), Medium (MN, 6mM) and High (HN, 12mM) and harvested at the 3-leaf, 5-leaf, 8-leaf and 10-leaf stages. The three genotypes are: wild type *Sorghum bicolor* (Sb), *totally cyanide deficient* mutants *tcd1*, and *adult cyanide deficient class* mutant (*acdc1*). Concentrations of nitrogen and HCN were calculated as the total mass of N or HCN in the plant divided by the total plant dry biomass.

**Table S1. Primers used for qPCR analysis for key genes in nitrogen uptake and transport and dhurrin synthesis and turnover (Fig 1b)**

Sequences obtained for each gene from Phytozome database (<https://phytozome.jgi.doe.gov/pz/portal.html#!search>) and primers designed using PerlPrimer (<http://perlprimer.sourceforge.net/>).

<b>Gene identifier</b>	<b>Size (bp)</b>	<b>Forward (5'-3')</b>	<b>Reverse (5'-3')</b>
<i>CYP79A1</i> (Sb01g001200)	162	CATGCTCTTCGGAAGGCTCCT	ATGGAGATGGACGGGTAGAGGT
<i>Nitrilase NIT4B2</i> (Sb04g026940)	152	CGTTACAGCTGACCTCGACCTT	TCTCTCCATCGGCTGAAGCA
<i>Nitrate reductase</i> (Sb07g022750)	172	CCCATGTACCAGGTCATCCA	CCTGGTCGATCACGTACCAC
<i>Glutamine synthetase</i> (Sb06g031460)	164	GCATCGAGACCTTCTCATGG	CCAGAGAATTGTTGTTTCAGC
<i>Ubiquitin</i> (Sb01g030340)	122	CGGAGGAGCTCTATGCCACA	AAGACGCTCCACTGCAGCAT

**Table S2. Growth parameters and descriptive statistics for three *Sorghum bicolor* genotypes grown at 2, 6 and 12 mM nitrate (LN, MN, HN) and harvested the (a) 3-leaf stage; (b) 5-leaf stage; (c) 8-leaf stage; and (d) 10-leaf stage**

Sb: wild type; *acdc1*: adult cyanide deficient mutants; *tcd1*: totally cyanide deficient mutants. Values are the mean of 7 or 4 (*tcd1*) replicates  $\pm$  s.e. Significance of one-way (3-leaf stage) or two-way ANOVAs (other leaf stages) are presented for each genotype (line, L) and treatment (T). Means with the same letter are not significantly different ( $P>0.05$ ; Tukey's Pairwise Comparison). Abbreviations: RGR, relative growth rate; LAR, leaf area ratio; SLA, specific leaf area, R:S ratio, root:shoot ratio, SLN, specific leaf nitrogen; \* $P<0.05$ ; \*\* $P<0.001$ ; \*\*\* $P<0.001$ ; ns, not significant

**Table S2. (a) 3 leaf stage (baseline)**

	Sb	<i>acdc1</i>	<i>tcd1</i>	One-way ANOVA L
Leaf area (cm <sup>2</sup> )	17 $\pm$ 1	13 $\pm$ 1	15 $\pm$ 1	ns
Height (cm)	7.2 $\pm$ 0.2 <sup>a</sup>	6.2 $\pm$ 0.3 <sup>b</sup>	5.1 $\pm$ 0.2 <sup>c</sup>	***
Leaf dry weight (g)	0.024 $\pm$ 0.002	0.022 $\pm$ 0.002	0.028 $\pm$ 0.001	ns
Sheath dry weight (g)	0.017 $\pm$ 0.002	0.015 $\pm$ 0.002	0.014 $\pm$ 0.001	ns
Root dry weight (g)	0.016 $\pm$ 0.003	0.019 $\pm$ 0.002	0.018 $\pm$ 0.001	ns
Total biomass	0.057 $\pm$ 0.006	0.055 $\pm$ 0.003	0.060 $\pm$ 0.001	ns
R:S ratio	0.39 $\pm$ 0.05	0.52 $\pm$ 0.06	0.42 $\pm$ 0.04	ns
LAR (cm <sup>2</sup> g <sup>-1</sup> )	312 $\pm$ 22	241 $\pm$ 28	243 $\pm$ 21	ns
SLA (cm <sup>2</sup> g <sup>-1</sup> )	751 $\pm$ 57	635 $\pm$ 85	521 $\pm$ 39	ns
SLN (g m <sup>-2</sup> )	0.65 $\pm$ 0.06	0.84 $\pm$ 0.13	0.93 $\pm$ 0.07	ns

**Table S2. (b) 5 leaf stage**

	Sb			<i>acdc1</i>			<i>tcd1</i>			2-way ANOVA		
	LN	MN	HN	LN	MN	HN	LN	MN	HN	L	T	L x T
Leaf area (cm <sup>2</sup> )	49 $\pm$ 2	56 $\pm$ 3	62 $\pm$ 5	53 $\pm$ 4	67 $\pm$ 3	55 $\pm$ 6	68 $\pm$ 13	65 $\pm$ 16	81 $\pm$ 10	**	ns	ns
Height (cm)	12.6 $\pm$ 0.4	13.5 $\pm$ 0.2	13.2 $\pm$ 0.4	12.2 $\pm$ 0.3	12.4 $\pm$ 0.3	12.4 $\pm$ 0.3	10.3 $\pm$ 0.6	10.2 $\pm$ 1.0	10.6 $\pm$ 0.3	***	ns	ns
Leaf DW (g)	0.073 $\pm$ 0.004	0.097 $\pm$ 0.001	0.110 $\pm$ 0.005	0.084 $\pm$ 0.009	0.130 $\pm$ 0.016	0.097 $\pm$ 0.009	0.098 $\pm$ 0.018	0.093 $\pm$ 0.029	0.120 $\pm$ 0.017	ns	ns	ns
Sheath DW (g)	0.045 $\pm$ 0.003	0.055 $\pm$ 0.002	0.064 $\pm$ 0.004	0.042 $\pm$ 0.003	0.050 $\pm$ 0.002	0.052 $\pm$ 0.004	0.061 $\pm$ 0.007	0.064 $\pm$ 0.014	0.064 $\pm$ 0.007	**	*	ns
Root DW (g)	0.025 $\pm$ 0.003	0.033 $\pm$ 0.002	0.040 $\pm$ 0.003	0.031 $\pm$ 0.004	0.036 $\pm$ 0.003	0.038 $\pm$ 0.004	0.041 $\pm$ 0.011	0.034 $\pm$ 0.011	0.041 $\pm$ 0.008	ns	ns	ns
Total biomass	0.14 $\pm$ 0.01	0.18 $\pm$ 0.00	0.21 $\pm$ 0.01	0.15 $\pm$ 0.01	0.22 $\pm$ 0.04	0.19 $\pm$ 0.02	0.21 $\pm$ 0.04	0.22 $\pm$ 0.05	0.22 $\pm$ 0.03	ns	ns	ns
R:S ratio	0.21 $\pm$ 0.02	0.22 $\pm$ 0.01	0.23 $\pm$ 0.02	0.25 $\pm$ 0.03	0.23 $\pm$ 0.03	0.25 $\pm$ 0.02	0.22 $\pm$ 0.04	0.18 $\pm$ 0.02	0.23 $\pm$ 0.03	ns	ns	ns
LAR (cm <sup>2</sup> g <sup>-1</sup> )	347 $\pm$ 19	303 $\pm$ 16	291 $\pm$ 17	356 $\pm$ 22	339 $\pm$ 31	299 $\pm$ 27	318 $\pm$ 16	346 $\pm$ 7	369 $\pm$ 6	ns	ns	ns
SLA (cm <sup>2</sup> g <sup>-1</sup> )	687 $\pm$ 49	574 $\pm$ 24	565 $\pm$ 30	689 $\pm$ 51	650 $\pm$ 78	577 $\pm$ 51	623 $\pm$ 20	754 $\pm$ 74	710 $\pm$ 20	ns	ns	ns
SLN (g m <sup>-2</sup> )	0.62 $\pm$ 0.04 <sup>b</sup>	0.81 $\pm$ 0.04 <sup>a</sup>	0.86 $\pm$ 0.04 <sup>a</sup>	0.71 $\pm$ 0.05 <sup>b</sup>	0.65 $\pm$ 0.01 <sup>b</sup>	0.90 $\pm$ 0.05 <sup>a</sup>	0.65 $\pm$ 0.02 <sup>b</sup>	0.63 $\pm$ 0.05 <sup>b</sup>	0.65 $\pm$ 0.02 <sup>b</sup>	*	**	*
RGR (g g <sup>-1</sup> d <sup>-1</sup> )	0.065 $\pm$ 0.004	0.084 $\pm$ 0.001	0.094 $\pm$ 0.003	0.077 $\pm$ 0.008	0.101 $\pm$ 0.010	0.093 $\pm$ 0.007	0.074 $\pm$ 0.013	0.059 $\pm$ 0.02	0.079 $\pm$ 0.008	*	ns	ns

**Table S2. (c) 8 leaf stage**

	<b>Sb</b>			<i>acdc1</i>			<i>tcd1</i>			<b>2-way ANOVA</b>		
	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>L</b>	<b>T</b>	<b>L×T</b>
<b>Leaf area (cm<sup>2</sup>)</b>	155 ± 6 <sup>d</sup>	172 ± 10 <sup>cd</sup>	208 ± 8 <sup>c</sup>	145 ± 8 <sup>d</sup>	232 ± 25 <sup>b</sup>	298 ± 14 <sup>a</sup>	191 ± 33 <sup>cd</sup>	130 ± 24 <sup>d</sup>	228 ± 29 <sup>c</sup>	***	***	***
<b>Height (cm)</b>	15.9 ± 0.3	17.1 ± 0.4	19.0 ± 1.0	17.1 ± 0.5	18.4 ± 0.8	19.9 ± 0.7	14.5 ± 0.2	15.7 ± 1.2	17.7 ± 0.8	***	***	ns
<b>Leaf DW (g)</b>	0.39 ± 0.02 <sup>e</sup>	0.38 ± 0.02 <sup>ef</sup>	0.53 ± 0.02 <sup>d</sup>	0.38 ± 0.03 <sup>e</sup>	0.55 ± 0.05 <sup>b</sup>	0.69 ± 0.04 <sup>ac</sup>	0.47 ± 0.13 <sup>e</sup>	0.30 ± 0.04 <sup>f</sup>	0.61 ± 0.09 <sup>cd</sup>	*	***	**
<b>Sheath DW (g)</b>	0.19 ± 0.01	0.20 ± 0.01	0.29 ± 0.0	0.20 ± 0.02	0.29 ± 0.03	0.32 ± 0.02	0.21 ± 0.05	0.18 ± 0.03	0.29 ± 0.04	*	***	ns
<b>Root DW (g)</b>	0.18 ± 0.01	0.13 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.24 ± 0.02	0.27 ± 0.02	0.15 ± 0.04	0.12 ± 0.03	0.18 ± 0.03	***	**	ns
<b>Total biomass</b>	0.76 ± 0.04 <sup>c</sup>	0.71 ± 0.04 <sup>c</sup>	1.00 ± 0.04 <sup>b</sup>	0.80 ± 0.05 <sup>c</sup>	1.08 ± 0.08 <sup>a</sup>	1.29 ± 0.06 <sup>a</sup>	0.84 ± 0.21 <sup>bc</sup>	0.59 ± 0.10 <sup>c</sup>	1.08 ± 0.16 <sup>ab</sup>	***	***	*
<b>R:S ratio</b>	0.32 ± 0.02	0.22 ± 0.02	0.24 ± 0.01	0.37 ± 0.02	0.29 ± 0.02	0.27 ± 0.01	0.23 ± 0.03	0.23 ± 0.03	0.20 ± 0.00	***	***	ns
<b>LAR (cm<sup>2</sup> g<sup>-1</sup>)</b>	206 ± 4 <sup>b</sup>	253 ± 9 <sup>a</sup>	200 ± 6 <sup>b</sup>	182 ± 10 <sup>b</sup>	217 ± 7 <sup>c</sup>	232 ± 9 <sup>c</sup>	215 ± 19 <sup>b</sup>	219 ± 11 <sup>bc</sup>	212 ± 6 <sup>b</sup>	ns	ns	*
<b>SLA (cm<sup>2</sup> g<sup>-1</sup>)</b>	404 ± 9	452 ± 13	396 ± 10	386 ± 24	427 ± 41	433 ± 20	489 ± 120	422 ± 26	379 ± 11	ns	ns	ns
<b>SLN (g m<sup>-2</sup>)</b>	0.58 ± 0.01 <sup>c</sup>	0.81 ± 0.03 <sup>b</sup>	1.04 ± 0.03 <sup>a</sup>	0.72 ± 0.04 <sup>b</sup>	0.91 ± 0.02 <sup>a</sup>	1.00 ± 0.04 <sup>a</sup>	0.73 ± 0.09 <sup>b</sup>	0.95 ± 0.06 <sup>a</sup>	1.03 ± 0.03 <sup>a</sup>	***	***	*
<b>RGR (g g<sup>-1</sup> d<sup>-1</sup>)</b>	0.050 ± 0.002	0.052 ± 0.002	0.067 ± 0.002	0.061 ± 0.003	0.063 ± 0.009	0.078 ± 0.003	0.064 ± 0.022	0.059 ± 0.011	0.081 ± 0.005	*	***	ns

**Table S2. (d) 10 leaf stage**

	<b>Sb</b>			<i>acdc1</i>			<i>tcd1</i>			<b>2-way ANOVA</b>		
	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>L</b>	<b>T</b>	<b>L x T</b>
<b>Leaf area (cm<sup>2</sup>)</b>	413 ± 29	529 ± 30	525 ± 30	277 ± 22	572 ± 64	561 ± 40	314 ± 44	533 ± 88	450 ± 38	ns	***	ns
<b>Height (cm)</b>	17.0 ± 0.2	20.9 ± 0.2	21.3 ± 0.6	20.4 ± 1.5	25.0 ± 1.5	24.1 ± 0.7	15.0 ± 0.3	21.4 ± 1.8	20.3 ± 0.6	***	***	ns
<b>Leaf DW (g)</b>	1.75 ± 0.18	1.50 ± 0.11	1.44 ± 0.10	1.24 ± 0.11	1.94 ± 0.28	1.70 ± 0.12	1.36 ± 0.22	1.60 ± 0.35	1.23 ± 0.11	ns	ns	ns
<b>Sheath DW (g)</b>	1.13 ± 0.13	0.73 ± 0.04	0.74 ± 0.05	0.91 ± 0.12	1.10 ± 0.22	0.84 ± 0.05	0.80 ± 0.13	0.75 ± 0.14	0.60 ± 0.14	ns	ns	ns
<b>Root DW (g)</b>	0.82 ± 0.06	0.59 ± 0.04	0.43 ± 0.03	0.69 ± 0.07	0.85 ± 0.16	0.67 ± 0.06	0.53 ± 0.10	0.51 ± 0.13	0.35 ± 0.05	**	*	ns
<b>Total biomass DW (g)</b>	3.71 ± 0.37	2.82 ± 0.19	2.60 ± 0.18	2.84 ± 0.25	3.89 ± 0.67	3.21 ± 0.23	2.68 ± 0.44	2.87 ± 0.61	2.17 ± 0.2	ns	ns	ns
<b>R:S ratio</b>	0.29 ± 0.01	0.26 ± 0.01	0.20 ± 0.01	0.33 ± 0.04	0.28 ± 0.01	0.26 ± 0.01	0.25 ± 0.02	0.21 ± 0.01	0.19 ± 0.01	***	***	ns
<b>LAR (cm<sup>2</sup> g<sup>-1</sup>)</b>	114 ± 5	189 ± 3	203 ± 3	98 ± 3	155 ± 8	175 ± 5	120 ± 7	196 ± 18	208 ± 6	***	***	ns
<b>SLA (cm<sup>2</sup> g<sup>-1</sup>)</b>	241 ± 11	356 ± 6	368 ± 6	225 ± 9	302 ± 6	332 ± 6	236 ± 11	349 ± 27	368 ± 9	***	***	ns
<b>SLN (g m<sup>-2</sup>)</b>	1.12 ± 0.06 <sup>c</sup>	1.70 ± 0.04 <sup>b</sup>	2.22 ± 0.11 <sup>a</sup>	1.66 ± 0.15 <sup>e</sup>	1.98 ± 0.10 <sup>bc</sup>	2.31 ± 0.12 <sup>ab</sup>	1.38 ± 0.09 <sup>ce</sup>	1.93 ± 0.13 <sup>bf</sup>	2.14 ± 0.09 <sup>af</sup>	**	***	ns
<b>RGR (g g<sup>-1</sup> d<sup>-1</sup>)</b>	0.040±0.001 <sup>b</sup>	0.056±0.002 <sup>a</sup>	0.047±0.003 <sup>ab</sup>	0.029±0.002 <sup>b</sup>	0.046±0.004 <sup>c</sup>	0.042±0.004 <sup>ac</sup>	0.026±0.001 <sup>b</sup>	0.075±0.012 <sup>d</sup>	0.034±0.005 <sup>ab</sup>	*	***	***
<b>Overall RGR (g g<sup>-1</sup> d<sup>-1</sup>)</b>	0.048±0.001 <sup>c</sup>	0.061±0.001 <sup>b</sup>	0.067±0.001 <sup>a</sup>	0.047±0.001 <sup>c</sup>	0.065±0.002 <sup>ab</sup>	0.068±0.002 <sup>a</sup>	0.055±0.007 <sup>c</sup>	0.069±0.004 <sup>a</sup>	0.064±0.002 <sup>a</sup>	ns	***	*

**Table S3. Allocation of nitrogen to dhurrin (dhurrin-N per total N) and nitrate (nitrate-N per total N) for leaves, sheaths and roots of three *Sorghum bicolor* genotypes different in dhurrin concentration and grown at 2, 6 and 12 mM nitrate (LN, MN, HN) and harvested the (a) 3-leaf stage; (b) 5-leaf stage; (c) 8-leaf stage; and (d) 10-leaf stage**

Sb: wild type; *acdc1*: adult cyanide deficient mutants; *tcd1*: totally cyanide deficient mutants. Values are the mean of 7 or 4 (*tcd1*) replicates  $\pm$  SE. Significance of a one-way (3-leaf stage) or two-way ANOVAs (other leaf stages) are presented for each genotype (line, L) and treatment (T). Means with the same letter are not significantly different ( $P>0.05$ ; Tukey's Pairwise Comparison)

**(a) Dhurrin-N per total N (%)**

(b)	Sb			<i>acdc1</i>			<i>tcd1</i>			2-way ANOVA		
	LN	MN	HN	LN	MN	HN	LN	MN	HN	L	T	L x T
<b>3-leaf stage</b>												
Leaf		3.58 $\pm$ 0.26 <sup>a</sup>			1.11 $\pm$ 0.37 <sup>ab</sup>			0.00 $\pm$ 0.00 <sup>c</sup>		***		
Sheath		1.80 $\pm$ 0.21 <sup>a</sup>			0.48 $\pm$ 0.11 <sup>b</sup>			0.00 $\pm$ 0.00 <sup>b</sup>		***		
Root		2.78 $\pm$ 0.39 <sup>a</sup>			1.83 $\pm$ 0.41 <sup>a</sup>			0.00 $\pm$ 0.00 <sup>b</sup>		***		
<b>5-leaf stage</b>												
Leaf	1.45 $\pm$ 0.08 <sup>b</sup>	0.99 $\pm$ 0.11 <sup>a</sup>	0.95 $\pm$ 0.10 <sup>a</sup>	0.26 $\pm$ 0.03 <sup>c</sup>	0.44 $\pm$ 0.1 <sup>c</sup>	0.22 $\pm$ 0.06 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>cd</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>cd</sup>	***	*	***
Sheath	0.47 $\pm$ 0.09 <sup>a</sup>	0.37 $\pm$ 0.06 <sup>a</sup>	0.43 $\pm$ 0.05 <sup>a</sup>	0.14 $\pm$ 0.05 <sup>b</sup>	0.18 $\pm$ 0.03 <sup>b</sup>	0.20 $\pm$ 0.03 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	***	ns	ns
Root	0.97 $\pm$ 0.21 <sup>a</sup>	1.34 $\pm$ 0.24 <sup>a</sup>	1.03 $\pm$ 0.10 <sup>a</sup>	0.30 $\pm$ 0.14 <sup>a</sup>	0.34 $\pm$ 0.18 <sup>a</sup>	0.46 $\pm$ 0.17 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	***	ns	ns
<b>8-leaf stage</b>												
Leaf	0.59 $\pm$ 0.02 <sup>b</sup>	0.46 $\pm$ 0.05 <sup>a</sup>	0.45 $\pm$ 0.03 <sup>ac</sup>	0.01 $\pm$ 0.00 <sup>d</sup>	0.02 $\pm$ 0.00 <sup>d</sup>	0.40 $\pm$ 0.03 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>de</sup>	0.00 $\pm$ 0.00 <sup>de</sup>	0.00 $\pm$ 0.00 <sup>e</sup>	***	***	***
Sheath	0.60 $\pm$ 0.07 <sup>c</sup>	0.21 $\pm$ 0.04 <sup>b</sup>	0.08 $\pm$ 0.02 <sup>a</sup>	0.10 $\pm$ 0.03 <sup>d</sup>	0.08 $\pm$ 0.02 <sup>d</sup>	0.06 $\pm$ 0.01 <sup>ad</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>ad</sup>	***	***	***
Root	1.94 $\pm$ 0.24 <sup>b</sup>	1.38 $\pm$ 0.22 <sup>ab</sup>	0.86 $\pm$ 0.15 <sup>a</sup>	1.23 $\pm$ 0.35 <sup>d</sup>	0.80 $\pm$ 0.25 <sup>cd</sup>	0.61 $\pm$ 0.21 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>f</sup>	0.00 $\pm$ 0.00 <sup>ef</sup>	0.00 $\pm$ 0.00 <sup>e</sup>	***	*	ns
<b>10-leaf stage</b>												
Leaf	1.87 $\pm$ 0.10 <sup>c</sup>	0.79 $\pm$ 0.06 <sup>b</sup>	0.43 $\pm$ 0.04 <sup>a</sup>	0.11 $\pm$ 0.10 <sup>d</sup>	0.09 $\pm$ 0.03 <sup>d</sup>	0.03 $\pm$ 0.01 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	***	***	***
Sheath	0.68 $\pm$ 0.05 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>ag</sup>	0.10 $\pm$ 0.01 <sup>aef</sup>	0.06 $\pm$ 0.01 <sup>ch</sup>	0.22 $\pm$ 0.11 <sup>cg</sup>	0.39 $\pm$ 0.21 <sup>ce</sup>	0.00 $\pm$ 0.00 <sup>dh</sup>	0.00 $\pm$ 0.00 <sup>dg</sup>	0.00 $\pm$ 0.00 <sup>df</sup>	***	ns	***
Root	2.07 $\pm$ 0.18 <sup>a</sup>	2.02 $\pm$ 0.21 <sup>a</sup>	1.49 $\pm$ 0.11 <sup>a</sup>	0.99 $\pm$ 0.20 <sup>b</sup>	1.01 $\pm$ 0.2 <sup>b</sup>	0.75 $\pm$ 0.07 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	***	ns	ns

**(c) Nitrate-N per total N (NO<sub>3</sub>-N per N %)**

	<b>Sb</b>			<i>acdc1</i>			<i>tcd1</i>			<b>2-way ANOVA</b>		
	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>LN</b>	<b>MN</b>	<b>HN</b>	<b>L</b>	<b>T</b>	<b>L x T</b>
<b>3-leaf stage</b>												
Leaf		12.75 ± 0.59			15.75 ± 3.32			19.79 ± 3.25		ns		
Sheath		37.82 ± 1.95			36.26 ± 3.61			42.38 ± 5.94		ns		
Root		25.17 ± 5.00			26.39 ± 5.27			26.08 ± 3.51		ns		
<b>5-leaf stage</b>												
Leaf	8.8 ± 0.8 <sup>b</sup>	12.1 ± 0.7 <sup>a</sup>	11.9 ± 0.6 <sup>a</sup>	8.8 ± 1.5 <sup>b</sup>	10.2 ± 0.8 <sup>a</sup>	11.1 ± 0.7 <sup>a</sup>	7.4 ± 2.9 <sup>b</sup>	10.3 ± 0.9 <sup>a</sup>	11.5 ± 0.6 <sup>a</sup>	ns	**	ns
Sheath	37.9 ± 1.7 <sup>b</sup>	40.4 ± 1.1 <sup>a</sup>	37.0 ± 3.4 <sup>ab</sup>	30.7 ± 4.9 <sup>b</sup>	40.3 ± 3.9 <sup>a</sup>	35.2 ± 3.2 <sup>ab</sup>	24.1 ± 9.2 <sup>b</sup>	41.8 ± 1.8 <sup>a</sup>	40.9 ± 2.9 <sup>ab</sup>	ns	*	ns
Root	16.1 ± 1.9 <sup>b</sup>	21.4 ± 1.2 <sup>a</sup>	23.4 ± 1.0 <sup>a</sup>	14.0 ± 2.3 <sup>bd</sup>	20.6 ± 2.3 <sup>ac</sup>	23.4 ± 1.8 <sup>ac</sup>	7.0 ± 4.4 <sup>d</sup>	13.0 ± 5.0 <sup>c</sup>	19.8 ± 0.6 <sup>c</sup>	*	***	ns
<b>8-leaf stage</b>												
Leaf	2.1 ± 0.2 <sup>bi</sup>	11.5 ± 0.6 <sup>ah</sup>	15.0 ± 0.8 <sup>af</sup>	2.4 ± 0.7 <sup>di</sup>	5.1 ± 0.4 <sup>cd</sup>	7.4 ± 0.5 <sup>cg</sup>	13.7 ± 6.1 <sup>e</sup>	13.4 ± 2.5 <sup>eh</sup>	11.1 ± 0.7 <sup>efg</sup>	***	***	***
Sheath	6.2 ± 1.9 <sup>b</sup>	42.7 ± 1.9 <sup>a</sup>	53.4 ± 3.0 <sup>a</sup>	3.9 ± 1.4 <sup>be</sup>	61.0 ± 8.0 <sup>d</sup>	45.3 ± 3.5 <sup>a</sup>	9.6 ± 3.4 <sup>bf</sup>	16.3 ± 1.6 <sup>f</sup>	20.2 ± 1.4 <sup>f</sup>	***	***	***
Root	0.2 ± 0.2 <sup>b</sup>	13.1 ± 2.4 <sup>a</sup>	23.5 ± 1.6 <sup>a</sup>	15.5 ± 7.4 <sup>d</sup>	3.2 ± 1.4 <sup>cd</sup>	10.8 ± 0.9 <sup>c</sup>	5.9 ± 4.2 <sup>f</sup>	16.6 ± 3.8 <sup>ef</sup>	24.2 ± 1.4 <sup>e</sup>	ns	***	***
<b>10-leaf stage</b>												
Leaf	10.2 ± 3.5 <sup>b</sup>	6.0 ± 1.2 <sup>b</sup>	11.6 ± 0.8 <sup>a</sup>	4.0 ± 0.5 <sup>d</sup>	4.3 ± 0.6 <sup>d</sup>	8.5 ± 0.9 <sup>c</sup>	5.8 ± 0.7 <sup>bd</sup>	4.3 ± 1.1 <sup>bd</sup>	10.4 ± 2.0 <sup>ac</sup>	*	***	ns
Sheath	23.3 ± 4.9 <sup>bh</sup>	24.4 ± 4.8 <sup>bg</sup>	52.0 ± 6.5 <sup>af</sup>	13.6 ± 5.6 <sup>dh</sup>	68.5 ± 15.1 <sup>c</sup>	43.8 ± 8.5 <sup>cf</sup>	11.3 ± 2.4 <sup>eh</sup>	23.5 ± 9.3 <sup>eg</sup>	42.9 ± 0.4 <sup>ef</sup>	*	***	**
Root	7.2 ± 1.0 <sup>bh</sup>	6.6 ± 1.3 <sup>bg</sup>	27.3 ± 4.4 <sup>a</sup>	7.3 ± 1.6 <sup>cdh</sup>	4.0 ± 1.0 <sup>dg</sup>	13.4 ± 1.8 <sup>c</sup>	5.3 ± 1.2 <sup>fh</sup>	4.5 ± 1.0 <sup>fg</sup>	22.1 ± 1.7 <sup>e</sup>	**	***	*



**Table S4: Chlorophyll and carotenoid concentration** of the three *Sorghum bicolor* genotypes grown at 2, 6 and 12 mM nitrate (LN, MN, HN) and harvested the 5-leaf, 8-leaf and 10-leaf stage of development. Sb: wild type; *acdc1*: adult cyanide deficient mutants; *tcd1*: totally cyanide deficient mutants. Values are the mean of 7 or 4 (*tcd1*) replicates  $\pm$  SE. Significance of two-way ANOVAs are presented for each genotype (line, L) and treatment (T). Means with the same letter are not significantly different ( $P > 0.05$ ; Tukey's Pairwise Comparison). \* $P < 0.05$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.001$ ; ns, not significant.

	Sb			<i>acdc1</i>			<i>tcd1</i>			2-way ANOVA		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	L	T	L x T
<b>5-leaf stage</b>												
Chl a/b leaf 1	2.7 $\pm$ 0.2 <sup>c</sup>	2.8 $\pm$ 0.2 <sup>c</sup>	3.1 $\pm$ 0.1 <sup>c</sup>	3.3 $\pm$ 0.1 <sup>b</sup>	3.2 $\pm$ 0.1 <sup>bc</sup>	3.3 $\pm$ 0.1 <sup>bc</sup>	4.2 $\pm$ 0.5 <sup>a</sup>	2.8 $\pm$ 0.3 <sup>c</sup>	2.7 $\pm$ 0.3 <sup>c</sup>	*	**	**
Chl a/b leaf 3	2.4 $\pm$ 0.1 <sup>a</sup>	2.9 $\pm$ 0.1 <sup>a</sup>	2.8 $\pm$ 0.1 <sup>ab</sup>	3.1 $\pm$ 0.1 <sup>c</sup>	3.1 $\pm$ 0.3 <sup>ac</sup>	3.0 $\pm$ 0.1 <sup>ac</sup>	3.7 $\pm$ 0.1 <sup>c</sup>	2.1 $\pm$ 0.2 <sup>b</sup>	2.1 $\pm$ 0.3 <sup>b</sup>	**	**	***
Carotenoids leaf 1	0.97 $\pm$ 0.06	0.90 $\pm$ 0.06	1.11 $\pm$ 0.06	1.10 $\pm$ 0.09	1.11 $\pm$ 0.05	1.13 $\pm$ 0.06	0.93 $\pm$ 0.07	0.91 $\pm$ 0.02	1.00 $\pm$ 0.07	**	ns	ns
Carotenoids leaf 3	0.88 $\pm$ 0.05	0.93 $\pm$ 0.03	0.96 $\pm$ 0.03	0.90 $\pm$ 0.06	0.95 $\pm$ 0.05	0.85 $\pm$ 0.04	0.72 $\pm$ 0.07	0.61 $\pm$ 0.03	0.71 $\pm$ 0.10	***	ns	ns
Total chl leaf 1	7.76 $\pm$ 0.39	7.11 $\pm$ 0.52	7.75 $\pm$ 0.39	7.99 $\pm$ 0.69	8.37 $\pm$ 0.37	7.93 $\pm$ 0.51	6.38 $\pm$ 0.51	7.24 $\pm$ 0.32	8.13 $\pm$ 0.32	ns	ns	ns
Total chl leaf 3	7.43 $\pm$ 0.32 <sup>a</sup>	6.58 $\pm$ 0.22 <sup>a</sup>	6.98 $\pm$ 0.30 <sup>a</sup>	6.22 $\pm$ 0.30 <sup>ab</sup>	7.21 $\pm$ 0.41 <sup>a</sup>	6.32 $\pm$ 0.33 <sup>a</sup>	5.37 $\pm$ 0.42 <sup>b</sup>	6.71 $\pm$ 0.99 <sup>ab</sup>	7.64 $\pm$ 0.79 <sup>a</sup>	ns	ns	**
<b>8-leaf stage</b>												
Chl a/b leaf 1	3.5 $\pm$ 0.1	3.4 $\pm$ 0.1	3.6 $\pm$ 0.2	3.4 $\pm$ 0.1	3.3 $\pm$ 0.2	3.4 $\pm$ 0.1	3.4 $\pm$ 0.2	3.6 $\pm$ 0.0	3.4 $\pm$ 0.1	ns	ns	ns
Chl a/b leaf 3	3.1 $\pm$ 0.2	3.7 $\pm$ 0.1	3.3 $\pm$ 0.1	3.6 $\pm$ 0.1	3.7 $\pm$ 0.1	3.5 $\pm$ 0.2	3.5 $\pm$ 0.1	3.4 $\pm$ 0.1	3.4 $\pm$ 0.1	ns	ns	ns
Carotenoids leaf 1	0.96 $\pm$ 0.04	0.87 $\pm$ 0.03	1.04 $\pm$ 0.03	0.85 $\pm$ 0.02	0.88 $\pm$ 0.02	0.99 $\pm$ 0.06	0.87 $\pm$ 0.06	1.08 $\pm$ 0.10	1.14 $\pm$ 0.06	*	***	ns
Carotenoids leaf 3	0.99 $\pm$ 0.04	0.96 $\pm$ 0.04	0.99 $\pm$ 0.03	0.96 $\pm$ 0.04	0.99 $\pm$ 0.06	1.05 $\pm$ 0.05	1.01 $\pm$ 0.05	1.11 $\pm$ 0.06	1.14 $\pm$ 0.13	*	ns	ns
Total chl leaf 1	6.83 $\pm$ 0.29	6.34 $\pm$ 0.22	7.36 $\pm$ 0.19	6.27 $\pm$ 0.26	6.71 $\pm$ 0.59	7.44 $\pm$ 0.49	6.57 $\pm$ 0.51	8.02 $\pm$ 0.71	8.23 $\pm$ 0.66	ns	*	ns
Total chl leaf 3	6.86 $\pm$ 0.27	6.65 $\pm$ 0.32	7.05 $\pm$ 0.24	6.33 $\pm$ 0.39	6.88 $\pm$ 0.42	7.25 $\pm$ 0.48	6.82 $\pm$ 0.42	8.07 $\pm$ 0.49	7.65 $\pm$ 0.79	ns	ns	ns
<b>10-leaf stage</b>												
Chl a/b leaf 1	3.3 $\pm$ 0.3	3.6 $\pm$ 0.1	3.6 $\pm$ 0.1	3.5 $\pm$ 0.1	3.5 $\pm$ 0.1	3.4 $\pm$ 0.1	3.2 $\pm$ 0.2	3.6 $\pm$ 0.1	3.2 $\pm$ 0.1	ns	ns	ns
Chl a/b leaf 3	3.1 $\pm$ 0.2	3.4 $\pm$ 0.1	3.4 $\pm$ 0.1	3.2 $\pm$ 0.1	3.4 $\pm$ 0.1	3.3 $\pm$ 0.1	3.3 $\pm$ 0.2	3.4 $\pm$ 0.1	3.4 $\pm$ 0.1	ns	ns	ns
Carotenoids leaf 1	0.91 $\pm$ 0.07	1.16 $\pm$ 0.04	1.16 $\pm$ 0.04	0.77 $\pm$ 0.04	1.23 $\pm$ 0.04	1.27 $\pm$ 0.05	0.79 $\pm$ 0.07	1.04 $\pm$ 0.08	1.06 $\pm$ 0.15	*	***	ns
Carotenoids leaf 3	0.73 $\pm$ 0.06	1.08 $\pm$ 0.04	1.16 $\pm$ 0.06	0.62 $\pm$ 0.05	1.12 $\pm$ 0.09	1.19 $\pm$ 0.05	0.58 $\pm$ 0.06	1.09 $\pm$ 0.02	1.19 $\pm$ 0.08	ns	***	ns
Total chl leaf 1	6.04 $\pm$ 0.39 <sup>b</sup>	7.80 $\pm$ 0.27 <sup>a</sup>	7.95 $\pm$ 0.39 <sup>a</sup>	4.78 $\pm$ 0.27 <sup>b</sup>	8.49 $\pm$ 0.26 <sup>a</sup>	8.86 $\pm$ 0.39 <sup>a</sup>	5.14 $\pm$ 0.40 <sup>b</sup>	7.03 $\pm$ 0.43 <sup>a</sup>	7.40 $\pm$ 0.89 <sup>a</sup>	*	***	*
Total chl leaf 3	4.59 $\pm$ 0.45	7.31 $\pm$ 0.39	7.98 $\pm$ 0.34	3.59 $\pm$ 0.39	7.57 $\pm$ 0.55	8.28 $\pm$ 0.29	2.98 $\pm$ 0.40	7.20 $\pm$ 0.15	8.58 $\pm$ 0.57	ns	***	ns