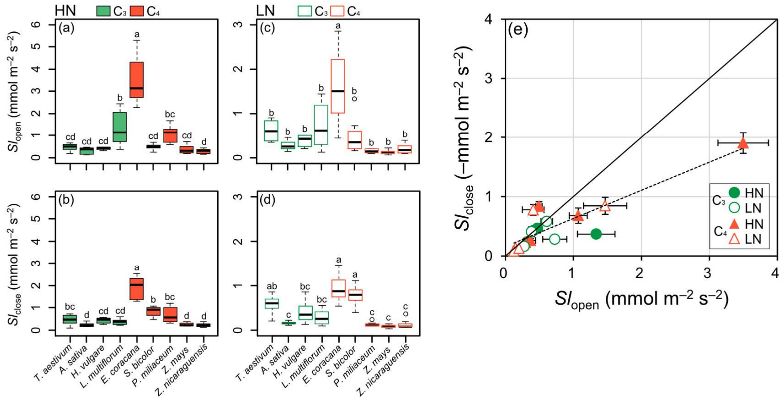
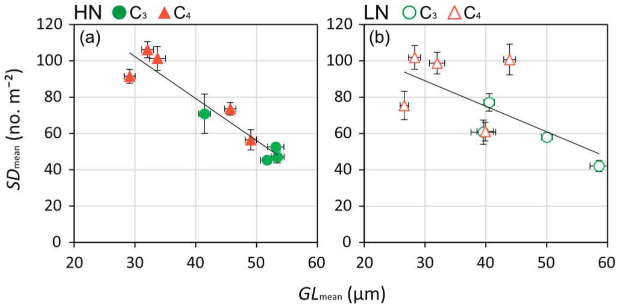
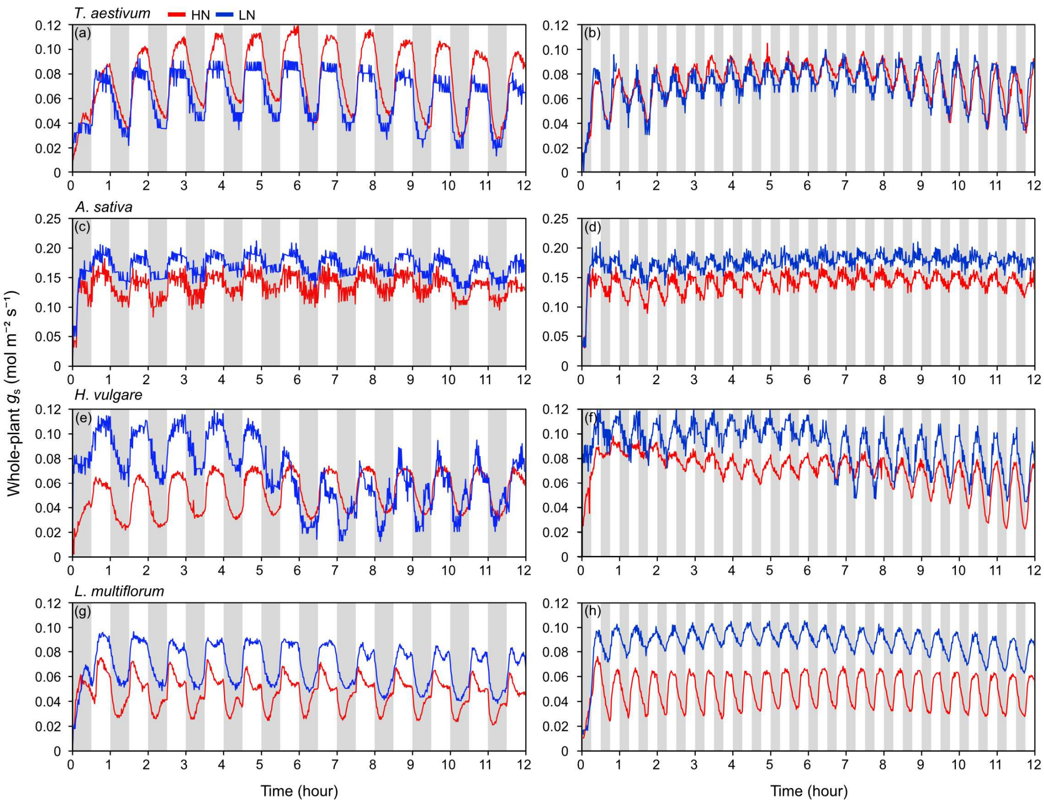
SUPPLEMENTAL DATA



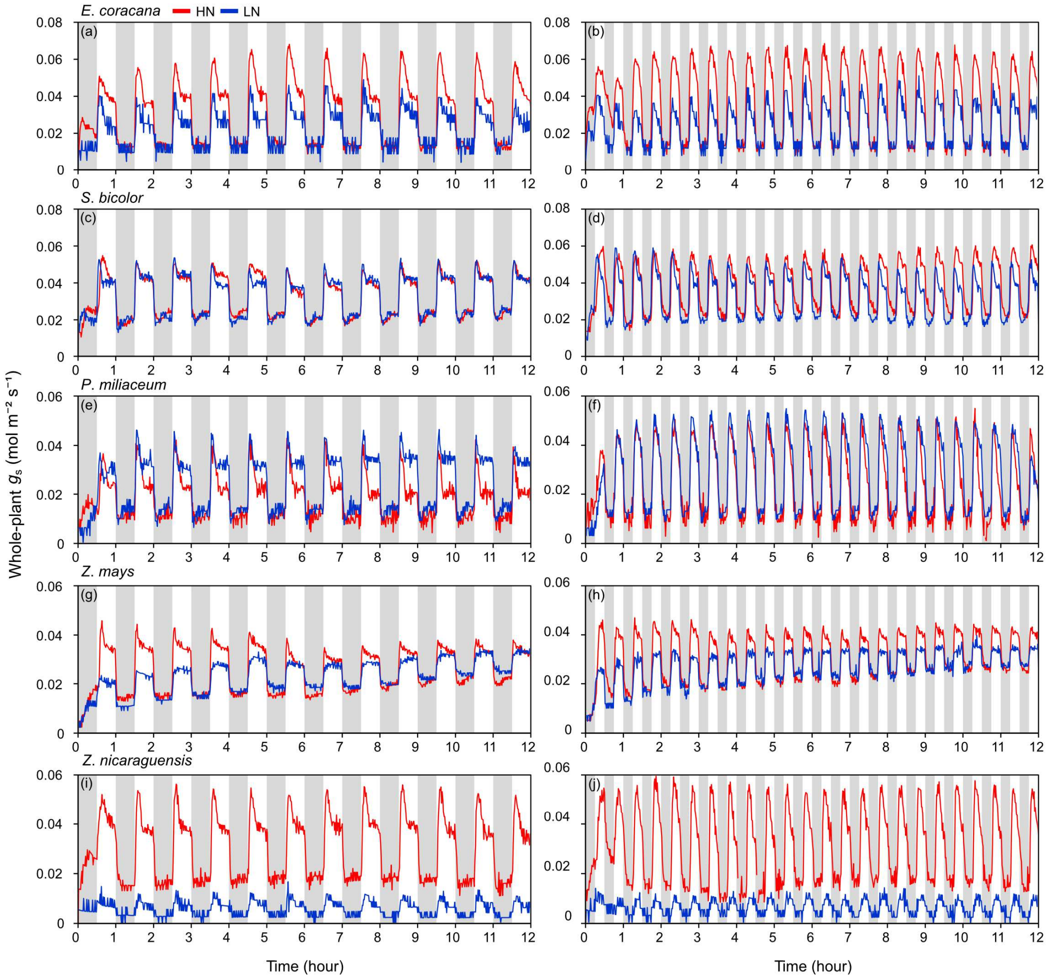
Supplemental Fig. S1. Maximum slope of the response of stomatal conductance (g_s) to the increased photosynthetically active photon flux density (PPFD) (Sl_{open}) and that to the decreased PPFD (Sl_{close}) in four C_3 and five C_4 species grown under high nitrogen (N) (HN) or low N (LN) conditions. (a-e) Green and orange boxes indicate C_3 and C_4 species, respectively. Significant differences between the species were determined using ANOVA followed by Tukey's test ($P < 0.05$) and are indicated by different letters. Data are the mean ± SE (n = 4). (e) In the relationship between Sl_{close} and Sl_{open}, solid and dotted lines represent regression line ($R^2 = 0.77$, $P < 0.01$) and 1:1 line, respectively.



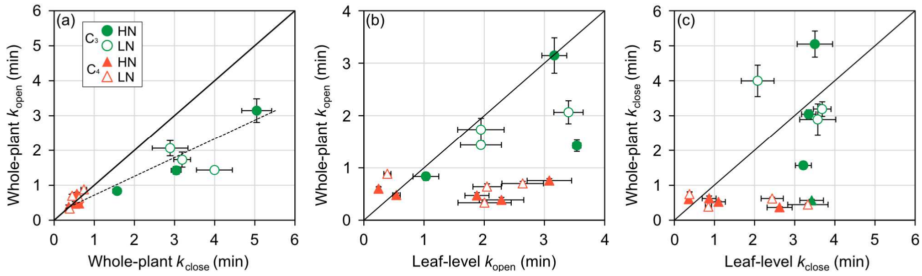
Supplemental Fig. S2. Relationship between mean stomatal density (SD_{mean}) and mean guard cell length (GL_{mean}) in four C_3 and five C_4 species grown under (a) high nitrogen (N) (HN) or (b) low N (LN) conditions. Green and orange symbols indicate C_3 and C_4 species, respectively, and filled and open symbols indicate plants grown under HN and LN conditions, respectively. Regression lines are shown for all C_3 and C_4 species. Values of R^2 are (a) 0.90 ($P < 0.01$) and (b) 0.45 ($P < 0.05$). Data are the mean ± SE (n = 4).



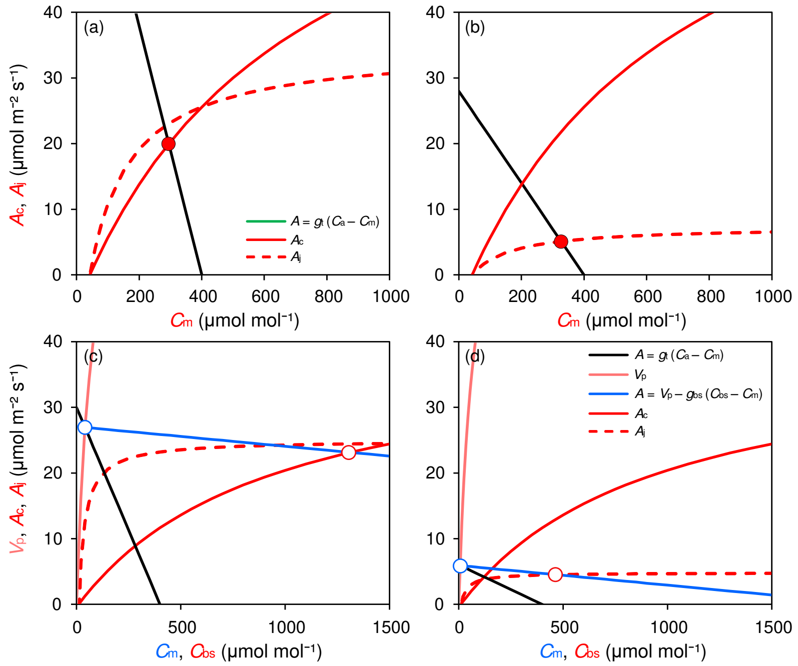
Supplemental Fig. S3. Dynamics of the whole-plant stomatal conductance (g_s) in the controlled fluctuating light environments in four C_3 species. Light intensity was alternately changed between low (80 µmol m^{-2} s^{-1} photosynthetically active photon flux density (PPFD) at 30 cm above the pot surface, grey area) and high (300 µmol m^{-2} s^{-1} PPFD at 30 cm above the pot surface, white area) at 30-min intervals (a, c, e, g) and 15-min intervals (b, d, f, h) for a total of 12 hours. Red and blue lines represent plants grown under high nitrogen (N) (HN) and low N (LN) conditions, respectively. Data are the mean of four pot plants on the balance.



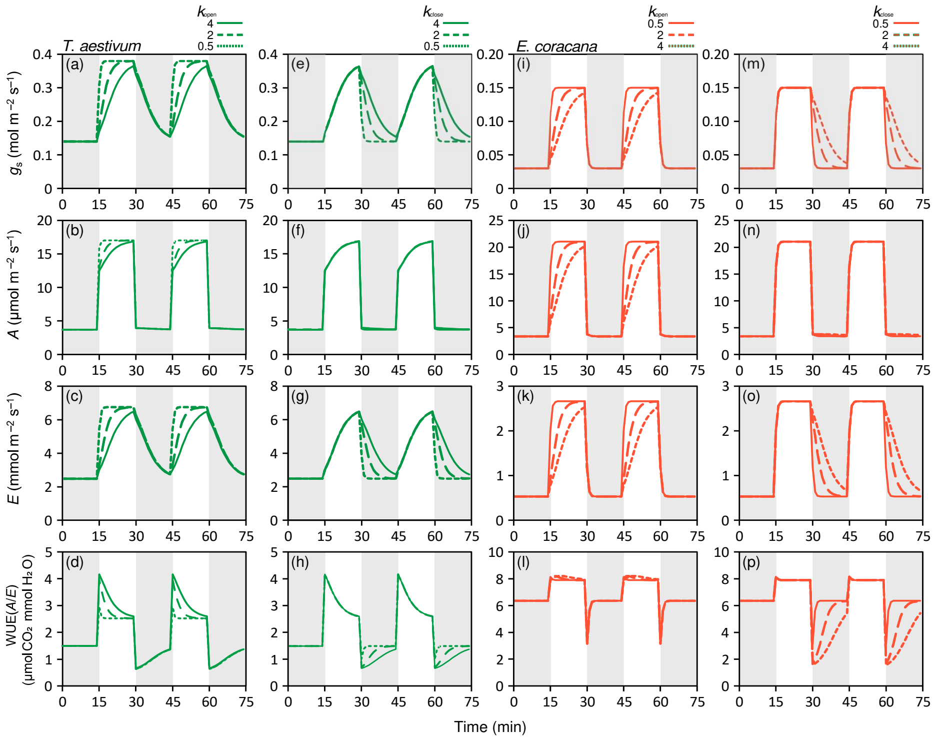
Supplemental Fig. S4. Dynamics of the whole-plant stomatal conductance (g_s) in the controlled fluctuating light environments in five C_4 species. Light intensity was alternately changed between low (80 µmol m^{-2} s^{-1} photosynthetically active photon flux density (PPFD) at 30 cm above the pot surface, grey area) and high (300 µmol m^{-2} s^{-1} PPFD at 30 cm above the pot surface, white area) at 30-min intervals (a, c, e, g, i) and 15-min intervals (b, d, f, h, j) for a total of 12 hours. Red and blue lines represent plants grown under high nitrogen (N) (HN) and low N (LN) conditions, respectively. Data are the mean of four pot plants on the balance.



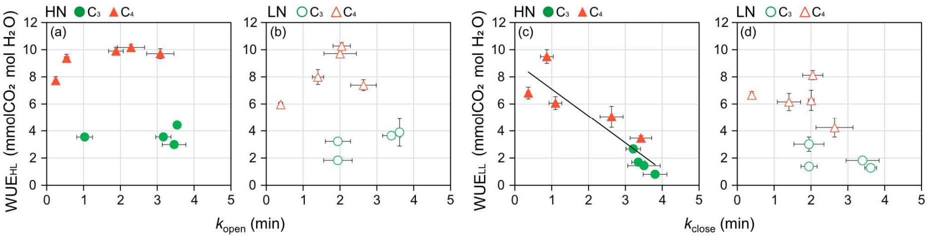
Supplemental Fig. S5. Relationship between whole-plant and leaf-level stomatal characteristics in four C_3 and five C_4 species grown under high nitrogen (N) (HN) or low N (LN) conditions. Green and orange symbols indicate C_3 and C_4 species, respectively, and filled and open symbols indicate plants grown under HN and LN conditions, respectively. (a) Relationships between whole-plant k_{open} and k_{close}, (b) whole-plant k_{open} and leaf-level k_{open}, and (c) whole-plant k_{close} and leaf-level k_{close}. Note that whole-plant k_{open} and k_{close} were obtained when photosynthetically active photon flux density (PPFD) varied between 80 and 300 μmol m^{-2} s^{-1}, whereas leaf-level k_{open} and k_{close} were obtained when PPFD varied between 100 and 1000 μmol m^{-2} s^{-1}. Regression line is shown for all C_3 and C_4 species. Value of R^2 is (a) 0.85 ($P < 0.01$). Data are the mean of twelve measurements during the 12-hour light period ± SE for whole-plant k_{open} and k_{close}. Data are the mean ± SE (n = 4) for leaf-level k_{open} and k_{close}.



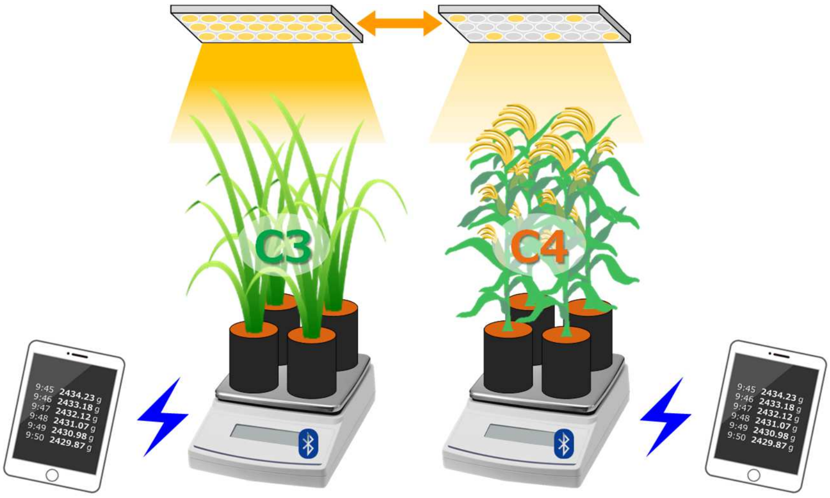
Supplemental Fig. S6. Relationship between the CO_2 assimilation rate (A_c, A_j, V_p) and CO_2 concentration in mesophyll cells (C_m) or bundle sheath cells (C_{bs}) described by the C_3 or C_4 photosynthesis models. In the C_3 photosynthesis model (a, b), the operating point of photosynthesis in mesophyll cells (filled red circles) is given by the intersection of the supply function ($A = g_t (C_a - C_m)$) and the demand function (minimum of either A_c or A_j). (a) g_s is 0.36 at the photosynthetically active photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1}, and (b) g_s is 0.15 at the PPFD of 100 μmol m^{-2} s^{-1}. In the C_4 photosynthesis model (c, d), PEP carboxylation rate (blue circle) is given by the intersection of the supply function ($A = g_t (C_a - C_m)$) and V_p, and the operating point of photosynthesis in the bundle cell (red circles) is given by the intersection of another supply function ($A = V_p - g_{bs} (C_{bs} - C_m)$ and the demand function (minimum of either A_c or A_j). (c) g_s is 0.16 at the PPFD of 1000 μmol m^{-2} s^{-1}, and (d) g_s is 0.02 at the PPFD of 100 μmol m^{-2} s^{-1}. Model parameters were listed in Table 1.



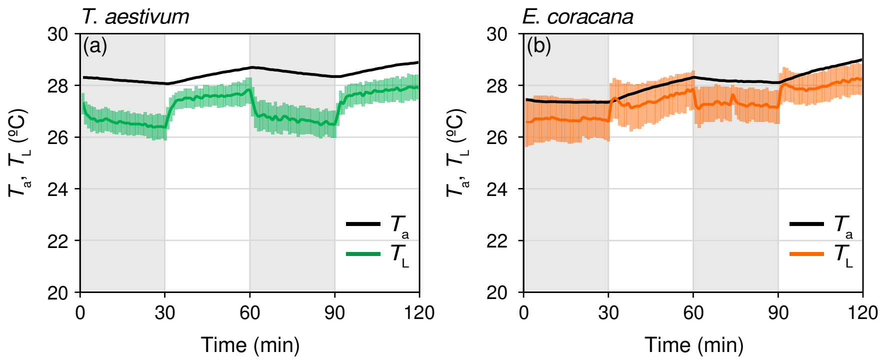
Supplemental Fig. S7. Changes in stomatal conductance (g_s), photosynthetic rate (A), transpiration rate (E), and water use efficiency (WUE, A/E) in the controlled fluctuating light environments simulated by the dynamic photosynthesis model in *T. aestivum* (a-h) and *E. coracana* (i-p) grown under high N conditions. In *T. aestivum*, solid, broken, and dotted lines are simulated results at k_{open} of 4, 2, and 0.5, respectively, at the constant k_{close} of 4 (a-d), and those are simulated results at k_{close} of 4, 2, and 0.5, respectively, at the constant k_{open} of 4 (e-h). In *E. coracana*, solid, broken, and dotted lines are simulated results at k_{open} of 0.5, 2, and 4, respectively, at the constant k_{close} of 0.5 (i-l), and those are simulated results at k_{close} of 0.5, 2, and 4, respectively, at the constant k_{open} of 0.5 (m-p). Other parameters used for the simulation were listed in Table 1. For each combination of k_{open} and k_{close}, A_{total} and E_{total} were calculated.



Supplemental Fig. S8. Relationship between water use efficiency during high (WUE_{HL}) and low light period (WUE_{LL}) and time constants for stomatal opening (k_{open}) and closure (k_{close}) in four C_3 and five C_4 species grown under (a, c) high nitrogen (N) (HN) or (b, d) low N (LN) conditions. Green and orange symbols indicate C_3 and C_4 species, respectively, and filled and open symbols indicate plants grown under HN and LN conditions, respectively. Regression line is shown for all C_3 and C_4 species. Value of R^2 is (c) 0.82 ($P < 0.01$). Data are the mean ± SE (n = 4).



Supplemental Fig. S9. Experimental design for evaluating whole-plant water consumption in the controlled fluctuating light environments. In the temperature-controlled growth room, light intensity was alternately changed between low (80 μmol m^{-2} s^{-1} photosynthetically active photon flux density (PPFD) at 30 cm above the pot surface) and high (300 μmol m^{-2} s^{-1} PPFD at 30 cm above the pot surface) at 30-min intervals on the first day and 15-min intervals on the second day. For each species, four pots were placed on a Bluetooth communication electronic balance with the minimum unit of 0.01 g, which are connected to a recording device with an automatic logging application.



Supplemental Fig. S10. Changes in leaf temperature (T_L) in the fluctuating light environments at 30-minute intervals in (a) *T. aestivum* and (b) *E. coracana*. Plants were grown under the same growth conditions as those used for the photosynthesis measurements. Measurements of T_L and air temperature (T_a) were conducted for 2 hours. Data are the mean ± SD (n = 3).

Supplemental Table S1. Stomatal characteristics determined by the photosynthesis measurements in four C_3 species and five C_4 species.

	Species	N	r_{open} (mol m^{-2} s^{-1})	G_{smax} (mol m^{-2} s^{-1})	k_{open} (min)	Sl_{open} (mmol m^{-2} s^{-2})	r_{close} (mol m^{-2} s^{-1})	G_{smin} (mol m^{-2} s^{-1})	k_{close} (min)	Sl_{close} (–mmol m^{-2} s^{-2})
C_3	*Triticum aestivum*	HN	0.14 ± 0.05	0.38 ± 0.04	3.17 ± 0.21	0.47 ± 0.06	0.38 ± 0.04	0.15 ± 0.05	3.50 ± 0.44	0.46 ± 0.08
		LN	0.06 ± 0.01	0.23 ± 0.02	1.94 ± 0.34	0.61 ± 0.09	0.21 ± 0.02	0.04 ± 0.02	2.08 ± 0.41	0.58 ± 0.07
	Avena sativa	HN	0.33 ± 0.05	0.49 ± 0.03	3.46 ± 0.32	0.32 ± 0.05	0.49 ± 0.03	0.35 ± 0.03	3.81 ± 0.33	0.24 ± 0.03
		LN	0.29 ± 0.03	0.45 ± 0.01	3.62 ± 0.11	0.28 ± 0.04	0.45 ± 0.01	0.33 ± 0.02	4.45 ± 0.16	0.16 ± 0.01
	Hordeum vulgare	HN	0.10 ± 0.01	0.34 ± 0.01	3.54 ± 0.07	0.42 ± 0.02	0.34 ± 0.01	0.11 ± 0.01	3.35 ± 0.18	0.43 ± 0.04
		LN	0.13 ± 0.03	0.34 ± 0.03	3.40 ± 0.24	0.38 ± 0.05	0.34 ± 0.03	0.14 ± 0.03	3.57 ± 0.45	0.41 ± 0.08
	Lolium multiflorum	HN	0.12 ± 0.01	0.29 ± 0.02	1.03 ± 0.21	1.34 ± 0.28	0.29 ± 0.02	0.10 ± 0.01	3.22 ± 0.20	0.37 ± 0.04
		LN	0.23 ± 0.03	0.39 ± 0.02	1.95 ± 0.39	0.73 ± 0.18	0.39 ± 0.02	0.23 ± 0.04	3.68 ± 0.22	0.28 ± 0.06
C_4	*Eleusine coracana*	HN	0.03 ± 0.00	0.16 ± 0.00	0.25 ± 0.03	3.50 ± 0.37	0.12 ± 0.01	0.02 ± 0.00	0.36 ± 0.05	1.90 ± 0.18
		LN	0.03 ± 0.00	0.10 ± 0.01	0.39 ± 0.06	1.47 ± 0.32	0.07 ± 0.01	0.02 ± 0.00	0.38 ± 0.05	0.85 ± 0.14
	Sorghum bicolor	HN	0.03 ± 0.00	0.18 ± 0.01	1.88 ± 0.20	0.49 ± 0.05	0.18 ± 0.01	0.04 ± 0.00	1.10 ± 0.17	0.84 ± 0.08
		LN	0.03 ± 0.00	0.12 ± 0.02	2.01 ± 0.44	0.41 ± 0.16	0.13 ± 0.01	0.03 ± 0.00	0.85 ± 0.08	0.78 ± 0.09
	Panicum miliaceum	HN	0.03 ± 0.00	0.11 ± 0.00	0.54 ± 0.06	1.07 ± 0.13	0.09 ± 0.00	0.02 ± 0.00	0.87 ± 0.17	0.68 ± 0.13
		LN	0.02 ± 0.00	0.07 ± 0.00	2.05 ± 0.24	0.15 ± 0.02	0.07 ± 0.00	0.02 ± 0.00	2.44 ± 0.28	0.13 ± 0.02
	Zea mays	HN	0.04 ± 0.00	0.15 ± 0.01	2.29 ± 0.37	0.37 ± 0.07	0.15 ± 0.01	0.04 ± 0.01	2.63 ± 0.31	0.26 ± 0.03
		LN	0.04 ± 0.00	0.09 ± 0.01	2.64 ± 0.35	0.13 ± 0.02	0.09 ± 0.01	0.05 ± 0.01	3.33 ± 0.50	0.09 ± 0.01
	Zea nicaraguensis	HN	0.04 ± 0.00	0.17 ± 0.01	3.08 ± 0.37	0.30 ± 0.04	0.17 ± 0.01	0.05 ± 0.00	3.42 ± 0.29	0.23 ± 0.03
		LN	0.02 ± 0.00	0.06 ± 0.00	1.40 ± 0.15	0.20 ± 0.04	0.05 ± 0.00	0.02 ± 0.00	2.04 ± 0.32	0.13 ± 0.04
	Two-way ANOVA	C_3, C_4	***	***	***	ns	***	***	***	**
		N	ns	***	ns	**	***	ns	ns	**
		C_3, C_4 × N	ns	ns	ns	ns	ns	ns	ns	*

See the main text for abbreviations. Data are the mean ± SE (n = 4). The effects of photosynthetic types (C_3 or C_4), nitrogen (N) conditions (HN or LN), and their interaction on the characteristics were determined by two-way ANOVA (*** P < 0.001, ** P < 0.01, * P < 0.05, ns P > 0.05).

Supplemental Table S2. Physiological and morphological characteristics of the leaves used for the photosynthesis measurements in four C_3 species and five C_4 species.

	Species	N	LMA ($g\ m^{-2}$)	N_{area} ($g\ N\ m^{-2}$)	A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	*SD* (adaxial) (mm^{-2})	*SD* (abaxial) (mm^{-2})	SD_{mean} (mm^{-2})	*GL* (adaxial) (µm)	*GL* (abaxial) (µm)	GL_{mean} (µm)
C_3	*Triticum aestivum*	HN	24.3 ± 1.4	1.02 ± 0.03	17.6 ± 1.0	54.3 ± 2.8	39.7 ± 1.7	47.0 ± 3.2	54.2 ± 1.2	52.6 ± 1.4	53.4 ± 0.9
		LN	29.4 ± 1.8	0.62 ± 0.09	11.8 ± 1.1	38.8 ± 2.9	45.4 ± 4.4	42.1 ± 3.0	56.9 ± 1.3	60.3 ± 2.0	58.6 ± 1.3
	Avena sativa	HN	22.9 ± 0.5	1.25 ± 0.02	22.3 ± 1.1	51.2 ± 3.2	53.9 ± 1.8	52.5 ± 2.0	52.2 ± 1.4	54.0 ± 1.8	53.1 ± 1.2
		LN	19.0 ± 0.6	0.57 ± 0.03	18.9 ± 0.5	53.1 ± 1.6	62.7 ± 2.7	57.9 ± 2.5	51.4 ± 1.0	48.5 ± 0.8	50.0 ± 0.8
	Hordeum vulgare	HN	30.1 ± 0.5	1.68 ± 0.07	18.7 ± 0.4	45.7 ± 1.8	44.9 ± 1.9	45.3 ± 1.4	50.2 ± 1.0	53.2 ± 1.3	51.7 ± 1.0
		LN	23.8 ± 0.9	0.36 ± 0.03	14.5 ± 1.1	78.1 ± 3.5	76.1 ± 8.2	77.1 ± 4.8	39.7 ± 0.9	41.3 ± 1.1	40.5 ± 0.8
	Lolium multiflorum	HN	30.1 ± 1.0	1.10 ± 0.05	15.0 ± 0.6	96.9 ± 8.0	44.7 ± 2.6	70.8 ± 10.	39.3 ± 0.1	43.5 ± 1.0	41.4 ± 0.8
		LN	26.8 ± 0.8	0.57 ± 0.05	10.6 ± 0.7	63.2 ± 7.5	58.1 ± 9.7	60.7 ± 6.6	40.1 ± 2.4	39.0 ± 2.9	39.5 ± 1.9
C_4	*Eleusine coracana*	HN	28.3 ± 2.1	0.98 ± 0.05	21.0 ± 1.0	114. ± 6.9	88.0 ± 3.9	101. ± 6.5	33.5 ± 2.2	33.8 ± 1.0	33.7 ± 1.2
		LN	27.3 ± 1.0	0.45 ± 0.04	10.6 ± 0.8	95.7 ± 9.7	101. ± 4.9	98.7 ± 5.9	33.1 ± 1.9	30.8 ± 1.0	31.9 ± 1.1
	Sorghum bicolor	HN	29.3 ± 1.0	0.94 ± 0.05	24.2 ± 0.6	98.3 ± 4.2	114. ± 4.6	106. ± 4.4	29.7 ± 0.6	34.3 ± 0.4	32.0 ± 0.9
		LN	29.0 ± 0.7	0.78 ± 0.05	19.7 ± 0.9	96.0 ± 5.1	107. ± 10.	101. ± 6.5	29.3 ± 0.6	27.2 ± 1.5	28.2 ± 0.9
	Panicum miliaceum	HN	28.2 ± 0.9	0.75 ± 0.07	15.5 ± 0.5	99.4 ± 3.7	83.5 ± 2.1	91.5 ± 3.7	27.7 ± 0.6	30.5 ± 1.2	29.1 ± 0.8
		LN	29.0 ± 1.5	0.31 ± 0.02	11.2 ± 0.6	64.4 ± 4.8	86.3 ± 11.	75.4 ± 7.7	26.6 ± 1.0	26.4 ± 0.7	26.5 ± 0.6
	Zea mays	HN	28.9 ± 0.8	0.91 ± 0.08	20.6 ± 1.2	65.5 ± 2.1	81.5 ± 2.3	73.5 ± 3.4	45.5 ± 0.5	45.7 ± 1.6	45.6 ± 0.8
		LN	26.9 ± 0.4	0.52 ± 0.04	10.1 ± 1.2	116. ± 3.2	85.4 ± 10.	100. ± 8.4	44.0 ± 1.0	43.7 ± 1.4	43.9 ± 0.9
	Zea nicaraguensis	HN	30.6 ± 0.8	1.06 ± 0.03	22.2 ± 0.5	42.1 ± 1.9	70.9 ± 1.6	56.5 ± 5.6	50.7 ± 1.1	47.3 ± 0.5	49.0 ± 0.8
		LN	27.8 ± 1.6	0.28 ± 0.02	6.9 ± 0.5	54.7 ± 5.6	67.4 ± 6.4	61.0 ± 5.1	38.5 ± 1.8	41.2 ± 1.4	39.8 ± 1.2
		C_3, C_4	***	***	ns	***	***	***	***	***	***
	Two-way ANOVA	N	ns	***	***	ns	ns	ns	ns	*	ns
		C_3, C_4 × N	ns	**	*	ns	ns	ns	ns	ns	ns

See the main text for abbreviations. Data are the mean ± SE (n = 4). The effects of photosynthetic types (C_3 or C_4), nitrogen (N) conditions (HN or LN), and their interaction on the characteristics were determined by two-way ANOVA (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns $P > 0.05$).

Supplemental Table S3. Whole-plant water use characteristics determined by the continuous measurements of pot weight in the controlled fluctuating environment in four C_3 species and five C_4 species.

	Species	N	r_{open} (mol m^{-2} s^{-1})	G_{smax} (mol m^{-2} s^{-1})	k_{open} (min)	Sl_{open} (mmol m^{-2} s^{-2})	r_{close} (mol m^{-2} s^{-1})	G_{smin} (mol m^{-2} s^{-1})	k_{close} (min)	Sl_{close} (–mmol m^{-2} s^{-2})
C_3	*Triticum aestivum*	HN	0.046 ± 0.007	0.103 ± 0.007	3.14 ± 1.16	0.125 ± 0.043	0.104 ± 0.008	0.043 ± 0.010	5.04 ± 1.23	0.079 ± 0.030
		LN	0.043 ± 0.008	0.080 ± 0.004	1.43 ± 0.23	0.159 ± 0.040	0.079 ± 0.006	0.033 ± 0.008	3.99 ± 1.48	0.081 ± 0.037
	Avena sativa	HN	–	–	–	–	–	–	–	–
		LN	–	–	–	–	–	–	–	–
	Hordeum vulgare	HN	0.037 ± 0.005	0.069 ± 0.003	1.42 ± 0.37	0.140 ± 0.022	0.065 ± 0.004	0.032 ± 0.003	3.04 ± 0.41	0.067 ± 0.009
		LN	0.052 ± 0.018	0.089 ± 0.015	2.06 ± 0.76	0.107 ± 0.037	0.079 ± 0.026	0.043 ± 0.023	2.89 ± 1.47	0.096 ± 0.043
	Lolium multiflorum	HN	0.043 ± 0.003	0.067 ± 0.003	0.83 ± 0.25	0.182 ± 0.025	0.053 ± 0.003	0.026 ± 0.001	1.56 ± 0.34	0.112 ± 0.026
		LN	0.055 ± 0.003	0.087 ± 0.004	1.73 ± 0.74	0.133 ± 0.056	0.083 ± 0.004	0.048 ± 0.005	3.18 ± 0.69	0.071 ± 0.018
C_4	*Eleusine coracana*	HN	0.013 ± 0.001	0.059 ± 0.004	0.59 ± 0.12	0.488 ± 0.111	0.038 ± 0.001	0.013 ± 0.000	0.59 ± 0.08	0.266 ± 0.035
		LN	0.012 ± 0.001	0.039 ± 0.003	0.88 ± 0.05	0.185 ± 0.027	0.026 ± 0.002	0.012 ± 0.000	0.74 ± 0.22	0.129 ± 0.048
	Sorghum bicolor	HN	0.023 ± 0.001	0.048 ± 0.002	0.47 ± 0.19	0.355 ± 0.073	0.039 ± 0.002	0.019 ± 0.001	0.52 ± 0.08	0.243 ± 0.035
		LN	0.021 ± 0.001	0.048 ± 0.001	0.33 ± 0.08	0.527 ± 0.107	0.041 ± 0.002	0.018 ± 0.001	0.38 ± 0.07	0.365 ± 0.064
	Panicum miliaceum	HN	0.011 ± 0.000	0.037 ± 0.001	0.47 ± 0.14	0.352 ± 0.093	0.022 ± 0.001	0.009 ± 0.000	0.62 ± 0.23	0.150 ± 0.104
		LN	0.013 ± 0.000	0.039 ± 0.002	0.63 ± 0.18	0.267 ± 0.073	0.033 ± 0.001	0.010 ± 0.000	0.61 ± 0.11	0.230 ± 0.044
	Zea mays	HN	0.018 ± 0.002	0.037 ± 0.003	0.38 ± 0.17	0.353 ± 0.155	0.032 ± 0.002	0.016 ± 0.002	0.36 ± 0.06	0.269 ± 0.072
		LN	0.017 ± 0.004	0.028 ± 0.003	0.69 ± 0.14	0.093 ± 0.014	0.027 ± 0.003	0.019 ± 0.004	0.44 ± 0.11	0.119 ± 0.025
	Zea nicaraguensis	HN	0.018 ± 0.002	0.051 ± 0.001	0.75 ± 0.12	0.278 ± 0.057	0.037 ± 0.001	0.016 ± 0.000	0.56 ± 0.11	0.234 ± 0.042
		LN	–	–	–	–	–	–	–	–
	Two-way ANOVA	C_3, C_4	***	***	***	***	***	***	***	***
		N	**	ns	ns	***	ns	*	ns	ns
		C_3, C_4 × N	***	ns	ns	**	*	**	ns	ns

See the main text for abbreviations. Data are the mean of twelve measurements during the 12-hour light period ± SE. The effects of photosynthetic types (C_3 or C_4), nitrogen (N) conditions (HN or LN), and their interaction on the characteristics were determined by two-way ANOVA (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns $P > 0.05$). The results obtained in the fluctuating light environments at 30-minute intervals are shown. Data for *A. sativa* grown under HN and *Z. nicaraguensis* grown under HN and LN were not shown since we could not fit the temporal responses of g_s to the equations due to small differences G_{smax} and G_{smin} (Figs. S4c, S5i).