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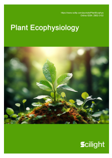

Supplementary Materials

Experimental Evidence for Coordinated Leaf Trait Responses to Elevated CO_2 in Five Common Crop Species

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Table S1. Statistical output per leaf trait, including the used statistical test, factor, degrees of freedom (dF), F-value, and P-value. Asterisk at P-value indicates level of significance with * = p < 0.05, ** = p < 0.01, and *** = p < 0.001.

Trait	Test	Factor	dF	F-Value	*p*-Value
A_{sat}(g400-m400) – A_{sat}(g1000-m1000)	ANOVA type III	Species	4	5.8464	0.000449 ***
		Treatment	1	42.1349	1.441e-08 ***
		Species × treatment	4	4.2068	0.004368 **
A_{sat} (g400-m400) – A_{sat} (g1000-m400)	ANOVA type III	Species	4	10.0218	4.136e-06 ***
		Treatment	1	69.0733	1.701e-10 ***
		Species × treatment	3	3.2408	0.03047 *
V_{cmax25}	ANOVA type II	Species	3	40.5367	3.59e-11 ***
		Treatment	1	16.3359	0.0002988 ***
		Species × treatment	3	2.5269	0.0743494
J_{1000}	ANOVA type II	Species	3	22.270	4.558e-08 ***
		Treatment	1	2.116	0.1552
		Species × treatment	3	0.752	0.5291
J_{1000}/V_{cmax25} ratio	ANOVA type II	Species	3	2.9803	0.045440 *
		Treatment	1	9.2364	0.004617 **
		Species × treatment	3	1.0900	0.367006
N_{area}	ANOVA type III	Species	4	185.695	< 2.2e-16 ***
		Treatment	1	43.012	8.32e-09 ***
		Species × treatment	4	2.539	0.04755 *
$g_{s(op)}$	ANOVA type II	Species	4	50.8830	< 2e-16 ***
		Treatment	1	4.8323	0.02578 *
		Species × treatment	4	1.6142	0.18052
$g_{s(avg)}$	ANOVA type III	Species	3	26.2244	1.649e-07 ***
		Treatment	1	5.0052	0.21946
		Species × treatment	3	6.7253	0.01984 *
Stomatal ratio	ANOVA type III	Species	4	1127.9030	<2.2e-16 ***
		Treatment	1	0.2491	0.619080
		Species × treatment	4	3.6186	0.009298 **
$g_{s(op)}$:g_{smax}	ANOVA type II	Species	4	121.3956	<2e-16 ***
		Treatment	1	1.2479	0.2679
		Species × treatment	4	1.0958	0.3657
$g_{s(avg)}$:g_{smax}	ANOVA type II	Species	3	52.9336	2.04e-09 ***
		Treatment	1	2.8335	0.1087
		Species × treatment	3	2.0378	0.1427
LMA	ANOVA type III	Species	4	169.0324	<2.2e-16 ***

		Treatment	1	7.8739	0.00649 **
		Species × treatment	4	7.8028	2.931e-05 ***
C:N ratio	ANOVA type III	Species	4	177.8860	<2.2e-16 ***
		Treatment	1	17.0856	0.0001059 ***
		Species × treatment	4	6.8924	0.0001118 ***
V_{pmax}	Welch T-test	Treatment	1		0.3808
A_{max}	Student's T-test	Treatment	1		0.8718
g_{smax}	Kruskal-Wallis test	Species			1.164e-12 ***
		Treatment			0.8626
Leaf size	Kruskal-Wallis test	Species			7.287e-14 ***
		Treatment			0.37

Leaf stable carbon isotopes

Leaves were finely ground using a Retsch MM400 ball-mill grinder (Verder Scientific, Inc., Newtown, PA, USA) and prepared for stable carbon isotope- and carbon and nitrogen content analysis, as a combined measurement. From the ground material 1 mg was weighed and the carbon isotope composition was measured using a Thermo Scientific™ Isotope Ratio Mass Spectrometer. Leaf carbon and nitrogen content (g g^{-1}) was measured using a Thermo Scientific™ Flash IRMS™ Elemental Analyser (Waltham, MA, USA). Leaf nitrogen per unit leaf area (Narea, gN m^{-2}) was calculated by multiplying leaf nitrogen content (N_{mass}; gN g^{-1}) with LMA (g m^{-2}). C:N was calculated as a mass-based trait. The carbon isotope composition of leaf tissue ($\delta^{13}C_{leaf}$) was calculated as:

$$\delta^{13}\mathrm{C}_{\mathrm{leaf}}\ (‰) = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) 1000 \quad \text{(S1)}$$

where, Rsample and Rstandard are the $^{13}C/^{12}C$ ratios of the leaf tissue and the V-PDB standard, respectively. δ13Cleaf was then converted to leaf carbon isotope discrimination (Δleaf) according to Farquhar and Richards (1984):

$$\Delta_{\mathrm{leaf}}\ (‰) = \frac{\delta^{13}\mathrm{C}_{\mathrm{air}} - \delta^{13}\mathrm{C}_{\mathrm{leaf}}}{1 + \delta^{13}\mathrm{C}_{\mathrm{leaf}}} \quad \text{(S2)}$$

δ^{13}Cair was taken as the average isotopic composition of air samples that were collected from the ambient air of each growth room using exetainers (Labco Limited, United Kingdom) (n = 5 for each room): -12.59‰ (s.d. = 2.3‰) and -25.14‰ (s.d. = 0.6‰), for the ambient and elevated growth rooms, respectively. The 13C to 12C ratios of the air samples were measured using a Thermo Scientific GasBench II coupled to a Thermo Finnigan Delta Plus XL isotope-ratio mass spectrometer. The purpose of using Δleaf rather than $\delta^{13}C_{leaf}$ is that it incorporates the isotopic composition of both the source (the atmosphere) and the product (leaf biomass) (Farquhar & Richards, 1984). We calculated the isotope derived χ ($\chi_{(iso)}$) for the C_3 species based on (Farquhar, O'Leary, & Berry, 1982):

$$\chi_{(\mathrm{iso})} = \frac{\Delta_{\mathrm{leaf}} - a}{b - a} \quad \text{(S3)}$$

where a is the discrimination associated with the diffusion of [CO_2] in air (4.4 ‰) and b is the discrimination due to carboxylation (27 ‰). Unfortunately, values of $\chi_{(iso)}$ were biologically unrealistically high (Figure S1B), and calculating $\chi_{(iso)}$ for the C_4 species maize using the method from Feng (1999) resulted in negative, biologically impossible values for the HC room. These issues were probably (partially) caused by the extremely low carbon air composition values of the HC room. We therefore decided to omit these results from further analyses.

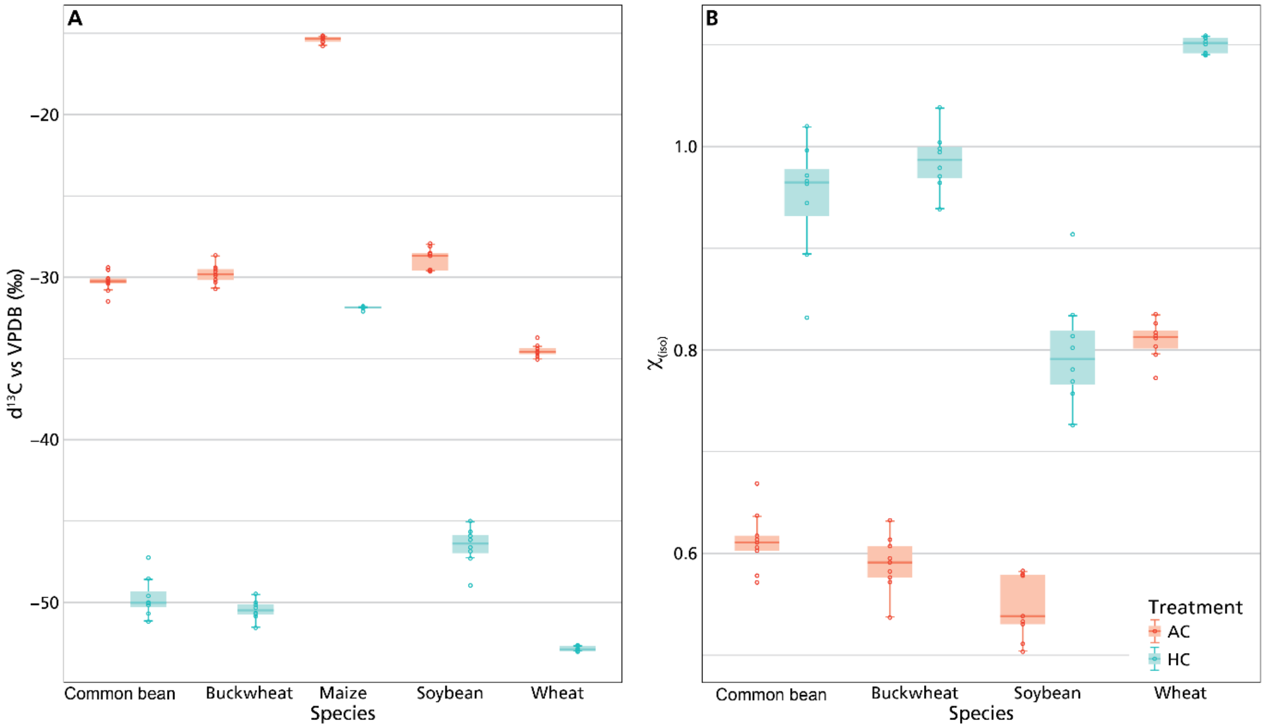


Figure S1. Boxplots of (**A**) $\delta^{13}C_{leaf}$ stable carbon isotope values, and (**B**) $\chi_{(iso)}$ values, per species and treatment. Boxes indicate median, first quartile, and third quartile of the observed data. Point represent individual datapoints. Whiskers are the furthest data point, no further than 1.5 times the inner quartile range.

Simulated c_i:c_a values using $g_{s(op)}$ or $g_{s(avg)}$

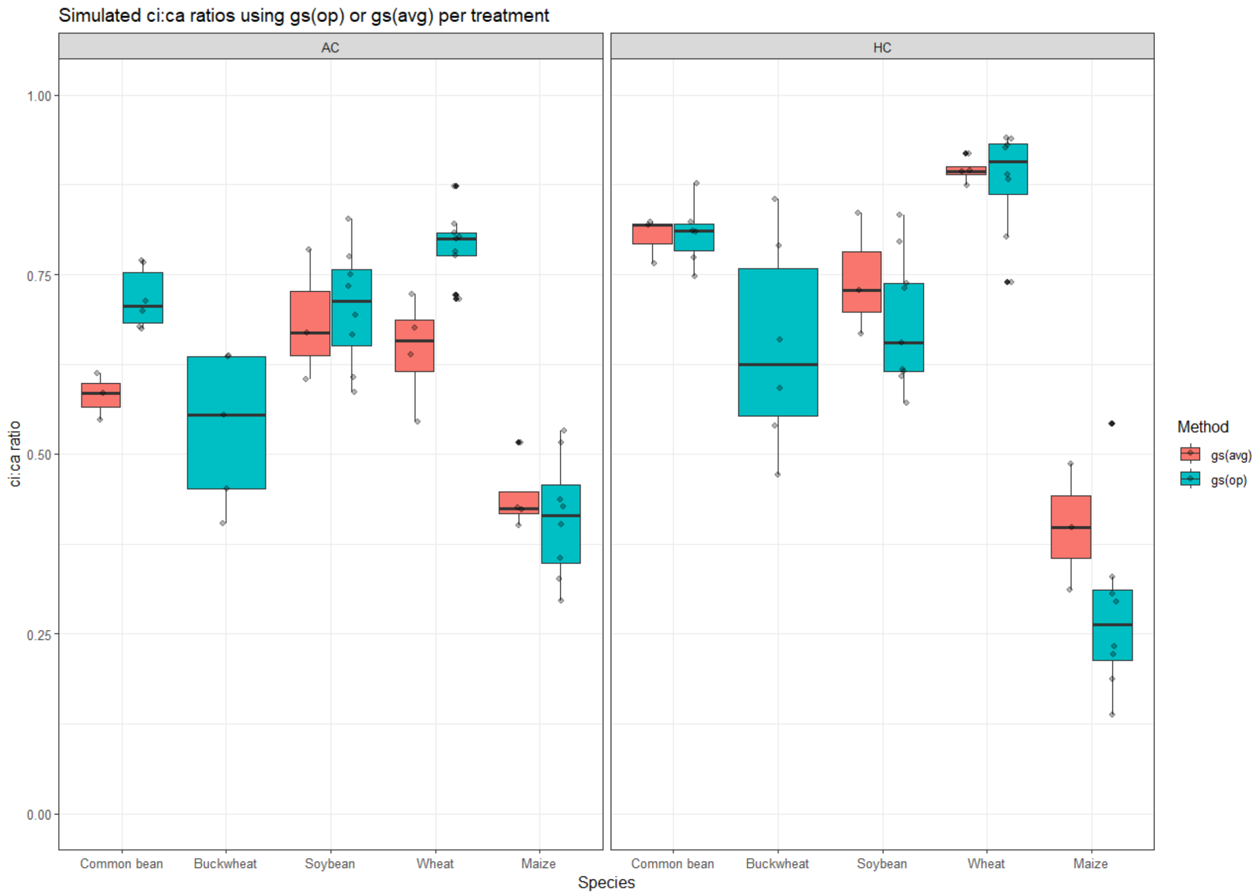
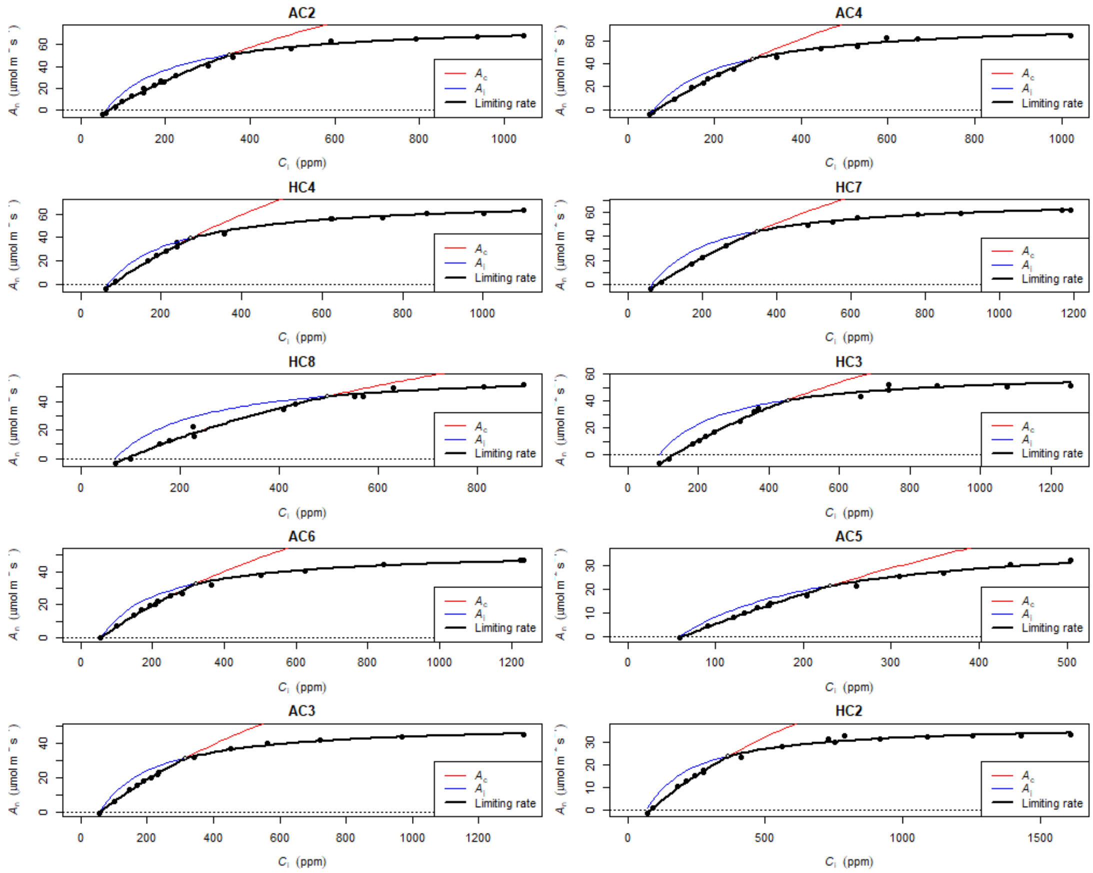


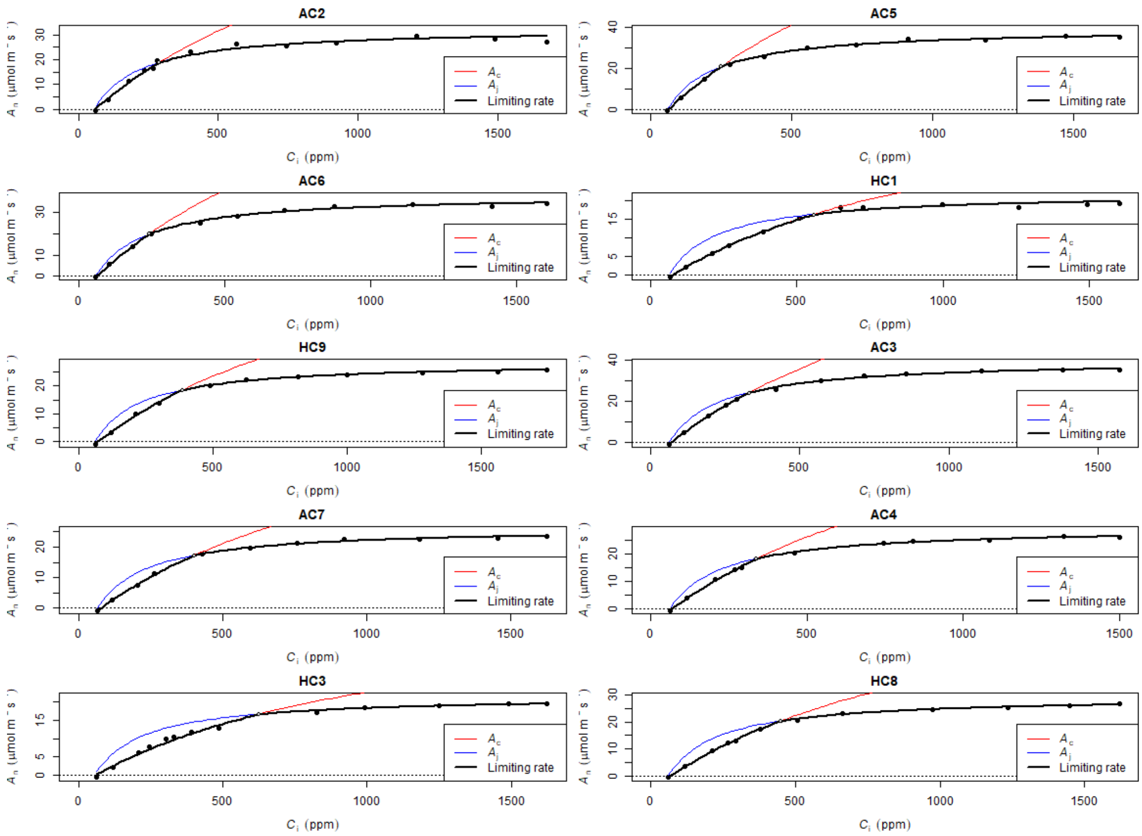
Figure S2. Boxplots of simulated ci:ca values using either $g_{s(op)}$ or $g_{s(avg)}$ values, per treatment. Boxes indicate median, first quartile, and third quartile of the observed data. Point represent individual datapoints. Whiskers are the furthest data point, no further than 1.5 times the inner quartile range.

A-C_i fitting plots

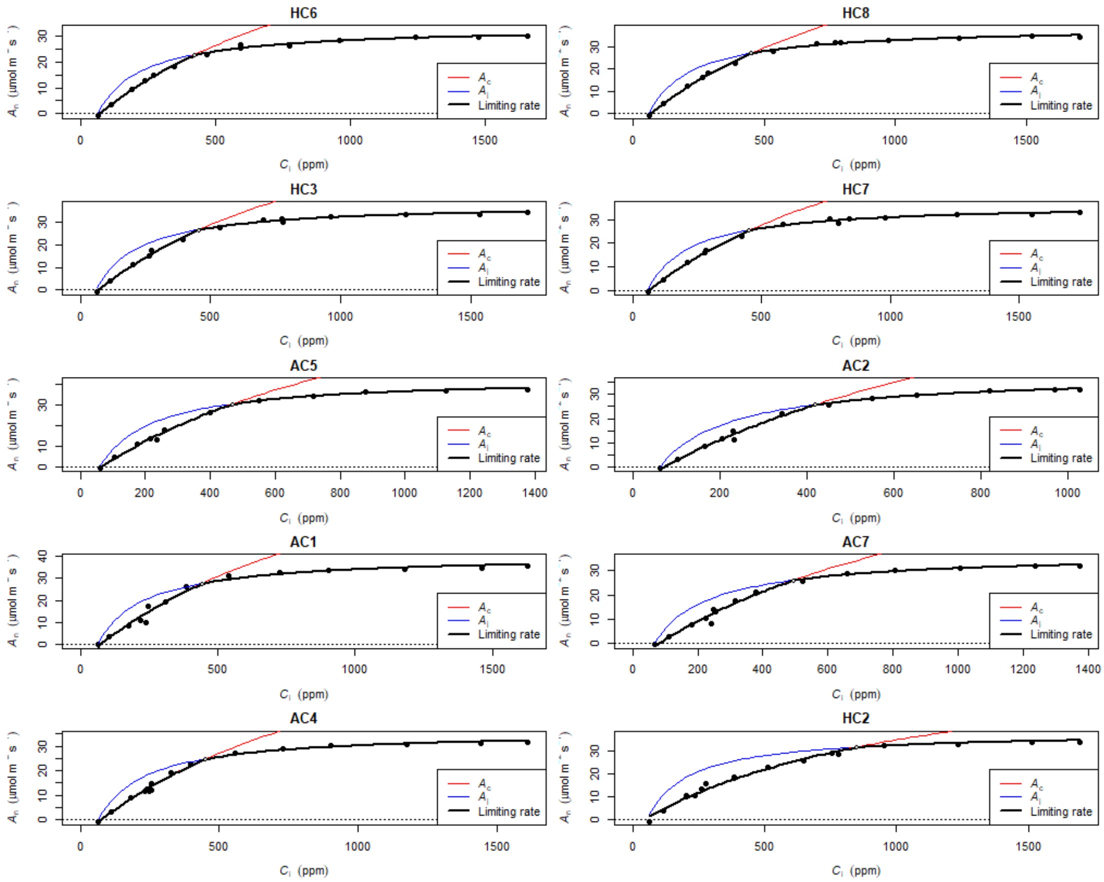
Buckwheat



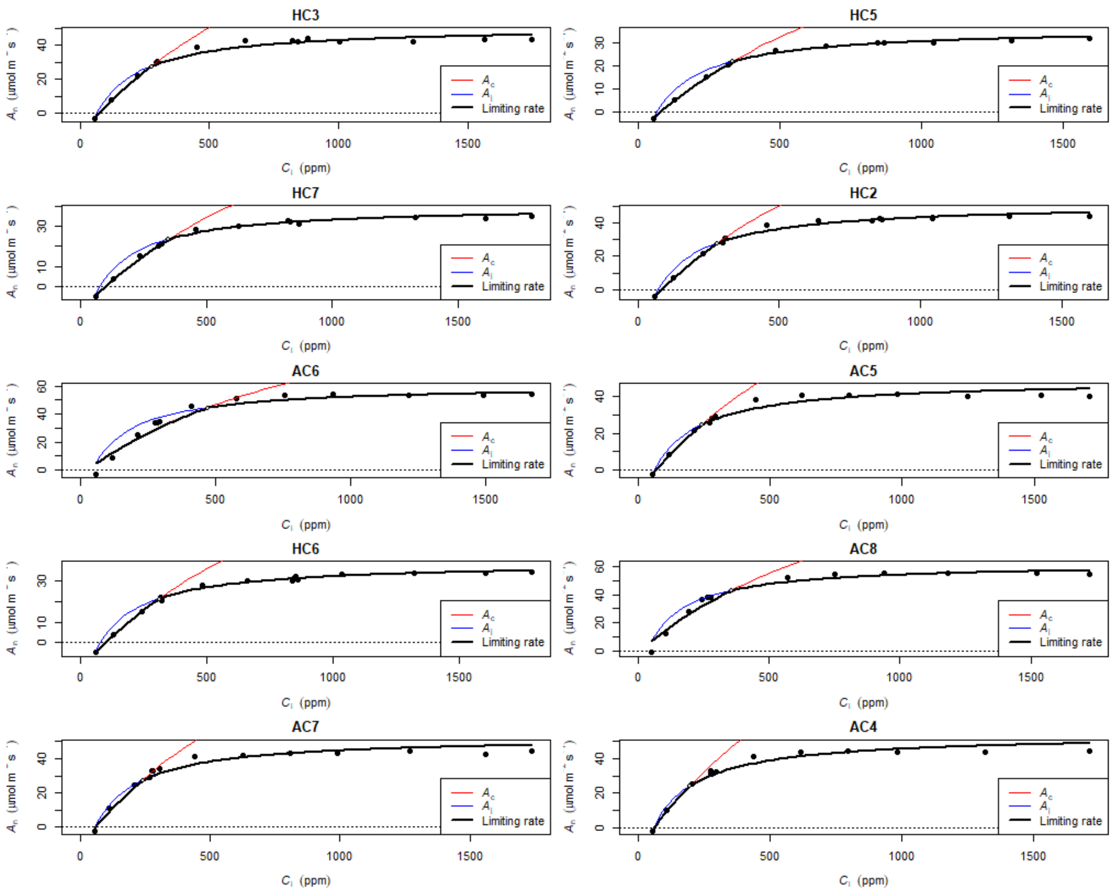
Common bean



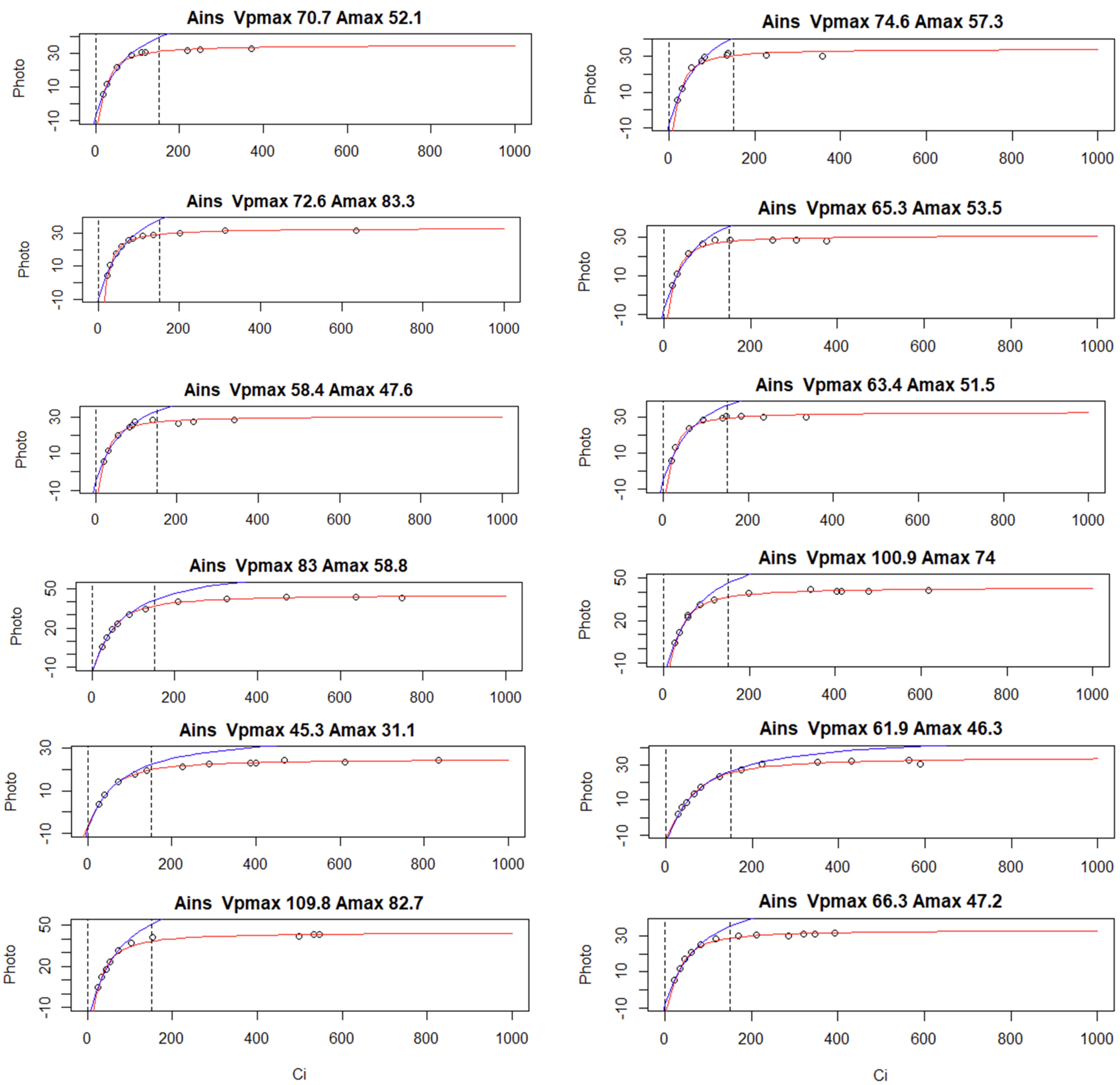
Soybean



Wheat



Maize



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