Amino acid-mediated impacts of elevated carbon dioxide and simulated root herbivory on aphids are neutralised by increased air temperatures

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Supplementary material

Table S1: Single-factor treatment effects of CO_2, temperature and herbivore damage on individual amino acids ($\mu mol\ g^{-1}$ dry mass). Mean values ($\pm$ standard errors) shown. EC = early-cut no aphids, C = control (no cut) no aphids, LC = late-cut no aphids, ECA = early-cut and aphids, A = no cut and aphids, LCA = late-cut and aphids.

Amino acid	CO_2		Temp (°C)		Treatment					
	a	e	26	30	EC	C	LC	ECA	A	LCA
Alanine (Ala)	2.276 ± 0.078	3.077 ± 0.137	2.548 ± 0.110	2.774 ± 0.115	2.730 ± 0.194	2.830 ± 0.238	2.220 ± 0.138	2.808 ± 0.237	3.022 ± 0.179	2.277 ± 0.141
Arginine (Arg)	1.140 ± 0.153	2.012 ± 0.390	2.182 ± 0.360	0.816 ± 0.088	2.175 ± 0.450	1.602 ± 0.804	1.252 ± 0.315	1.424 ± 0.427	1.019 ± 0.170	1.817 ± 0.560
Aspartic acid (Asp)	4.633 ± 0.244	6.433 ± 0.667	6.992 ± 0.587	3.720 ± 0.206	5.239 ± 0.488	5.969 ± 1.326	3.772 ± 0.393	5.757 ± 0.528	6.051 ± 0.541	5.982 ± 1.212
Cysteine (Cys)	0.813 ± 0.031	0.809 ± 0.030	0.817 ± 0.030	0.805 ± 0.030	0.828 ± 0.041	0.768 ± 0.041	0.771 ± 0.051	0.875 ± 0.048	0.846 ± 0.070	0.762 ± 0.055
Glutamic acid (Glu)	1.262 ± 0.039	1.493 ± 0.058	1.485 ± 0.053	1.238 ± 0.041	1.390 ± 0.096	1.252 ± 0.077	1.116 ± 0.063	1.355 ± 0.081	1.619 ± 0.093	1.467 ± 0.081
Glycine (Gly)	0.372 ± 0.014	0.451 ± 0.022	0.441 ± 0.019	0.372 ± 0.016	0.422 ± 0.033	0.400 ± 0.031	0.365 ± 0.039	0.423 ± 0.034	0.444 ± 0.025	0.398 ± 0.027
Histidine (His)	0.194 ± 0.017	0.316 ± 0.034	0.337 ± 0.031	0.152 ± 0.013	0.241 ± 0.044	0.159 ± 0.024	0.271 ± 0.041	0.281 ± 0.059	0.174 ± 0.032	0.383 ± 0.059
Isoleucine (Iso)	0.963 ± 0.036	1.117 ± 0.073	1.033 ± 0.060	1.037 ± 0.049	1.006 ± 0.089	0.929 ± 0.072	0.937 ± 0.095	1.048 ± 0.130	0.988 ± 0.061	1.297 ± 0.112
Leucine (Leu)	1.326 ± 0.065	1.645 ± 0.091	1.528 ± 0.083	1.415 ± 0.072	1.554 ± 0.147	1.507 ± 0.140	1.351 ± 0.121	1.461 ± 0.149	1.498 ± 0.121	1.477 ± 0.140
Lysine (Lys)	0.986 ± 0.083	1.523 ± 0.136	1.274 ± 0.111	1.197 ± 0.112	1.203 ± 0.148	1.147 ± 0.244	1.102 ± 0.159	0.969 ± 0.103	1.094 ± 0.153	1.901 ± 0.271
Methionine (Met)	0.350 ± 0.027	0.395 ± 0.026	0.410 ± 0.024	0.325 ± 0.030	0.423 ± 0.053	0.238 ± 0.027	0.354 ± 0.040	0.350 ± 0.044	0.416 ± 0.063	0.439 ± 0.038
Phenylalanine (Phe)	0.695 ± 0.047	0.977 ± 0.076	0.822 ± 0.059	0.834 ± 0.067	0.809 ± 0.098	0.753 ± 0.068	0.784 ± 0.100	0.787 ± 0.122	0.717 ± 0.071	1.111 ± 0.160
Proline (Pro)	2.001 ± 0.229	3.341 ± 0.405	2.742 ± 0.316	2.501 ± 0.332	2.773 ± 0.644	1.581 ± 0.215	2.012 ± 0.409	3.490 ± 0.771	2.397 ± 0.364	3.493 ± 0.696
Serine (Ser)	1.466 ± 0.057	2.040 ± 0.143	1.718 ± 0.119	1.755 ± 0.087	1.889 ± 0.249	1.498 ± 0.129	1.528 ± 0.185	1.963 ± 0.225	1.561 ± 0.103	1.964 ± 0.169
Threonine (Thr)	0.745 ± 0.032	0.926 ± 0.054	0.840 ± 0.043	0.818 ± 0.044	0.864 ± 0.080	0.767 ± 0.067	0.714 ± 0.071	0.903 ± 0.092	0.824 ± 0.053	0.900 ± 0.084
Tyrosine (Tyr)	0.578 ± 0.033	0.632 ± 0.042	0.547 ± 0.032	0.668 ± 0.043	0.543 ± 0.050	0.568 ± 0.053	0.615 ± 0.072	0.540 ± 0.061	0.600 ± 0.055	0.753 ± 0.087
Valine (Val)	0.998 ± 0.045	1.364 ± 0.100	1.225 ± 0.085	1.106 ± 0.061	1.194 ± 0.139	1.052 ± 0.106	1.023 ± 0.134	1.233 ± 0.164	1.148 ± 0.078	1.360 ± 0.150

Table S2: Results from multivariate permutational analysis (PERMANOVA) of temperature, CO_2 and plant-damage (p-d) treatment effects on different groups of amino acids. For the purpose of PERMANOVA, ungrouped individuals (Glu, Met, Cys and Tyr) were grouped with the highest correlated individual amino acid. * indicates significance ($P<0.01$) adjusted for absence of mixed effects.

	df	SS	MS	Pseudo-F	P(perm)
Group 1 (Ala, Gly, Iso, Leu, Lys, Phe, Pro, Ser, Thr, Val)					
Temp	1	0.171	0.171	2.793	0.055
CO_2	1	0.801	0.801	13.050	0.001*
P-d treatment	5	0.499	0.099	1.627	0.072
Temp $\times$ CO_2	1	0.301	0.301	4.911	0.011
Temp $\times$ P-d treatment	5	0.309	0.062	1.006	0.414
CO_2 $\times$ P-d treatment	5	0.184	0.037	0.601	0.867
Temp $\times$ CO_2 $\times$ P-d treatment	4	0.096	0.024	0.390	0.969
Residuals	255	15.647	0.061		
Total	277	18.008			
Group 2 (Arg, Asp, His, Lys)					
Temp	1	2.657	2.656	31.501	0.001*
CO_2	1	0.290	0.290	3.442	0.025
P-d treatment	5	1.429	0.286	3.390	0.001*
Temp $\times$ CO_2	1	0.256	0.256	3.039	0.030
Temp $\times$ P-d treatment	5	0.580	0.116	1.374	0.145
CO_2 $\times$ P-d treatment	5	0.401	0.080	0.950	0.450
Temp $\times$ CO_2 $\times$ P-d treatment	4	0.167	0.042	0.495	0.908
Residuals	255	21.504	0.084		
Total	277	27.283			
Group 3 (Glu, Lys)					
Temp	1	0.721	0.720	17.234	0.001*
CO_2	1	0.435	0.435	10.404	0.001*
P-d treatment	5	0.612	0.122	2.930	0.004*
Temp $\times$ CO_2	1	0.258	0.258	6.167	0.004*
Temp $\times$ P-d treatment	5	0.171	0.034	0.816	0.598
CO_2 $\times$ P-d treatment	5	0.315	0.063	1.505	0.130
Temp $\times$ CO_2 $\times$ P-d treatment	4	0.048	0.012	0.288	0.975
Residuals	255	10.661	0.042		
Total	277	13.220			
Group 4 (Met, Cys)					
Temp	1	0.639	0.639	12.143	0.001*
CO_2	1	0.103	0.103	1.953	0.124
P-d treatment	5	0.283	0.057	1.077	0.403
Temp $\times$ CO_2	1	0.538	0.538	10.226	0.001*
Temp $\times$ P-d treatment	5	0.161	0.032	0.612	0.810
CO_2 $\times$ P-d treatment	5	0.233	0.047	0.885	0.559
Temp $\times$ CO_2 $\times$ P-d treatment	4	0.187	0.047	0.889	0.554
Residuals	255	13.412	0.053		
Total	277	15.555			
Group 5 (Tyr, Lys)					
Temp	1	0.635	0.635	9.264	0.001*
CO_2	1	0.497	0.497	7.248	0.002*
P-d treatment	5	0.433	0.087	1.264	0.229
Temp $\times$ CO_2	1	0.388	0.388	5.666	0.006*
Temp $\times$ P-d treatment	5	0.426	0.085	1.242	0.216
CO_2 $\times$ P-d treatment	5	0.201	0.040	0.588	0.878
Temp $\times$ CO_2 $\times$ P-d treatment	4	0.067	0.017	0.246	0.997
Residuals	255	17.471	0.069		
Total	277	20.118			

Supplementary material

Figure legends

Figure S1: Principal component analysis of foliar amino acid data with attribute loadings on the first two components PC 1 and PC 2. Amino acid abbreviations are denoted in Table 1. Sample scores on PC 1 and PC 2 explain, respectively, 50% and 12% of the variation in the dataset. Plots were coloured according to chamber (i.e. temperature and CO_2) conditions (A) and plant-damage treatment (B).

Figure S2: Temperature, CO_2 and plant-damage treatment effects on average amino acid concentrations (mean ± standard error) of group 1 (A and B) and group 2 (C and D) amino acids. Significant treatment factors according to PERMANOVA indicated * ($P < 0.01$).
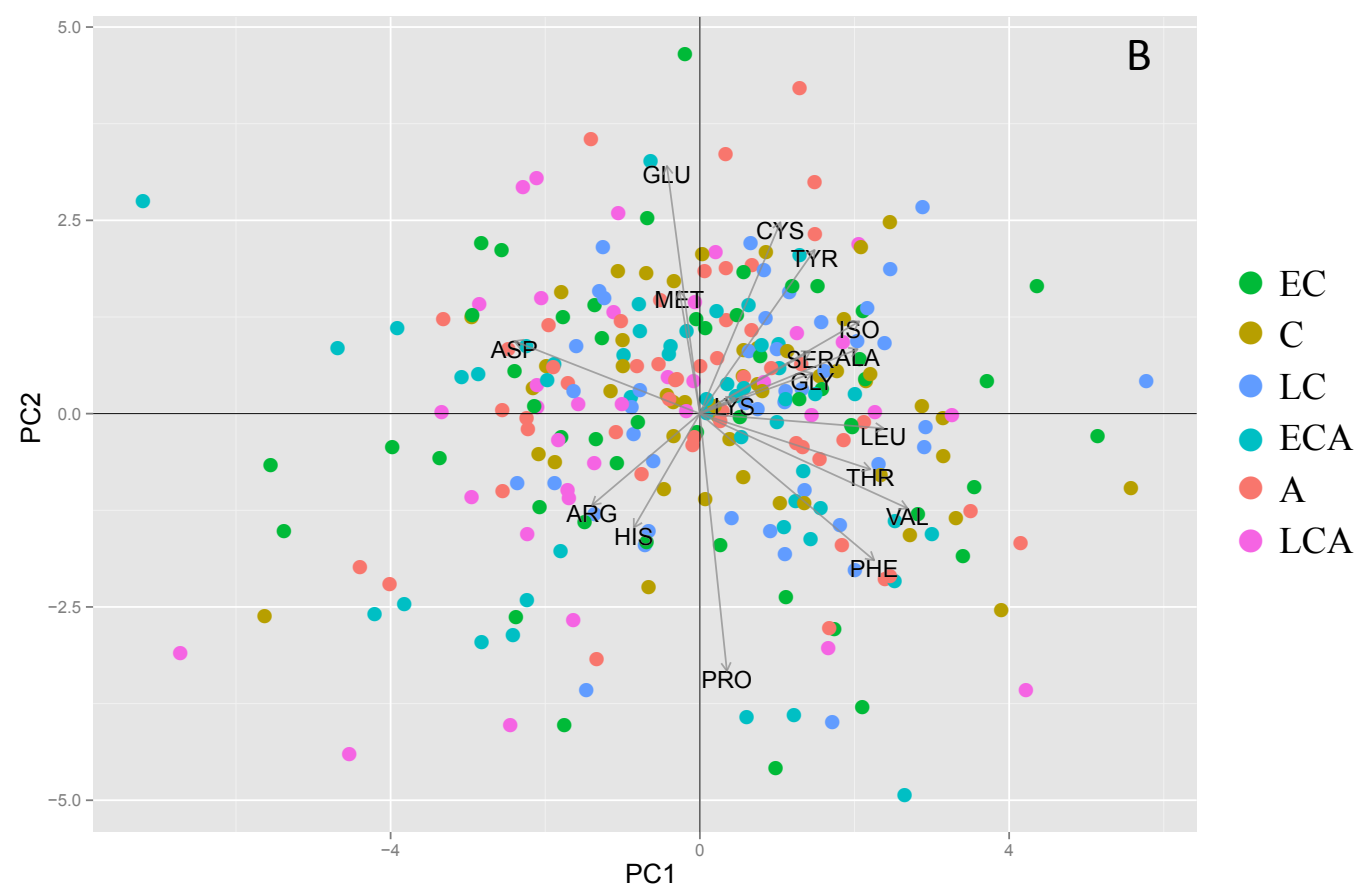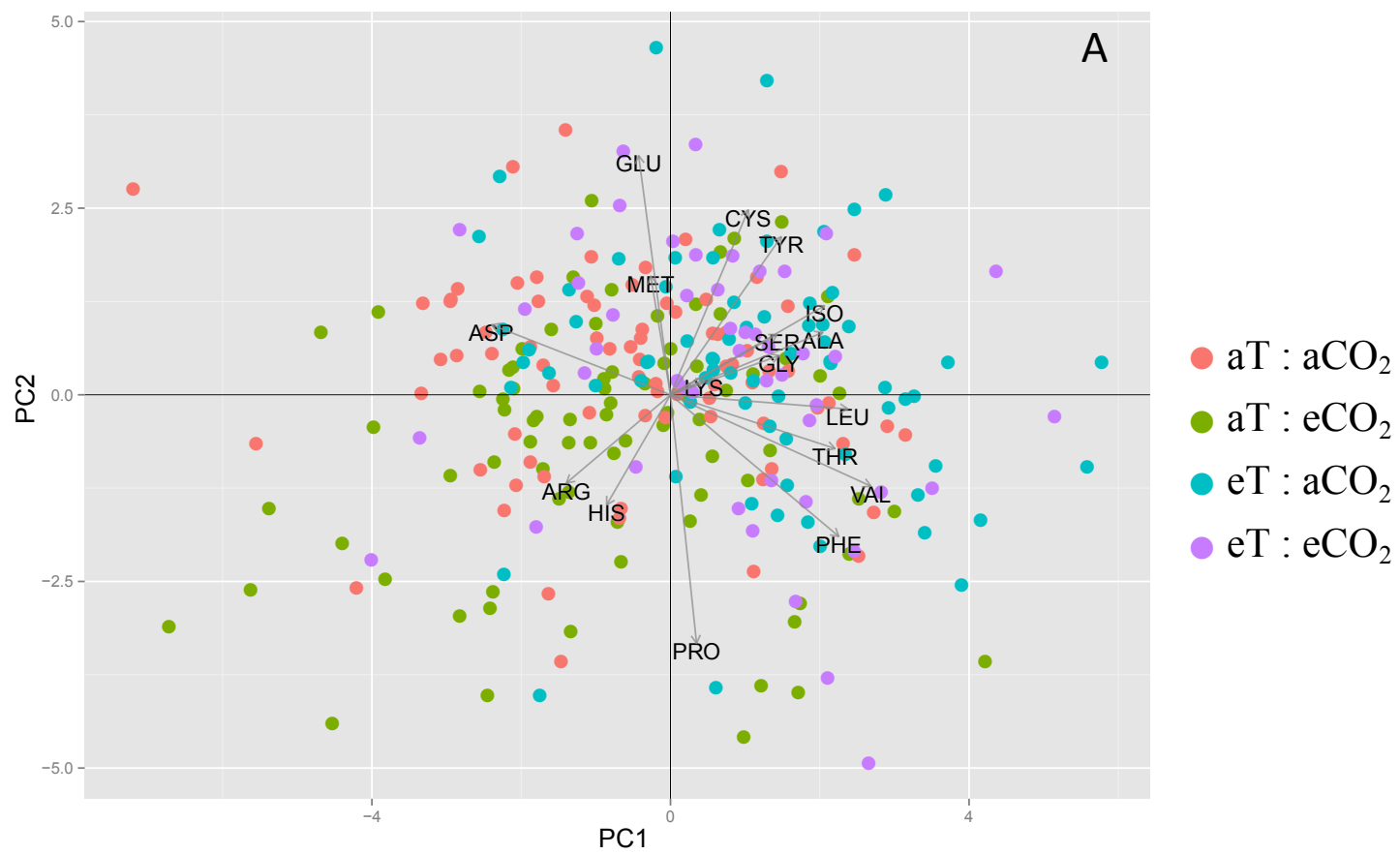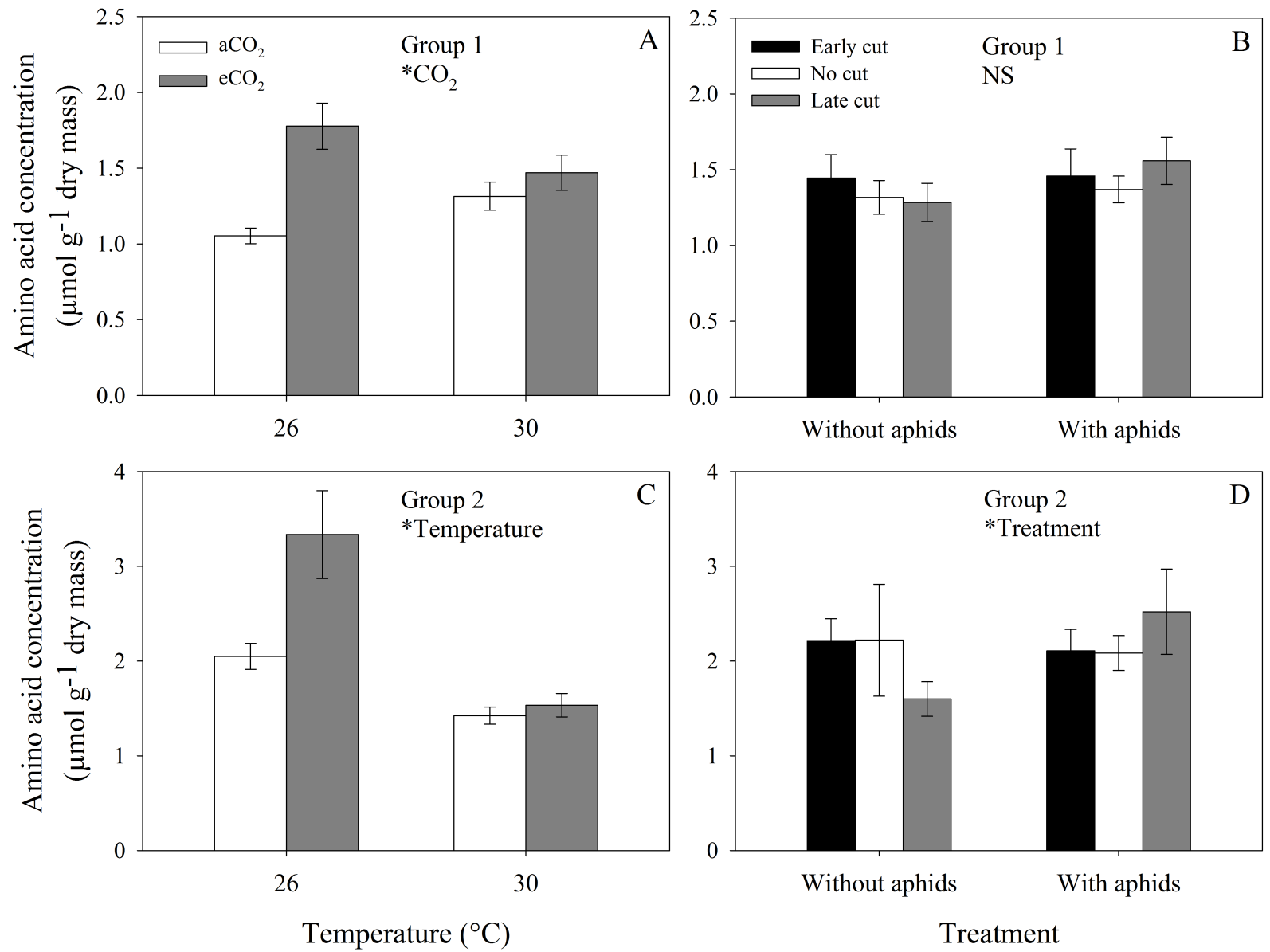
Figure S1

Figure S2