

Supplementary dataset

Systematic biology analysis on photosynthetic carbon metabolism of maize leaf following sudden heat shock under elevated CO₂

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Running title: Effects of CO₂ on C₄ metabolism recovering from heat shock

Serine	****	****	****	ns	ns	****	ns
Valine	****	****	****	****	****	****	****
Threonine	ns	ns	ns	ns	ns	ns	ns
Proline	**	****	****	****	ns	**	ns
Putrescine	ns	ns	ns	ns	ns	ns	ns
Asparagine	****	****	****	****	****	****	****
Glutamine	****	****	**	****	****	**	****
Phenylalanine	ns	*	ns	ns	ns	ns	ns

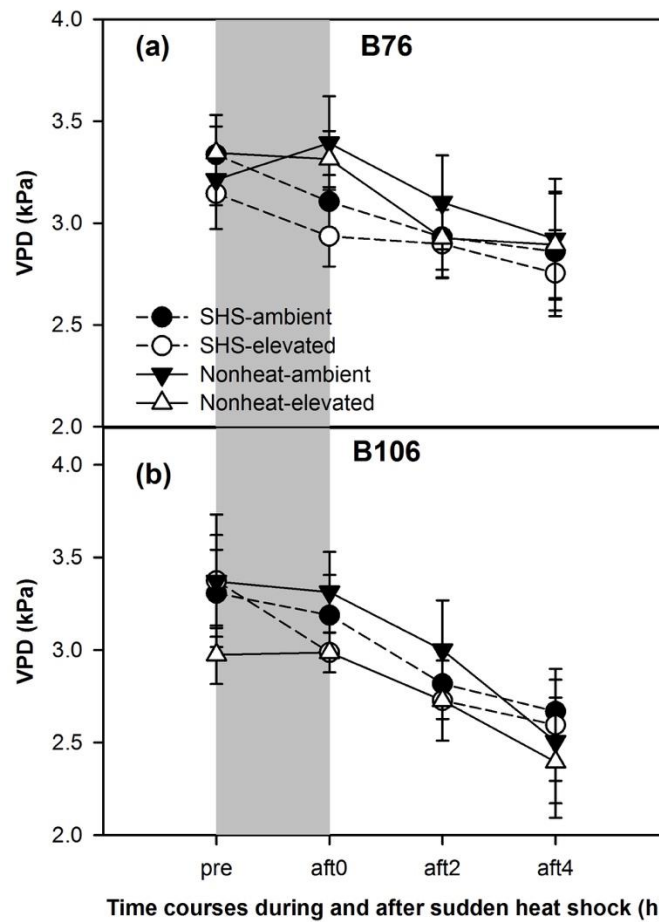
Note: Symbols “*”, “**”, “****”, “*****” represent significant levels at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively. “ns”: not significant.

Supplementary Table S2. Analysis of variance (*ANOVA*) for various carbohydrates, organic acids and amino acids in response to CO₂, sudden heat shock (SHS), recovery from SHS and their interactions in B106.

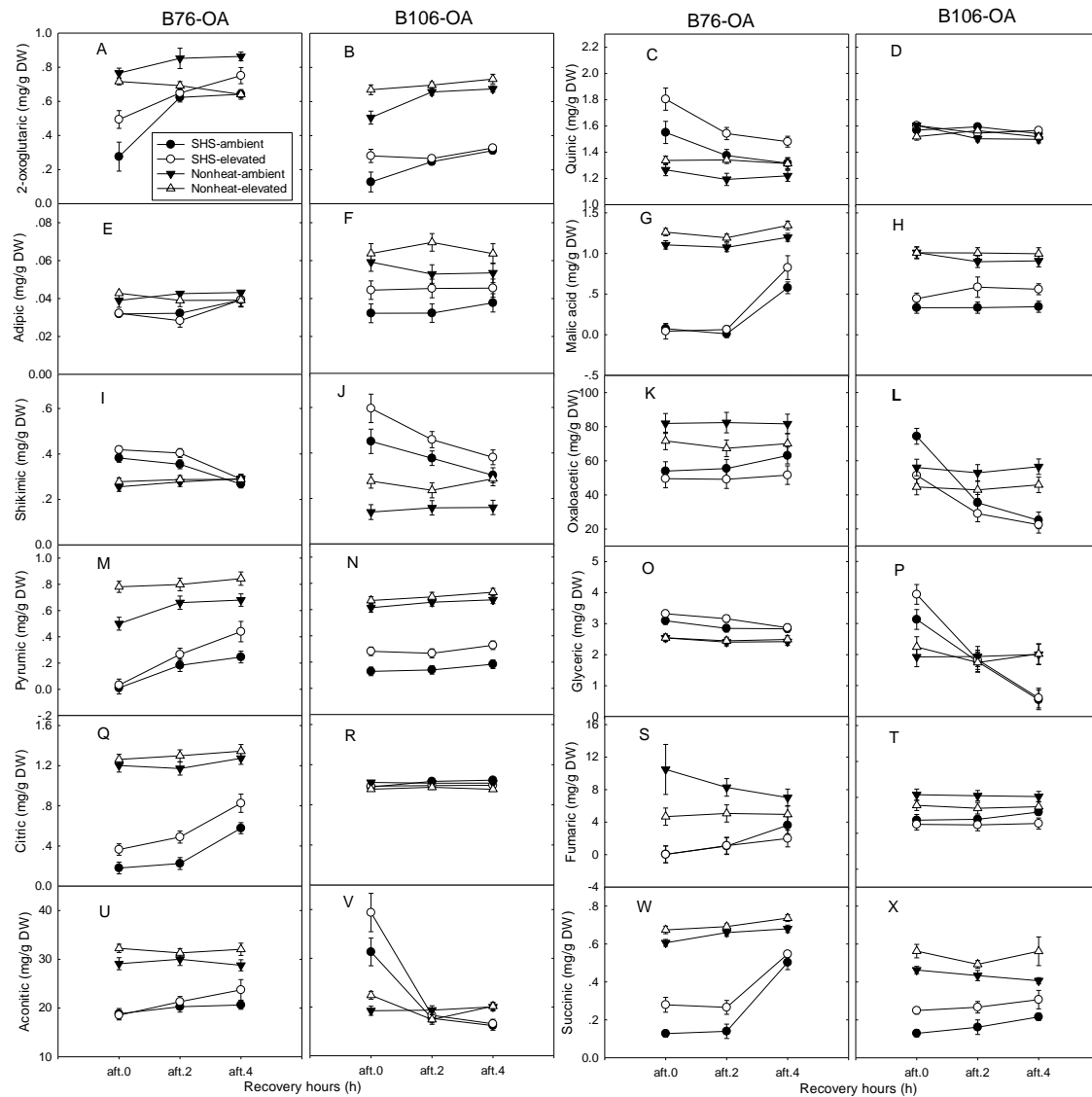
B106	CO ₂	SHS	Recover	CO ₂ *SHS	CO ₂ *Recover	SHSt*Recover	CO ₂ *SHS*Recover
Ribose	****	****	****	****	****	****	****
Fructose	****	****	****	**	*	ns	*
Glucose	****	****	****	ns	*	****	ns
My-inositol	**	****	ns	ns	ns	ns	ns
Surcose	*	****	*	ns	ns	*	ns
Maltose	ns	****	****	****	****	**	****
Trehalose	****	****	****	****	****	****	****
Raffinose	ns	ns	ns	ns	ns	ns	ns
Starch	****	****	****	****	**	****	**
2-oxoglutaric	****	****	****	ns	****	ns	ns
Quinic	ns	**	*	ns	ns	ns	**
Adipic	****	****	ns	ns	*	ns	ns
Shikimic	****	****	****	ns	**	****	ns
Pyruvic	****	****	****	****	ns	ns	ns
Citric	***	ns	ns	ns	ns	ns	ns
Aconitic	**	****	****	*	****	****	ns
Maleic	ns	ns	ns	ns	ns	ns	ns
Malic acid	****	****	**	****	**	****	ns
Oxaloacetic	****	****	****	ns	****	****	**
Malonic	ns	ns	ns	ns	ns	ns	ns
Alyceric	****		****	****	****	****	****
Fumuric	**	****	ns	ns	ns	ns	ns
Succinic	****	****	*	ns	ns	**	ns
Leucine	****	****	****	ns	****	****	****
Isoleucine	****	****	*	ns	ns	*	ns
Alanine	ns	**	**	ns	ns	**	ns
Glycine	****	****	****	****	****	****	****
Serine	ns	****	****	****	**	****	****

Valine	****	****	****	****	***	****	****
Threonine	ns	ns	ns	ns	ns	ns	ns
Proline	***	****	****	ns	**	****	ns
Putrescine	ns	*	ns	ns	ns	ns	ns
Asparagine	****	****	****	****	ns	****	ns
Glutamine	****	**	****	****	****	****	****
Phenylalanine	ns	ns	ns	ns	ns	ns	ns

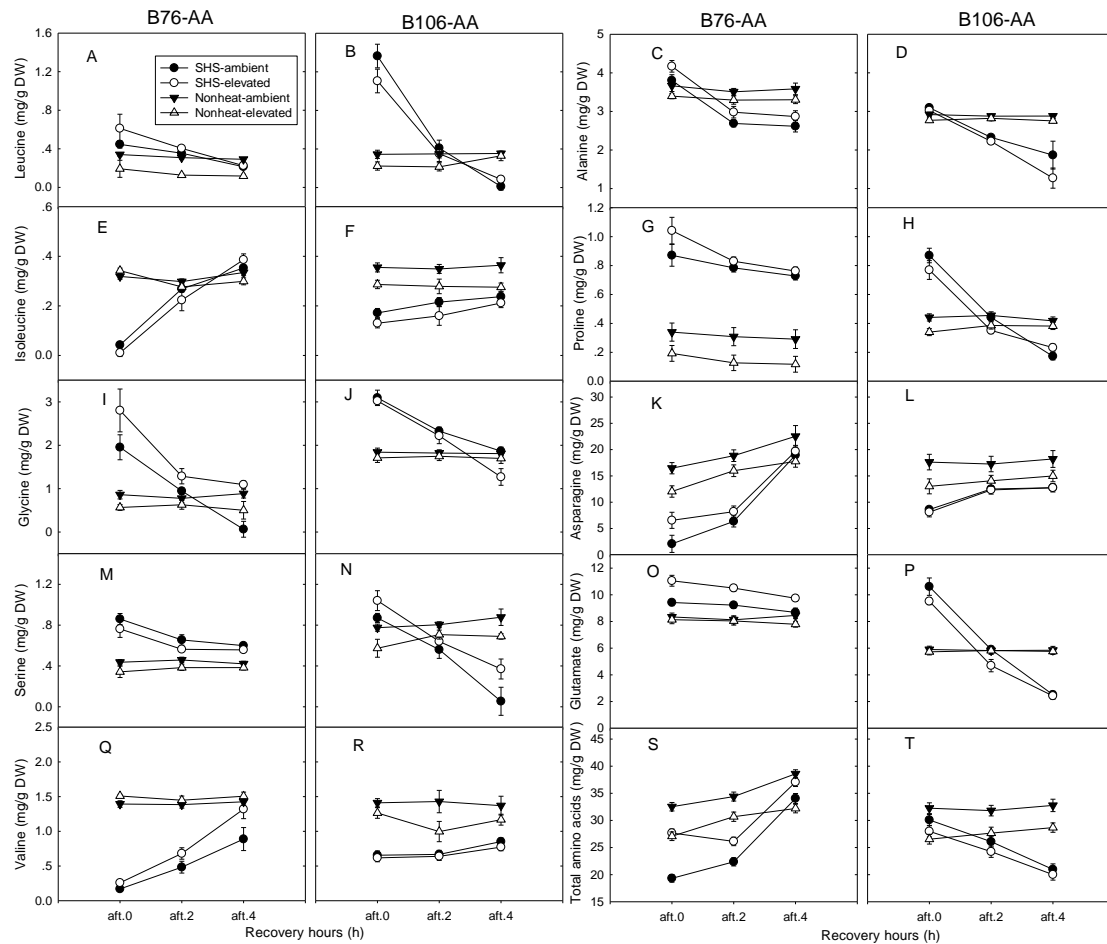
Note: Symbols “*”, “**”, “***”, “****” represent significant levels at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively. “ns”: not significant.



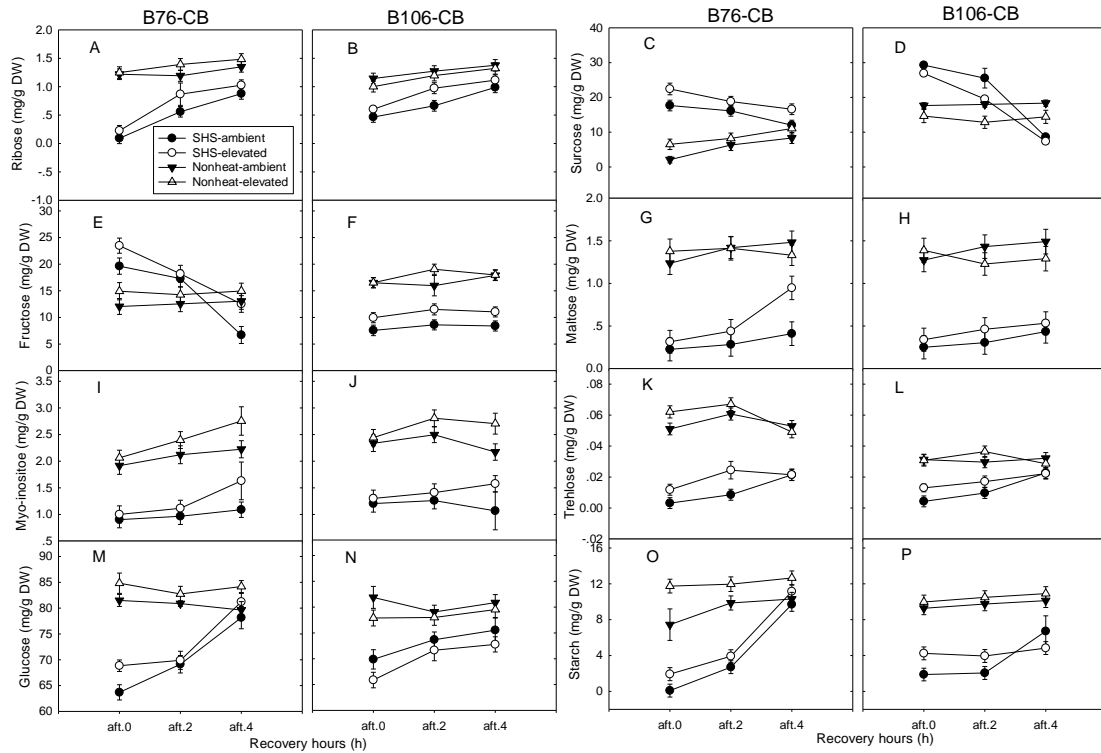
Supplementary Fig. S1. Sudden heat shock (SHS) induced decrease and recovery of vapor pressure deficit (VPD) in two maize genotypes grown under elevated CO₂ conditions. Shapes of circle in solid line and triangle in dotted line represent SHS treated leaves and nonheated leaves, respectively, while black and white symbols mean ambient CO₂ and elevated CO₂, respectively. The grey area represents the period during 2h SHS. Vertical bars represent at two sides of each panel represent significance scale regarding each combination of CO₂ and SHS treatments across during and after SHS from one-way ANOVA analysis, ($P < 0.05$). n=5



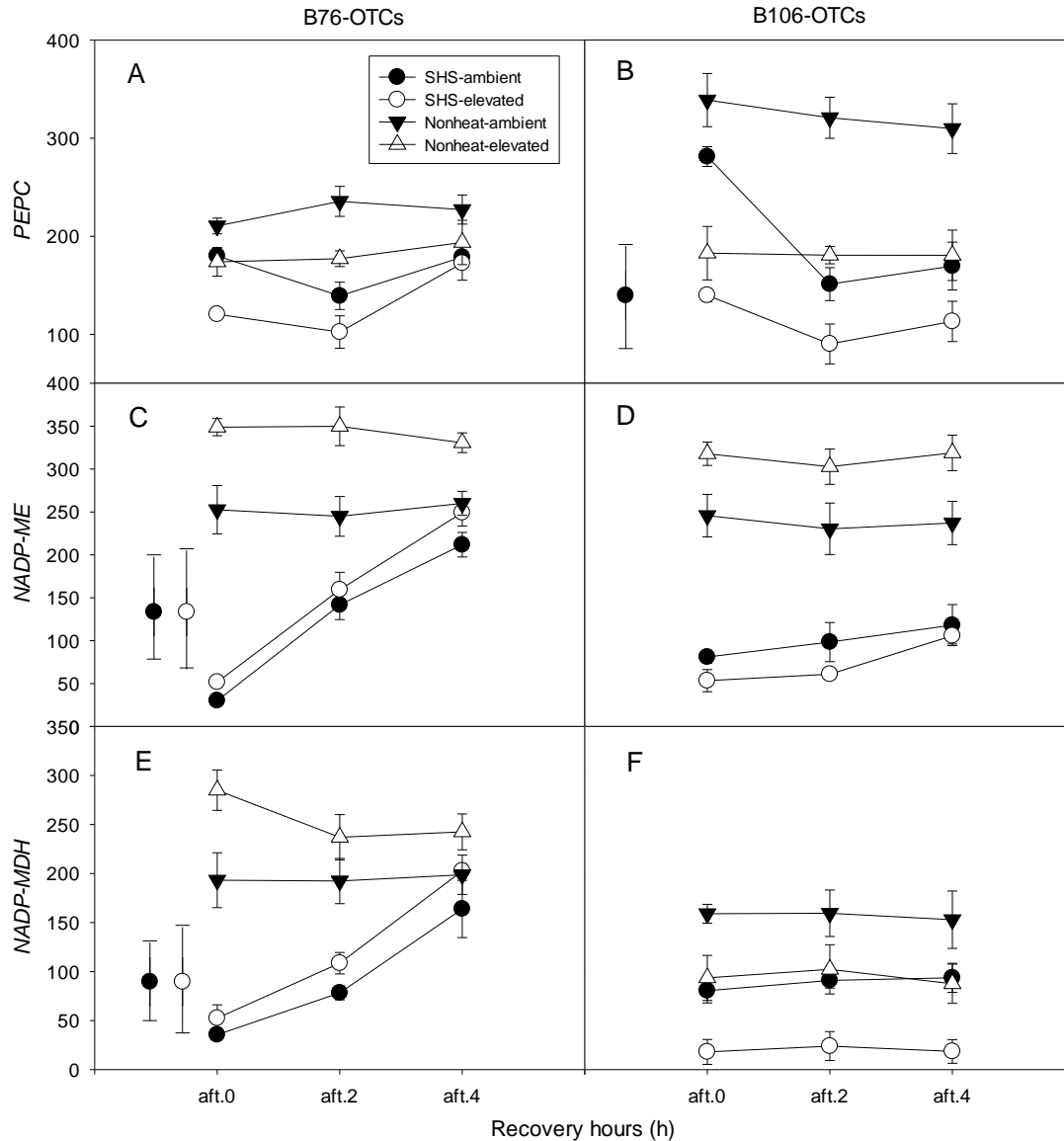
Supplementary Fig. S2. Recovery profiling of organic acids (OA) after sudden heat shock (SHS) at different hours in two maize genotypes grown under different CO₂. SHS-ambient and SHS-elevated represent response of metabolites in SHS leaves grown under ambient and elevated CO₂, respectively. Nonheat-ambient and Nonheat-elevated represent response of metabolites in nonheated leaves grown under ambient and elevated CO₂, respectively



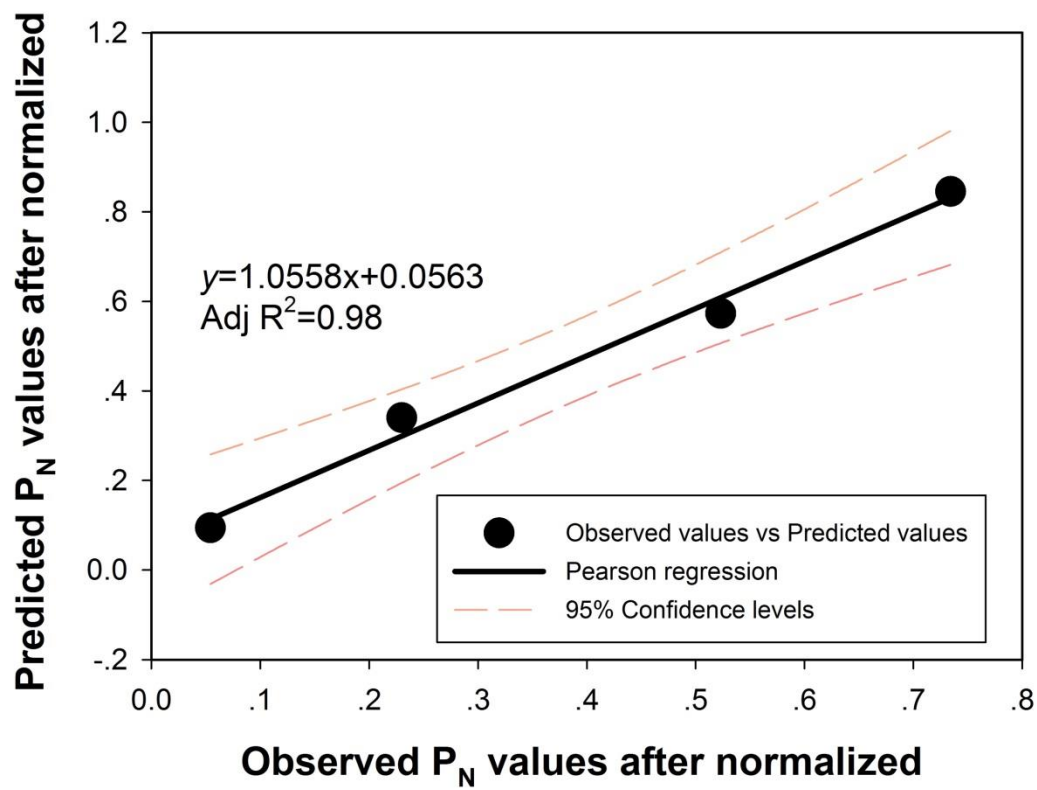
Supplementary Fig. S3. Recovery profiling of amino acids (AA) after sudden heat shock (SHS) at different hours in two maize genotypes grown under different CO₂. SHS-ambient and SHS-elevated represent response of metabolites in SHS leaves grown under ambient and elevated CO₂, respectively. Nonheat-ambient and Nonheat-elevated represent response of metabolites in nonheated leaves grown under ambient and elevated CO₂, respectively.



Supplementary Fig. S4. Recovery profiling of carbohydrates (CB) after sudden heat shock (SHS) at different hours in two maize genotypes grown under different CO₂. SHS-ambient and SHS-elevated represent response of metabolites in SHS leaves grown under ambient and elevated CO₂, respectively. Nonheat-ambient and Nonheat-elevated represent response of metabolites in nonheated leaves grown under ambient and elevated CO₂, respectively.



Supplementary Fig S5. Gene expression of three C₄ decarboxylating enzymes in response to recovery from sudden heat shock (SHS) in two maize genotypes grown under two CO₂ levels. Shapes of circle and triangle represent SHS treated leaves and nonheated leaves, respectively, while black and white symbols mean ambient CO₂ and elevated CO₂, respectively. The grey area represents the period during 2h SHS. Vertical bars represent at two sides of each panel represent significance scale regarding each combination of CO₂ and SHS treatments across during and after SHS from one-way ANOVA analysis, ($P < 0.05$). n=3-5.



Supplementary Fig. S6. Prediction of photosynthetic rates (P_N) from test dataset using linear regression model. The values of P_N was normalized between 0~1.