Supplementary Material

1 Supplementary Figures and Tables

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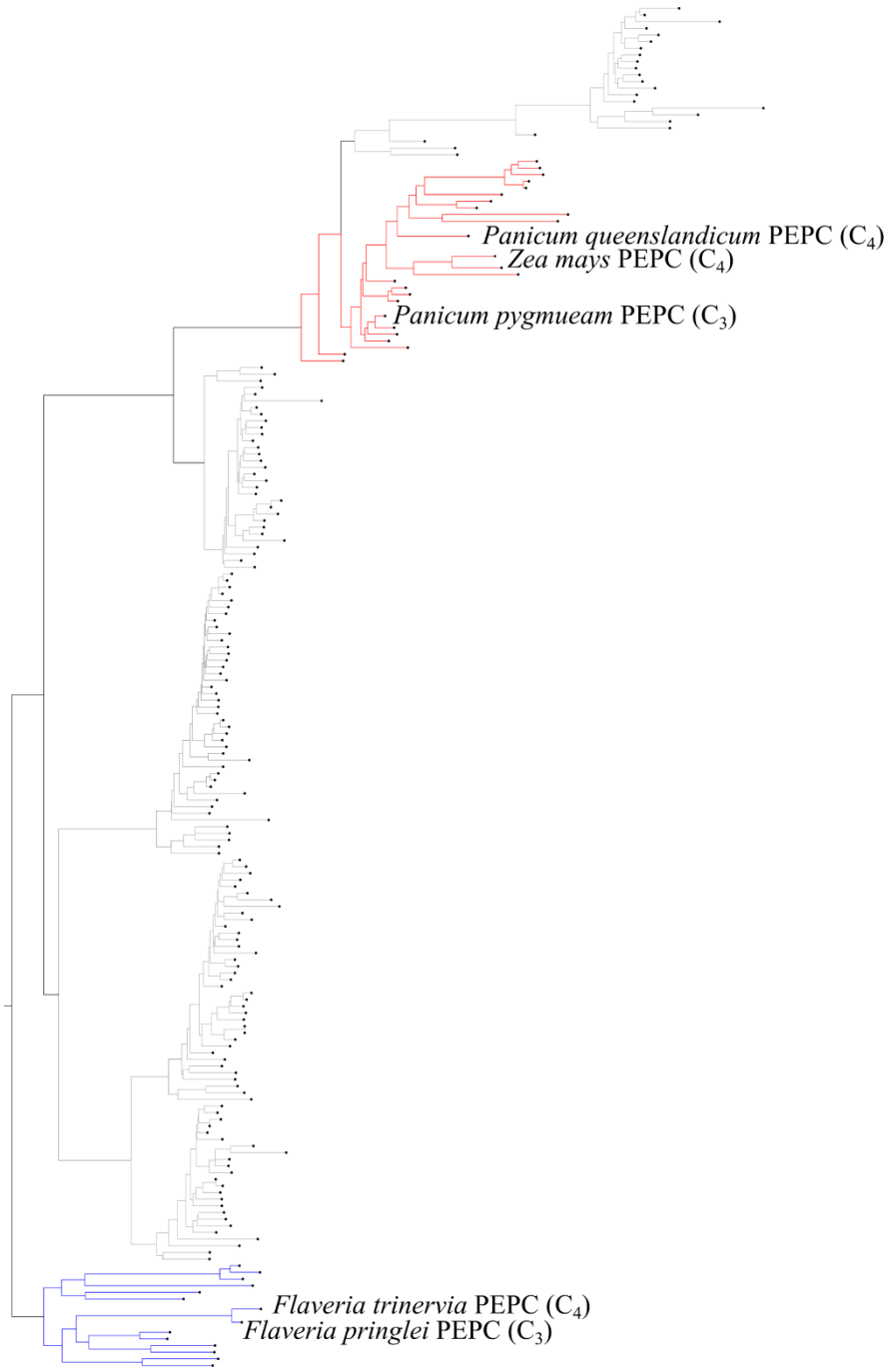
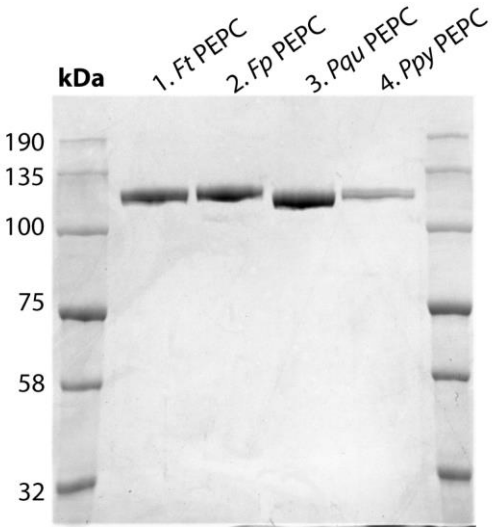
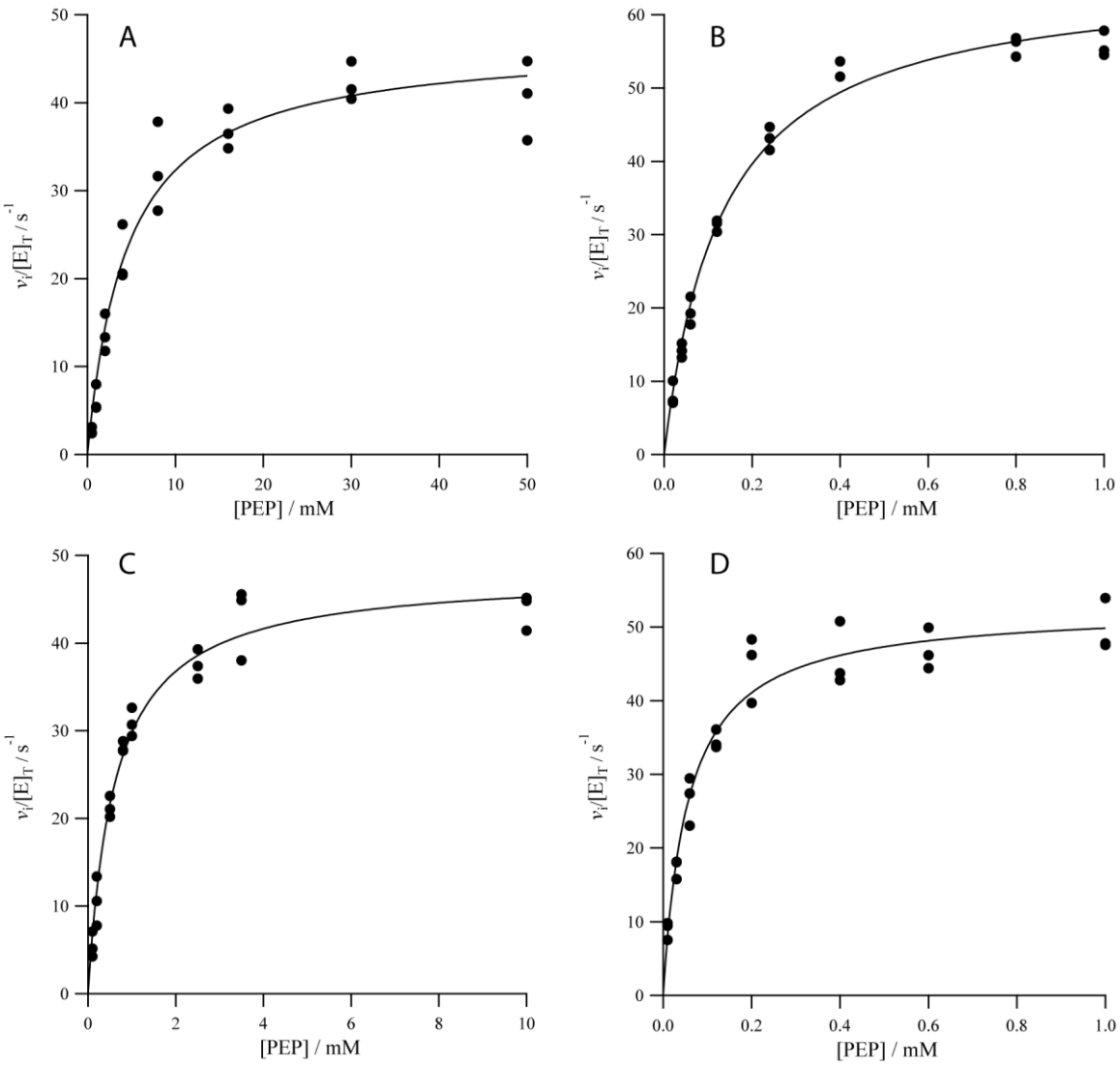
1.1 Supplementary Figures



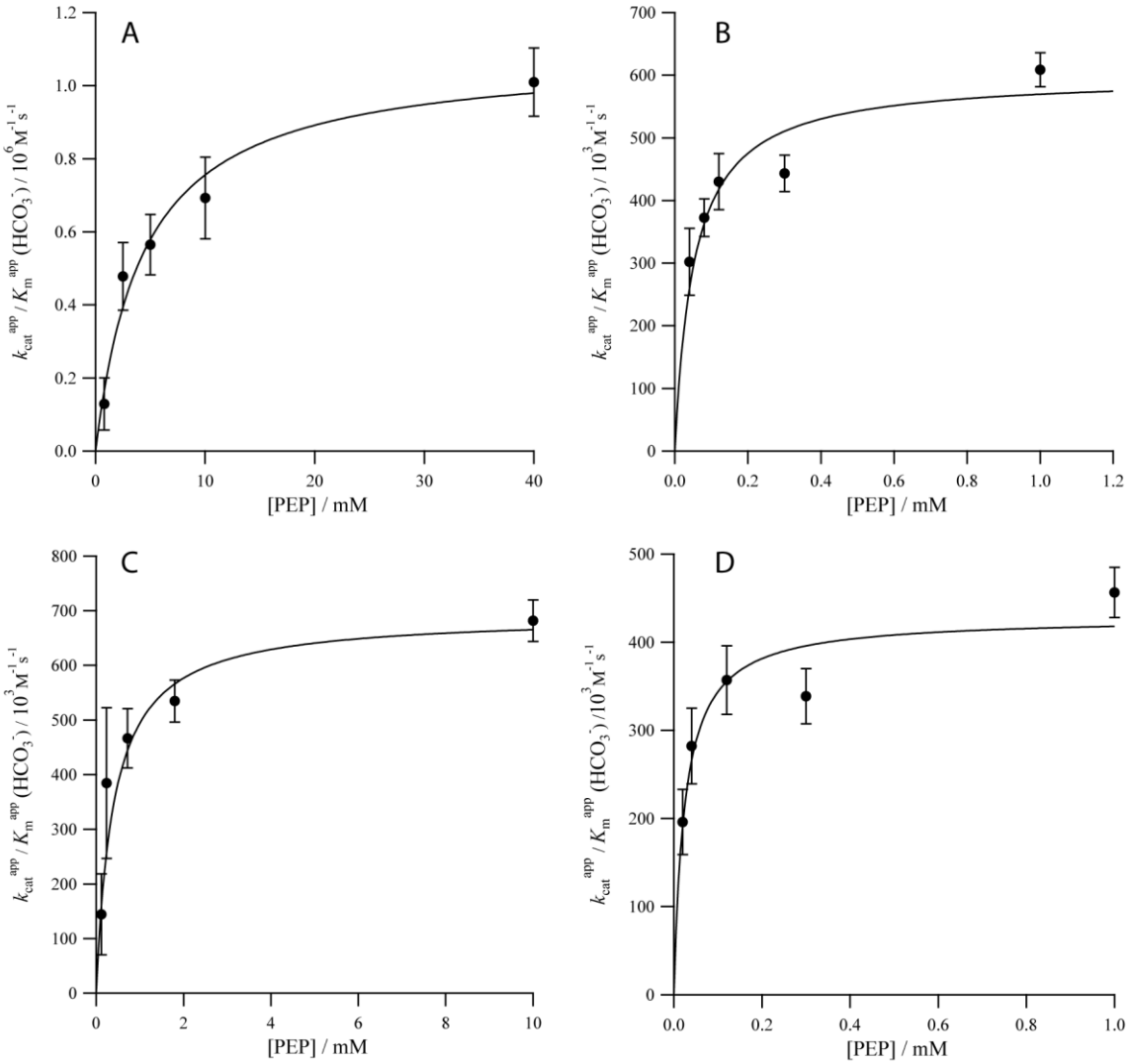
Supplementary Figure 1. Maximum likelihood tree of orthologous PEPC genes. In red are the ppc1P3 isoforms. In blue are the ppc1E2 isoforms. Sequences for *Zea mays* (C_4) taken from (Dong et al., 1998).



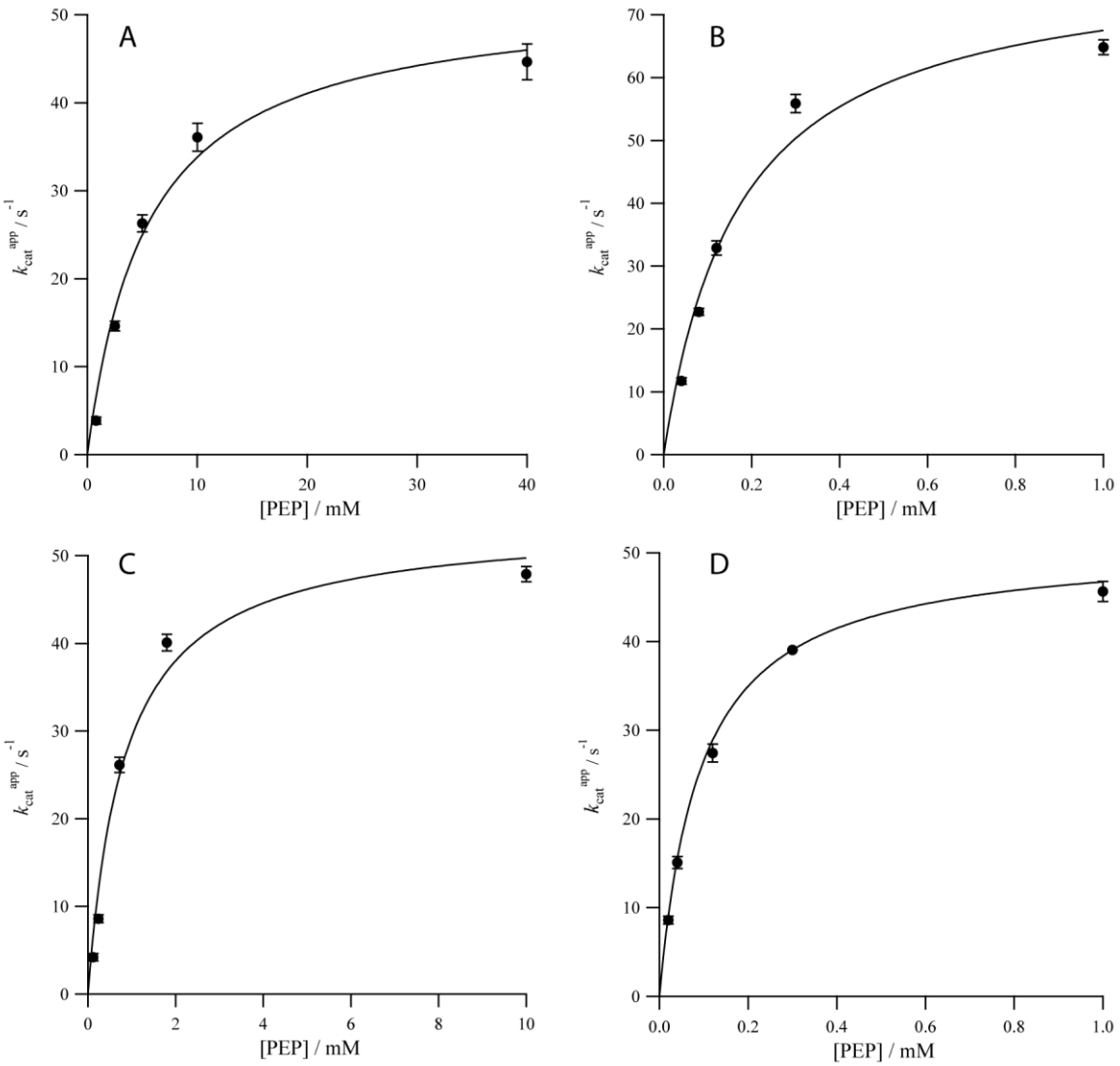
Supplementary Figure 2. SDS PAGE gel of purified PEPC Enzymes. 8 % acrylamide SDS PAGE analysis of PEPC proteins (5 µg by BCA assay) compared in this study. Gel visualised with instant blue. PEPC from 1. *Flaveria trinervia*, 2. *Flaveria pringlei*, 3. *Panicum queenslandicum* and 4. *Panicum pygmaeum* PEPC.



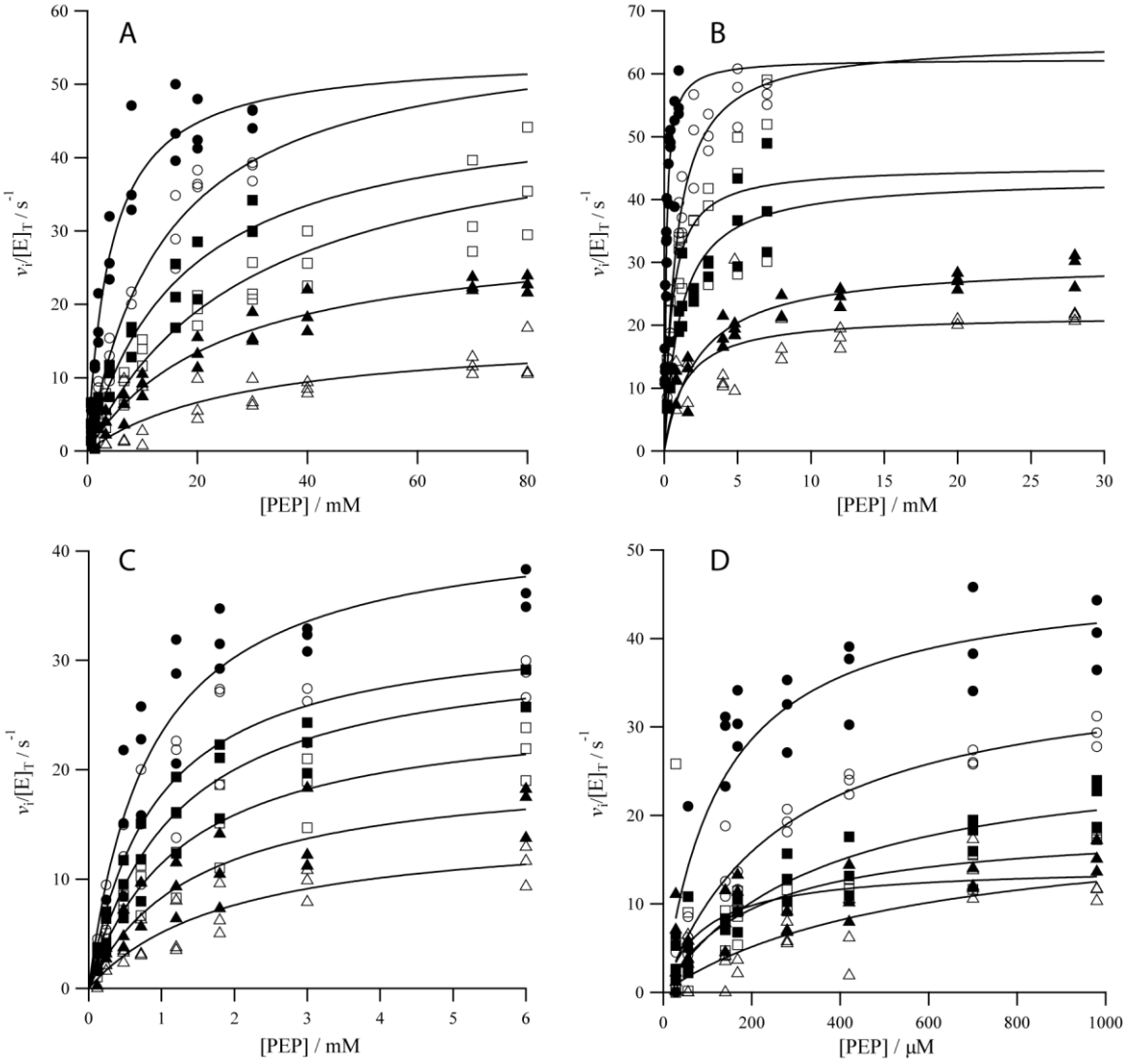
Supplementary Figure 3. **PEP Michaelis-Menten Assays (v_i vs PEP).** Assays conditions were 50 mM Tricine.KOH pH 8.0, 10 mM $MgCl_2$, 0.2 mM NADH, 0.01 Uμl^{-1} malate dehydrogenase, initiated by 10 nM PEPC. Assays were repeated (n = 3) for each concentration of PEP. Lines are described by equation 1. (A) Markers experimental data for *Panicum queenslandicum* PEPC, the line is characterised by the parameters k_{cat} = 46.96 ± 1.74 s^{-1}, K_m^{PEP} = 4.53 ± 0.59 mM and k_{cat}/K_m^{PEP} = 0.10 × 10^5 ± 1.08 × 10^3 $M^{-1}s^{-1}$; (B) Markers represent data for *Panicum pygmaeum* PEPC, the line is characterised by the parameters k_{cat} = 65.59 ± 1.26 s^{-1}, K_m^{PEP} = 0.13 ± 0.01 mM and k_{cat}/K_m^{PEP} = 5.01 × 10^5 ± 2.44 × 10^4 $M^{-1}s^{-1}$; (C) Markers represent experimental data for *Flaveria trinervia* PEPC, the line is characterised by the parameters k_{cat} = 47.99 ± 1.21 s^{-1}, K_m^{PEP} = 0.60 ± 0.05 mM, and k_{cat}/K_m^{PEP} = 0.79 × 10^5 ± 5.43 × 10^3 $M^{-1}s^{-1}$; (D) Markers represent experimental data for *Flaveria pringlei* PEPC, the line is characterised by the parameters k_{cat} = 52.65 ± 1.37 s^{-1}, K_m^{PEP} = 0.06 ± 0.01 mM and k_{cat}/K_m^{PEP} = 9.35 × 10^5 ± 8.49 × 10^4 $M^{-1}s^{-1}$.



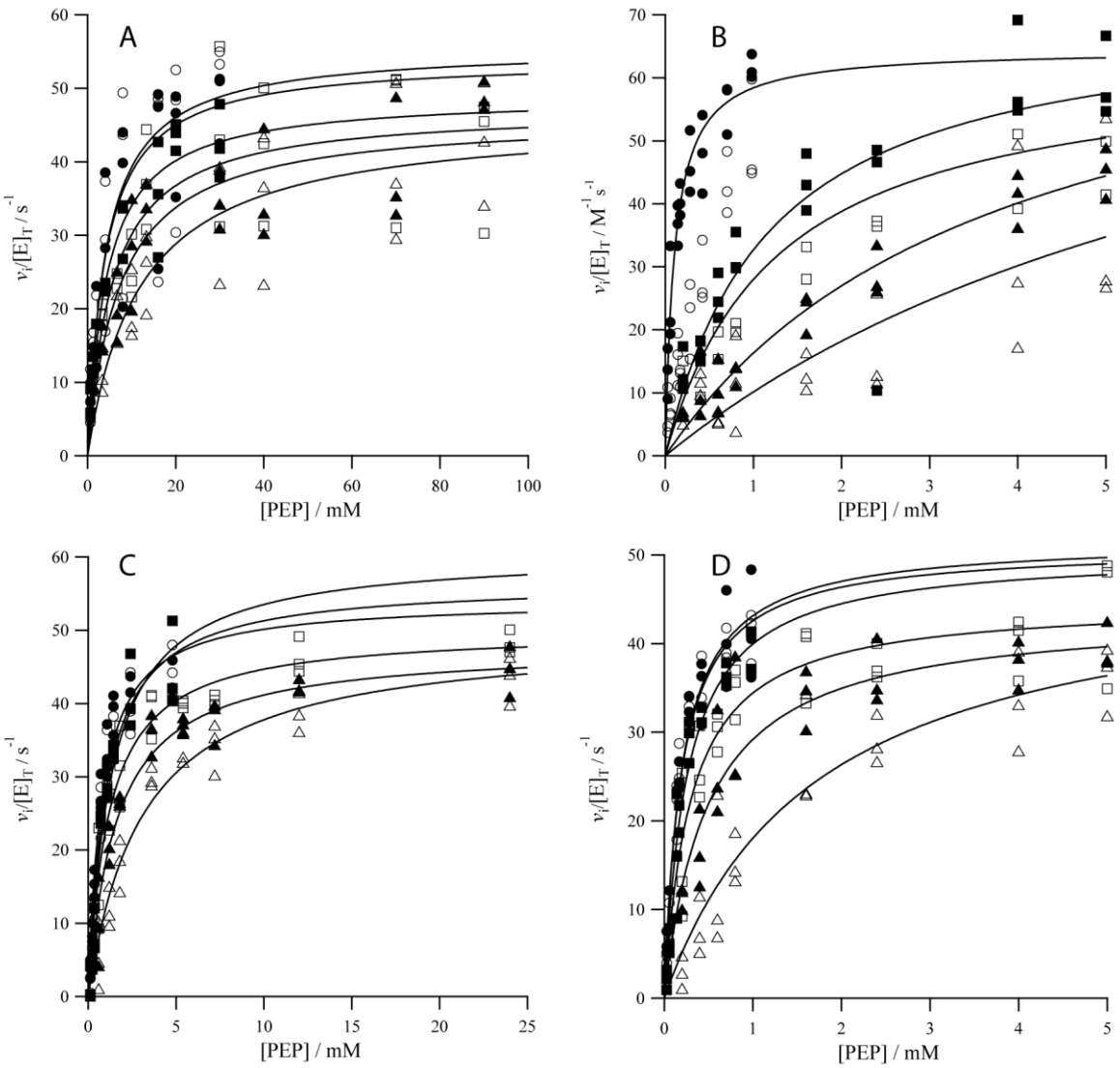
Supplementary Figure 4. Secondary analysis of bicarbonate Michaelis-Menten behaviour (k_{cat}^{app}/K_m^{app} vs PEP). Secondary plot of the $k_{cat}^{app}/K_m^{app\ HCO3-}$ parameter from fitting data shown in Figure 1, the lines are described by equation 2 and error bars represent standard errors. Markers represent the $k_{cat}^{app}/K_m^{app\ HCO3-}$ for PEPC from (A) *Panicum queenslandicum*, the line is characterised by the parameters K_i^{PEP} = 4.39 ± 1.10 mM and k_{cat}/K_m^{HCO3-} = 1.09 × 10^6 ± 8.88 × 10^4 $M^{-1}s^{-1}$; (B) *Panicum pygmaeum*, the line is characterised by the parameters K_i^{PEP} = 0.02 ± 0.01 mM and k_{cat}/K_m^{HCO3-} = 0.60 × 10^6 ± 2.93 × 10^4 $M^{-1}s^{-1}$; (C) *Flaveria trinervia*, the line is characterised by the parameters K_i^{PEP} = 0.40 ± 0.13 mM and k_{cat}/K_m^{HCO3-} = 0.69 × 10^6 ± 4.17× 10^4 $M^{-1}s^{-1}$. (D) *Flaveria pringlei*, the line is characterised by the parameters K_i^{PEP} = 0.02 ± 0.01 mM and k_{cat}/K_m^{HCO3-} = 0.44 × 10^6 ± 2.17 × 10^4 $M^{-1}s^{-1}$.



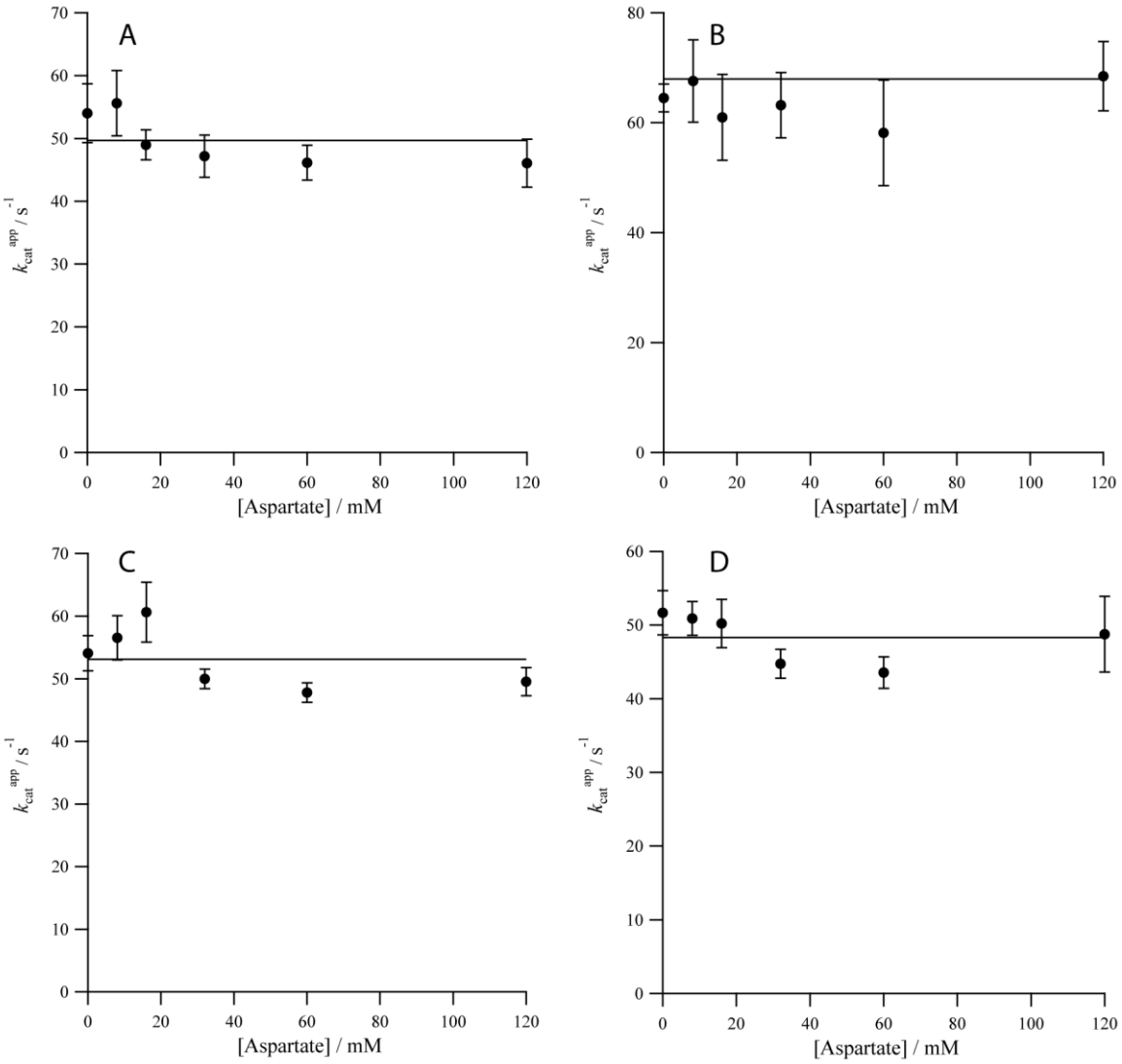
Supplementary Figure 5. Secondary analysis of bicarbonate Michaelis-Menten behaviour (k_{cat}^{app} vs PEP). Secondary plot of the k_{cat}^{app} parameter from fitting data shown in Figure 1. The line described by equation 2, error bars represent standard errors from fit of lines. Markers represent the k_{cat}^{app} parameter for PEPC from (A) *Panicum queenslandicum*, the line is characterised by the parameters k_{cat} = 52.25 ± 3.72 s^{-1} K_m^{PEP} = 5.46 ± 1.12 mM; (B) *Panicum pygmaeum*, the line is characterised by the parameters k_{cat} = 79.06 ± 6.64 s^{-1} and K_m^{PEP} = 0.17 ± 0.04 mM; (C) *Flaveria trinervia*, the line is characterised by the parameters k_{cat} = 53.89 ± 4.12 s^{-1} and K_m^{PEP} = 0.84 ± 0.02 mM; (D) *Flaveria pringlei*, the line is characterised by the parameters k_{cat} = 51.01 ± 0.05 s^{-1}, K_m^{PEP} = 0.08 ± 0.01 mM.



Supplementary Figure 6. Primary plots of Malate Inhibition Assays (v_i vs PEP). Assays conditions were 50 mM Tricine.KOH pH 8.0, 10 mM $MgCl_2$, 0.2 mM NADH, 0.01 Uμl^{-1} malate dehydrogenase, PEP concentration as shown on the axis. Lines are described by equation one. Assays were repeated (n = 3) for each point. Markers (in order of increasing malate: ●, ○, ■, □, ▲, △) show individual data points for the following PEPC with enzyme concentration in brackets (A) *P. queenslandicum* (10 nM), with 0, 8 mM, 16 mM, 32 mM, 60 mM and 120 mM malate; (B) *P. pygmaeum* (5 nM), with 0, 4 mM, 12 mM, 24 mM, 32 mM and 60 mM malate; (C) *F. trinervia* (10 nM), with 0, 8 mM, 16 mM, 32 mM, 60 mM and 120 mM malate; (D) *F. pringlei* (5 nM), with 0, 4 mM, 12 mM, 24 mM, 32 mM and 60 mM malate.



Supplementary Figure 7. Primary plots of Michaelis-Menten Aspartate Inhibition Assays (v_i vs PEP). Assays conditions were 50 mM Tricine.KOH pH 8.0, 10 mM $MgCl_2$, 0.2 mM NADH, 0.01 Uμl^{-1} malate dehydrogenase, PEP concentration as shown. Lines are described by equation one. Assays were repeated (n = 3) for each point. Markers (in order of increasing aspartate: ●, ○, ■, □, ▲, △) show individual data points for the following PEPC with enzyme concentration in brackets (A) *P. queenslandicum* (10 nM) with 0, 8 mM, 16 mM, 32 mM, 60 mM and 120 mM aspartate, 60 mM and 120 mM aspartate; (B) *P. pygmaeum* (5 nM) with 0, 8 mM, 16 mM, 32 mM; (C) *F. trinervia* (10 nM) with 0, 8 mM, 16 mM, 32 mM, 60 mM and 120 mM aspartate; (B) *P. pygmaeum* (5 nM) with 0, 8 mM, 16 mM, 32 mM; (D) *F. pringlei* (5 nM), performed in the presence of 0, 8 mM, 16 mM, 32 mM, 60 mM and 120 mM aspartate.



Supplementary Figure 8. Secondary plots of Aspartate Inhibition (k_{cat}^{app} vs Aspartate). Assays conditions were 50 mM Tricine.KOH pH 8.0, 10 mM $MgCl_2$, 0.2 mM NADH, 0.01 Uμl^{-1} malate dehydrogenase, in the presence of varied aspartate (0-120 mM). Lines are illustrative and error bars represent standard errors. Secondary plot of k_{cat}^{app} against aspartate concentration for PEPC from (A) *P. queenslandicum*, (B) *P. pygmaeum*, (C) *F. trinervia*, and (D) *F. pringlei*.

Supplementary Table 1 Summary of primers used in this study. Primers were used for cloning and sequencing.

Primer	Sequence
FlvFor1B	TACTTCCAATCCAATGCAATGGCTAACCGGAAT
FlvRev1B	TTATCCACTTCCAATGTTATTACTAACCGGTGTTCTGC
Flav_1303_Seq_For	AGACAAGTGTCGACTT
Flav_1832_Seq_Rev	TTGTAGAGCTGCCATG
PquFor1B	GACGACGACAAGATGGCGTCCTCCGAGCGCCACC
PquRev1B	GAGGAGAAGCCCGGTTAGCCCGTGTTCTGCATGCC
PpyFor1B	TACTTCCAATCCAATGCAATGGCAAGCAG
PpyRev1B	TTATCCACTTCCAATGTTATTATTAACCGGTATTC
Pqu_1323_Seq_For	CGTGAAGCTGGACAT
Pqu_1752_Seq_Rev	ATGACCTGCTGCTTG
Ppy_1291_Seq_For	GATGGTAGTCTGCTGG
Ppy_1791_Seq_Rev	GCTATCGCTATAACCA
T7 Promotor	TAATACGACTCACTATAGGG
T7 Terminator	GCTAGTTATTGCTCAGCGG