Table 1. Summary of protein associated with primary CNS metabolism and significantly affected by nutrient conditions (K, Ca or putrescine). This table is a summary combining two two-way ANOVA analyses (K and Ca, or K and putrescine as factors) and shows proteins with a *P*-value lower than the Bonferroni threshold to minimize the false discovery rate. Protein not involved in metabolism are listed in Table S2. The loading value indicate the normalized weight in multivariate analysis and is positive when the protein content increase when the availability in the nutrient considered increases. Proteins associated with a significant interaction effect (K *x* Ca or K *x* putrescine) are displayed in Fig. S5.

Protein	**Statistics**	
	-log(*P*)	**Loading**
Proteins associated with a significant K effect (in combination with either Ca or putrescine)		
in leaves		
Oxalate-CoA ligase	10.55	–0.94
Aconitase	10.39	–0.90
NADP-dependent isocitrate dehydrogenase	10.31	–0.91
Glucose/ribitol dehydrogenase	9.94	–0.89
Pyruvate kinase	8.50	0.95
Rubisco activase	8.11	0.96
Glucose 6-phosphate dehydrogenase	7.95	–0.86
Fructose 1,6-bisphosphate aldolase	7.63	0.81
Carbamoyl phosphate synthase	7.47	0.93
Phosphoglycerate mutase	7.46	–0.94
Fructokinase	7.20	–0.90
Aspartate aminotransferase	7.12	–0.92
NADP-dependent malic enzyme	7.06	–0.90
Phospho*enol*pyruvate carboxykinase	6.72	0.92
Pyruvate Pi dikinase	6.45	0.89
Dihydrolipoamide acetyl transferase	6.17	–0.89
Methionine synthase	6.06	0.92
NAD-dependent malic enzyme	5.99	–0.85
Rubisco, small chain	5.92	0.77
Phosphoribulokinase	5.81	0.89
Acetyl-CoA carboxylase	5.78	0.96
Pyruvate dehydrogenase (E1 subunit)	5.44	0.85
S-adenosylmethionine synthase	5.42	0.81
Phosphofructokinase (PPi dependent)	5.41	0.87
Serine hydroxymethyltransferase	5.40	0.78
Ornithine aminotransferase	5.39	–0.90
Glyceraldehyde 3-phosphate dehydrogenase	5.36	0.91
Starch synthase 1	5.35	0.77
6-phosphogluconate dehydrogenase	5.08	–0.83
Carbonic anhydrase γ	5.06	0.87
Succinate dehydrogenase	5.04	–0.86
Phosphoglycerate kinase (chloroplastic)	4.97	0.86
Triose phosphate isomerase	4.94	0.90
Serine glyoxylate aminotransferase	4.89	0.90
Cysteine synthase	4.83	–0.90
Alanine aminotransferase	4.72	–0.90
in roots		
Glucose/ribitol dehydrogenase	8.74	0.90
Pyruvate kinase	7.34	0.51
Phenylalanine ammonia lyase	6.71	–0.29
Glutamine synthetase	6.67	0.57
Phospho*enol*pyruvate carboxykinase	6.56	–0.87
Nitrate transporter 2.3	5.82	0.52
High affinity nitrate transporter 3.1	5.61	–0.64
Aspartate aminotransferase (cytosolic)	5.46	0.59

Oxalate-CoA ligase	5.43	–0.70
S-adenosylmethionine synthase	5.42	–0.46
High affinity nitrate transporter family protein	5.40	0.46
Cysteine synthase	5.38	0.78
ADP-glucose pyrophosphorylase	5.22	–0.76
Proteins associated with a significant Ca effect		
in leaves		
Glucose/ribitol dehydrogenase	7.63	0.10
Pyruvate kinase	7.53	–0.21
Aconitase	7.48	0.04
Carbonic anhydrase (chloroplastic)	6.30	–0.42
Fructose 1,6-bisphosphate aldolase	6.19	–0.19
Rubisco, small chain	6.09	–0.28
Carbamoyl phosphate synthase	5.93	–0.19
Pyruvate dehydrogenase (E1 subunit)	5.91	–0.37
Glycerate dehydrogenase	5.59	–0.19
Glutamine synthetase	5.49	0.48
Cystathionine β-synthase	5.14	–0.22
Phosphofructokinase (PPi dependent)	4.98	–0.29
Glucose 6-phosphate dehydrogenase	4.92	0.03
Succinate dehydrogenase	4.86	0.23
Rubisco activase	4.68	–0.01
in roots		
Glucose/ribitol dehydrogenase	5.91	–0.56
Pyruvate kinase	5.72	–0.79
Proteins associated with a significant putrescine effect		
in leaves		
1-deoxy-D-xylulose 5-phosphate reductoisomerase	7.64	0.06
Glucose/ribitol dehydrogenase	7.45	–0.17
Glycerate dehydrogenase	7.31	0.22
Phosphoribulokinase	5.77	0.34
Fructose 1,6-bisphosphate aldolase	5.56	0.14
Aconitase	5.34	0.02
Rubisco activase	5.36	0.32
Glucose 6-phosphate dehydrogenase	4.85	–0.16
Rubisco, small chain	4.79	0.19
in roots		
Aspartate aminotransferase	8.39	-0.86
High affinity nitrate transporter 3.1	8.16	–0.58
Phenylalanine ammonia lyase	7.94	0.77
6-phosphogluconate dehydrogenase	6.41	–0.90
Phospho*enol*pyruvate carboxykinase	6.39	0.91
Carbonic anhydrase X2	6.33	–0.93
ADP-glucose pyrophosphorylase	6.11	0.93
Succinyl-CoA ligase (succinate thiokinase)	5.93	–0.83
Cysteine synthase	5.87	–0.89
3-phosphoglycerate dehydrogenase	5.65	0.61
Malate dehydrogenase	5.52	–0.86
Nitrate transporter 2.3	5.50	–0.83
Glutamate decarboxylase	5.44	0.90
Sucrose synthase	4.97	0.72
Enolase	4.97	–0.83
Glutamine synthetase	4.79	–0.75
Phosphofructokinase	4.73	0.89

Figures

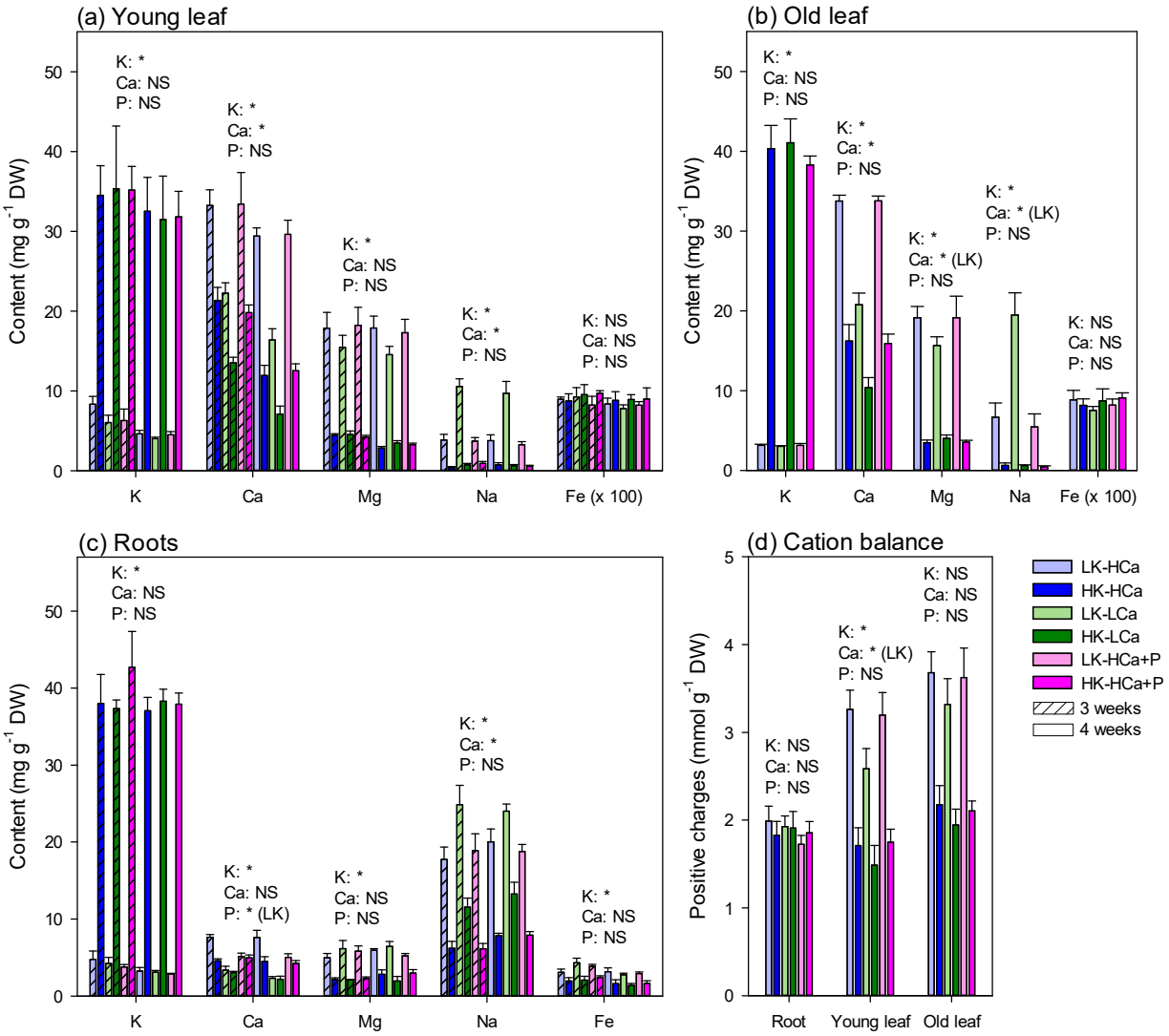

Fig. 1. Cation composition in sunflower roots and leaves after 3 (dashed) or 4 (plain) weeks under different K/Ca/putrescine nutrient conditions: (a) young (i.e., developing) leaf, (b) old (i.e., mature) leaf, (c) roots. Cations were quantified by ICP coupled to atomic absorption (OES). Minor cations (Cu, Mn, Zn) are not shown (always < 0.12 mg g^{-1} DW) in this figure. In (d), the total cation balance (sum of contents in moles, weighted by the carried positive charge) is shown. Statistics (ANOVA) are summarized on top of each bar group, showing the effect of K, Ca and putrescine (asterisk, $P < 0.05$; NS, $P > 0.05$). When there is a specific effect, conditions are indicated between parentheses. In (a) and (b), the iron (Fe) content has been multiplied by 100 to facilitate reading.

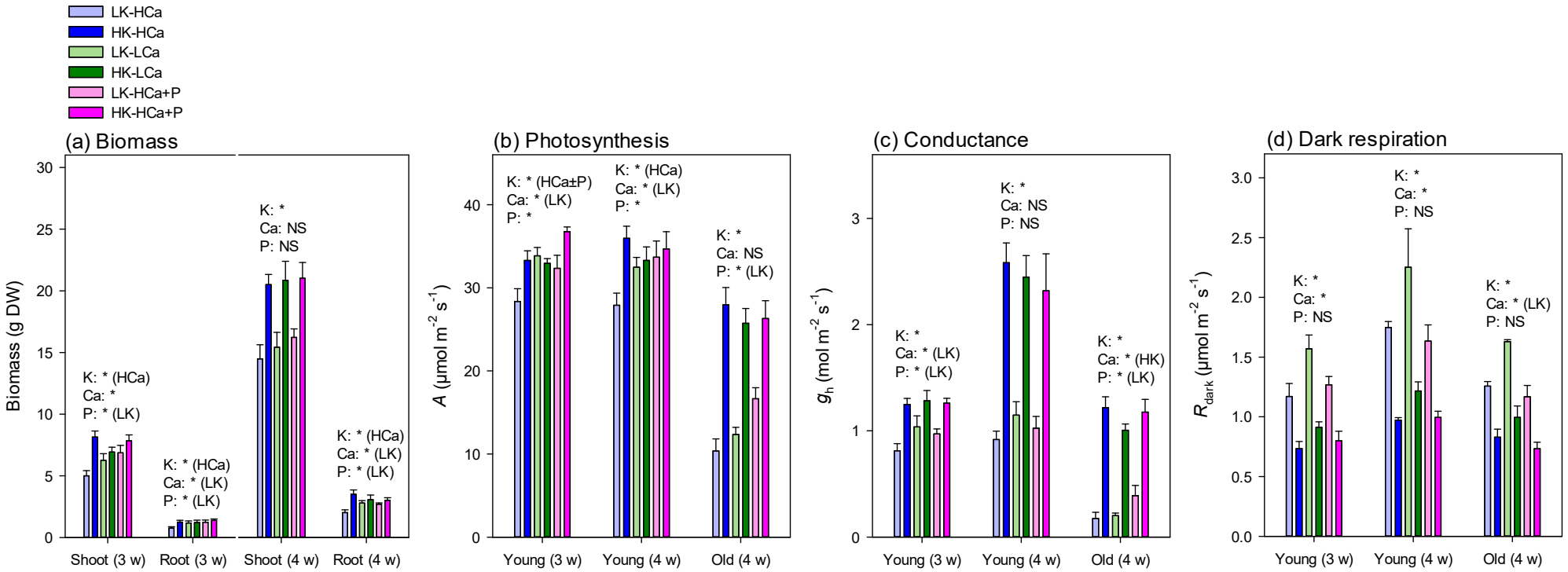

Fig. 2. Biomass and carbon assimilation in sunflower plants after 3 or 4 weeks under different K/Ca/putrescine nutrient conditions. (a) shoot and root biomass (in g dry weight), (b-d) net CO_2 assimilation, conductance for water vapor (under 21% O_2, 400 µmol mol^{-1} CO_2, saturating light) and CO_2 evolution in darkness measured by gas exchange. Statistics as in Fig. 1.

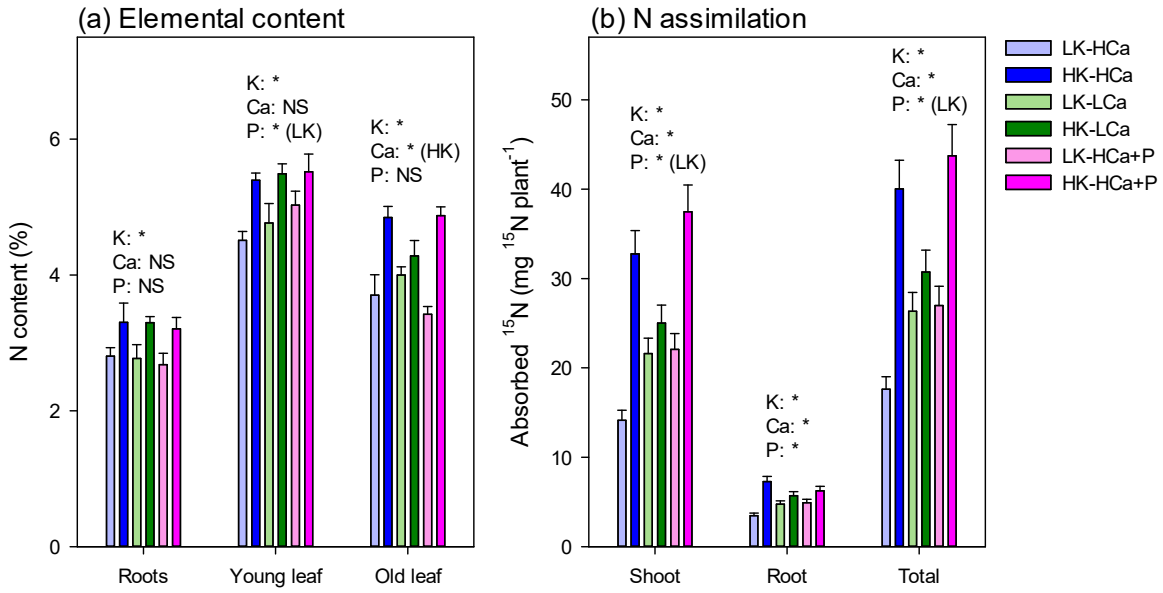

Fig. 3. Nitrogen metabolism in sunflower plants after 4 weeks under different K/Ca/putrescine nutrient conditions. (a) N elemental content (% dry weight), and (b) N assimilation in 72 h assessed with ^{15}N-nitrate labelling. Statistics as in Fig. 1.

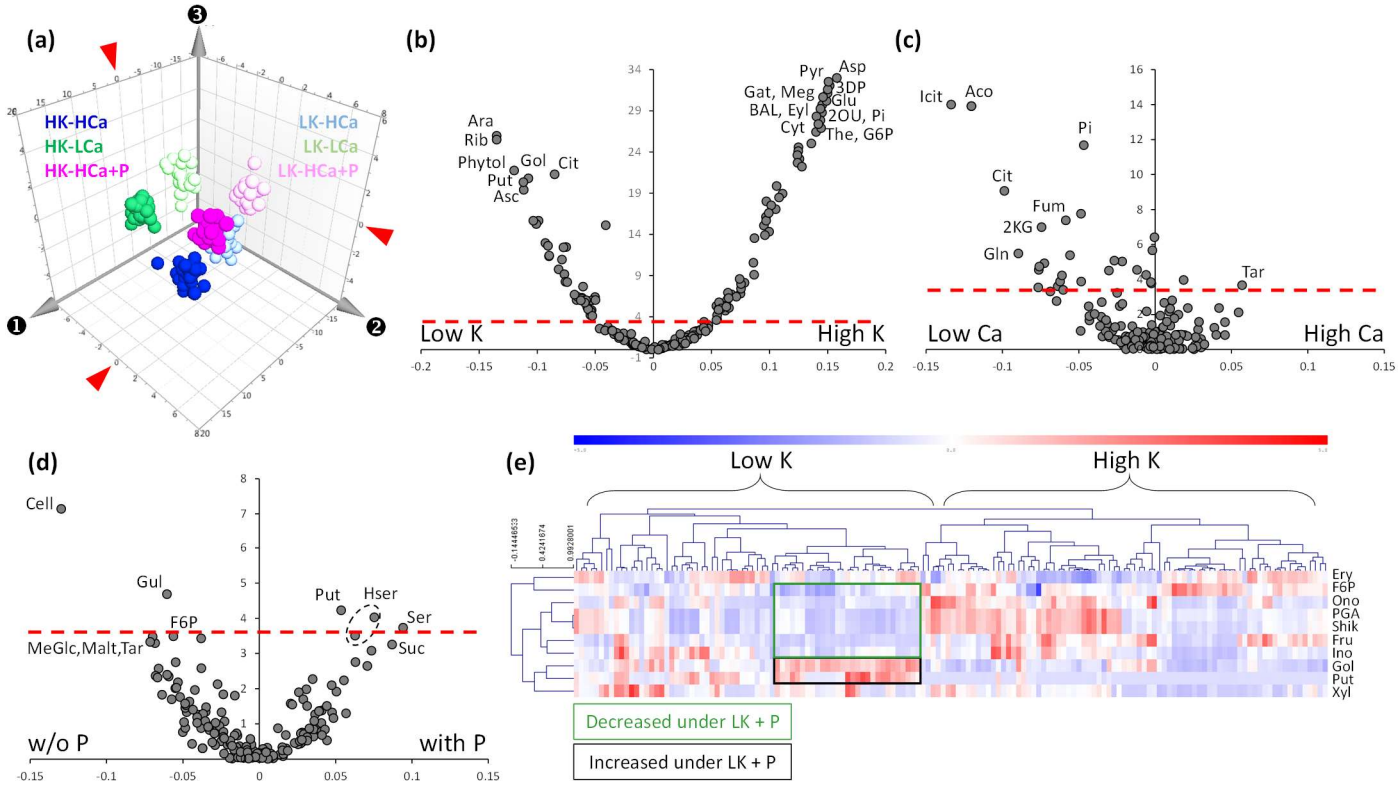

Fig. 4. Metabolomics pattern of leaves under different K/Ca/putrescine nutrient conditions. (a) 3D scatter plot of the OPLS analysis, showing the excellent discrimination of sample groups, with K conditions discriminated along axis 1, both calcium and putrescine effect along axis 2, and the putrescine effect along axis 3. (b-d) Volcano plot (–log(*P*) vs. p_{corr}) depicting discriminant metabolites for the effect of K (b), Ca (c) and putrescine (d) conditions. (e) Heatmap showing metabolites significant for the K *x* putrescine effect ($P < 0.01$). Abbreviations: 2KG, 2-ketoglucose; 2OU, 2-oxogulonate; 3DP, 3-deoxypentitol; Aco, aconitate; Asc, ascorbate; Ara, arabinose; Asp, aspartate; BAL, β-alanine; Cell, cellobiose; Cit, citrate; Cyt, cystathionine; Ery, erythrose; Eyl, erythritol; F6P, fructose 6-phosphate; Fum, fumarate; Gat, glycerate; G6P, glucose 6-phosphate; Gol, glycolate; Gln, glutamine; Glu, glutamate; Gul, gulonate; Hser, homoserine; Icit, isocitrate; Ino, inositol; Malt, maltose; Meg, methylglutarate; MeGlc, methylglucose; Ono, ononitol; PGA, 3-phosphoglycerate; Pi, phosphate; Put, putrescine; Pyr, pyroglutamate; Rib, ribose; Ser, serine; Shik, shikimate; Suc, sucrose; Tar, tartarate; The, threonate; Xyl, xylose. In (b-d), the horizontal red dashed line stands for the Bonferroni *P*-value threshold. In (a), the red arrowheads locate the origin (zero) of each axis. In this figure, old and young leaves have been pooled together.

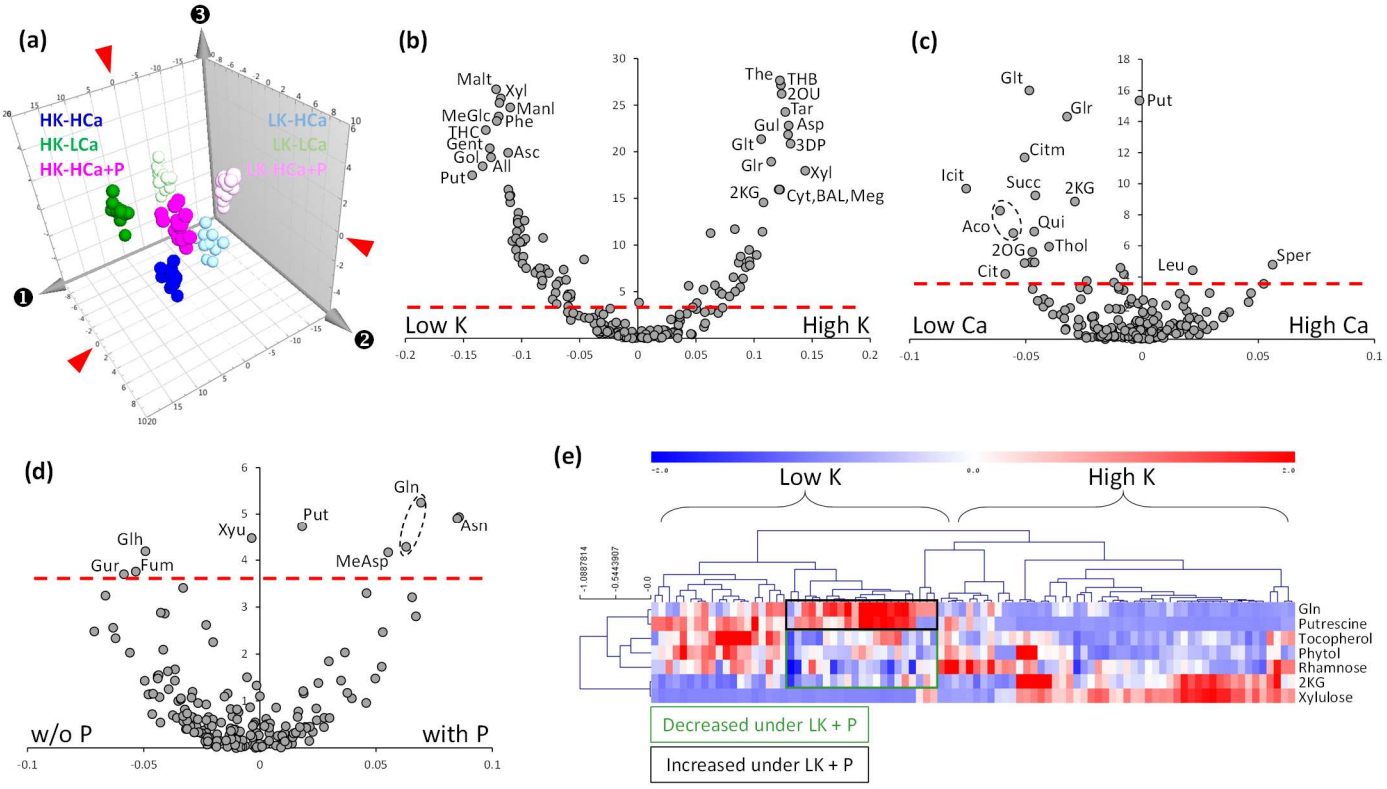

Fig. 5. Metabolomics pattern of roots under different K/Ca/putrescine nutrient conditions. (a) 3D scatter plot of the OPLS analysis, showing the excellent discrimination of sample groups, with K conditions discriminated along axis 1, both calcium and putrescine effect along axis 2, and the putrescine effect along axis 3. (b-d) Volcano plot (–log(*P*) vs. p_{corr}) depicting discriminant metabolites for the effect of K (b), Ca (c) and putrescine (d) conditions. (e) Heatmap showing metabolites significant for the K *x* putrescine effect ($P < 0.01$). Abbreviations: 2KG, 2-ketogluconate; 2OG, 2-oxoglutarate; 2OU, 2-oxogulonate; 3DP, 3-deoxypentitol; Aco, aconitate; All, allose; Asc, ascorbate; Asn, asparagine; Asp, aspartate; BAL, β-alanine; Cit, citrate; Citm, citramalate; Cyt, cystathionine; F6P, fructose 6-phosphate; Fum, fumarate; Gat, glycerate; Gent, gentiobiose; G6P, glucose 6-phosphate; Glr, glucarate; Glt, glutarate; Gol, glycolate; Glh, glyceraldehyde; Gln, glutamine; Glu, glutamate; Gul, gulonate; Gur, glucuronate; Icit, isocitrate; Leu, leucine; Malt, maltose; Manl, mannitol; MeAsp, methylaspartate; Meg, methylglutarate; MeGlc, methylglucose; Phe, phenylalanine; Pi, phosphate; Put, putrescine; Qui, quinate; Sper, spermidine; Succ, succinate; Tar, tartarate; THB, trihydroxybenzoate; THC, transhydroxycinnamate; The, threonate; Thol, threitol; Xyl, xylose; Xyu, xylulose. In (b-d), the horizontal red dashed line stands for the Bonferroni *P*-value threshold. In (a), the red arrowheads locate the origin (zero) of each axis.

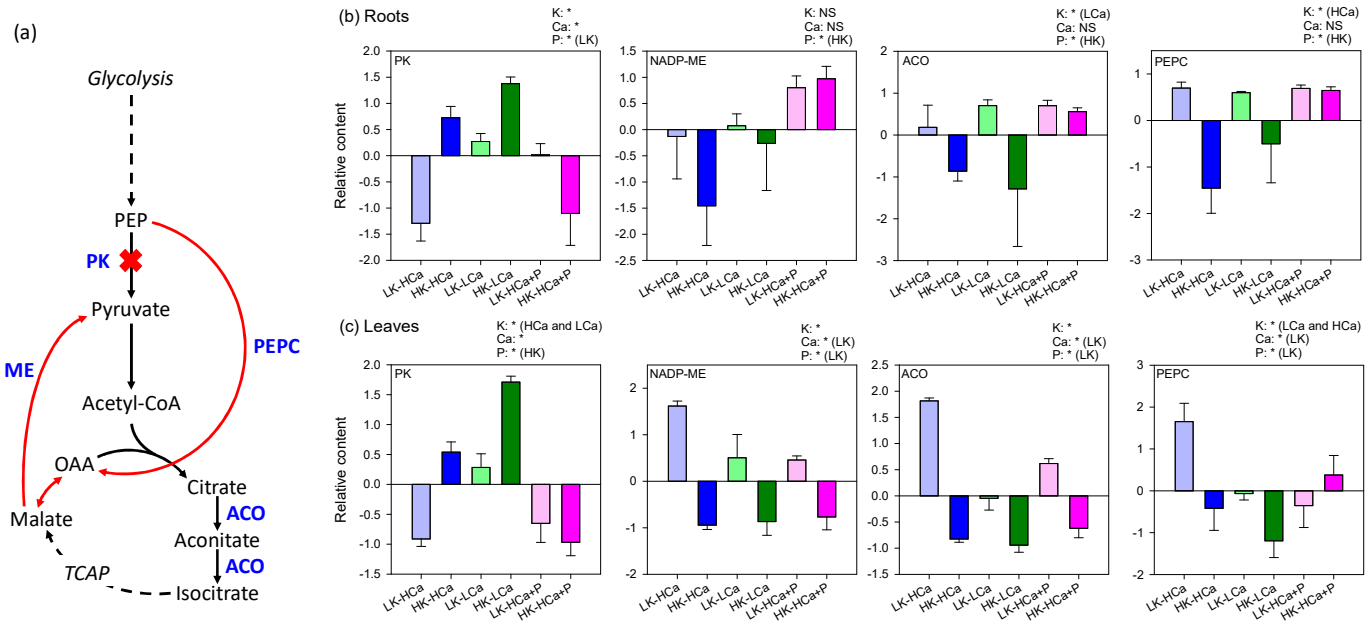

Fig. 6. Relative content in selected enzymes in leaves and roots under different K/Ca/putrescine nutrient conditions. (a), general metabolic scheme to replace the enzymes of interest, showing in red the potential alternative pathway to synthesize pyruvate when pyruvate kinase (PK) activity is inhibited by K deficiency (red cross). (b) and (c), relative contents in roots (b) and leaves (c). Abbreviations: ACO, aconitase; NADP-ME, NADP-dependent malic enzyme; OAA, oxaloacetate; PEP, phospho*enol*pyruvate; PEPC, phospho*enol*pyruvate carboxylase; PK, pyruvate kinase; TCAP, tricarboxylic acid pathway. This figure only shows enzyme isoforms with significant changes (with either K, Ca or putrescine) and does not show proteins that do not change significantly. In this figure, significance means $P<0.01$ (one-way ANOVA). On top, summary that indicates significant differences. In roots, there are also significant changes in PEP carboxykinase (see Table 1).