Supporting information for publication

Integrating proteomics, metabolomics and typical analysis to investigate the uptake and oxidative stress of graphene oxide and polycyclic aromatic hydrocarbons

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Page number: 19

Table number: 4

Figure number: 8

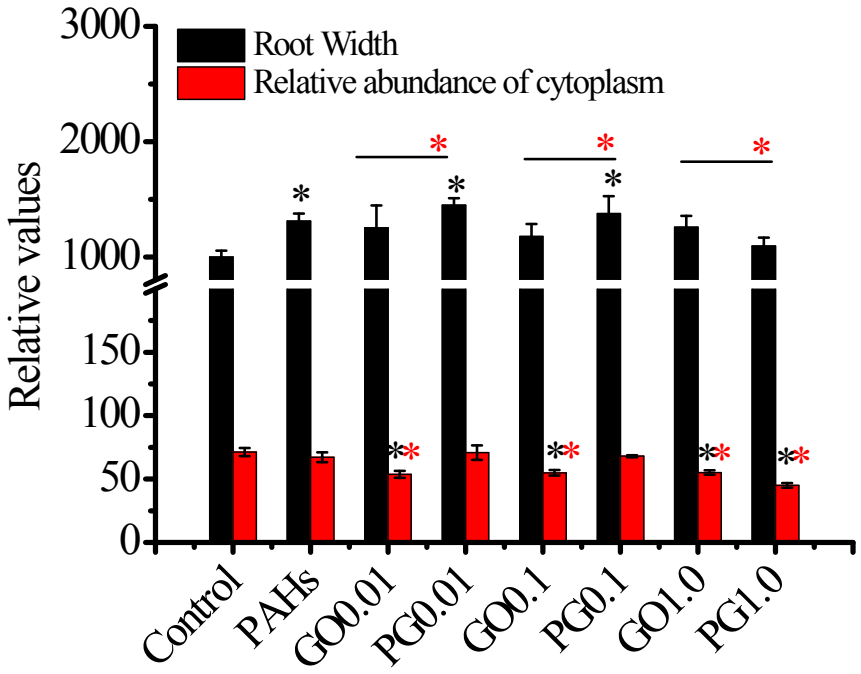

Fig. S1 Development (root width and relative abundance of cytoplasm) of rice roots exposed to PAHs, GO and PG. The relative values of root width and abundance of cytoplasm were analyzed using ImageJ 1.43. The tested concentrations of GO were 0.01-1 mg/L; for example, GO0.1 represents 0.1 mg/L GO. The tested concentration of PAHs was 10 μg/L. Black and red "*" denote significant differences compared with the control and PAHs groups, respectively, at $p < 0.05$. "—" denotes comparisons between pristine GO and PG.

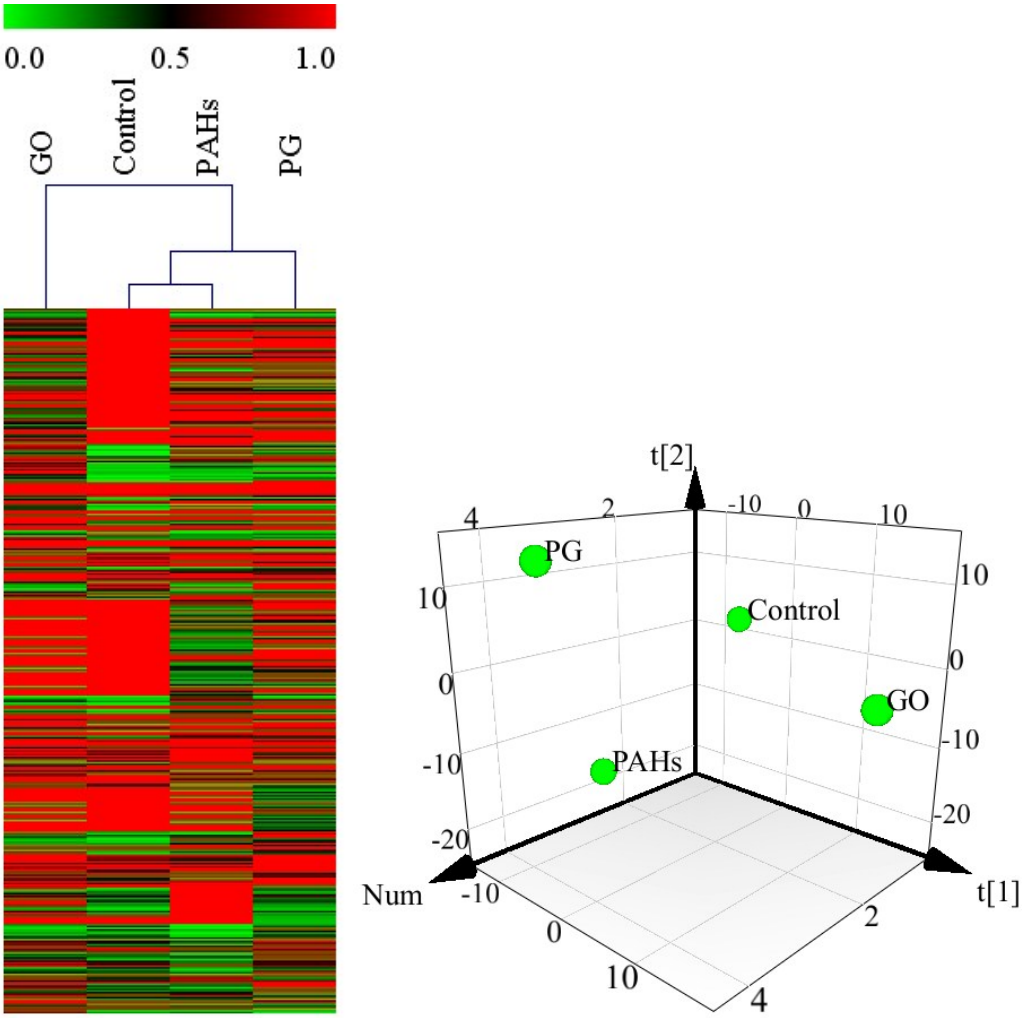

Fig. S2 Proteomic analysis of rice roots exposed to GO, PAHs and PG. The tested concentrations of GO and PAHs were 1.0 mg/L and 10 μg/L, respectively. The heat map represents the relative levels of the differentially expressed proteins. The cluster analysis of the proteins was conducted using a hierarchical clustering model.

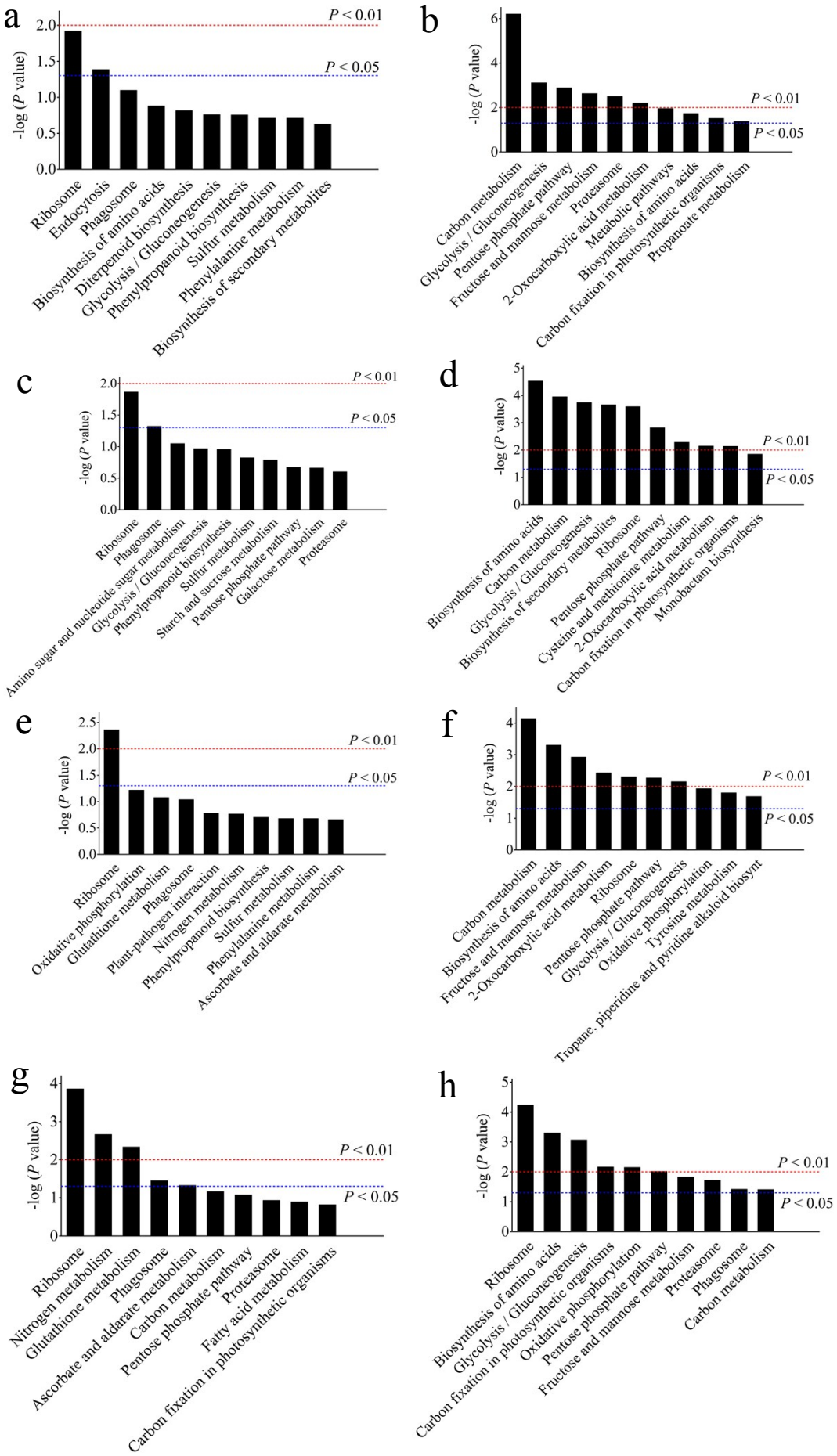
a
-log (P value)
P < 0.01
P < 0.05
Ribosome
Endocytosis
Phagosome
Biosynthesis of amino acids
Diterpenoid biosynthesis
Glycolysis / Gluconeogenesis
Phenylpropanoid biosynthesis
Sulfur metabolism
Phenylalanine metabolism
Biosynthesis of secondary metabolites
b
-log (P value)
P < 0.01
P < 0.05
Carbon metabolism
Glycolysis / Gluconeogenesis
Pentose phosphate pathway
Fructose and mannose metabolism
Proteasome
2-Oxocarboxylic acid metabolism
Metabolic pathways
Biosynthesis of amino acids
Carbon fixation in photosynthetic organisms
Propanoate metabolism
c
-log (P value)
P < 0.01
P < 0.05
Ribosome
Phagosome
Amino sugar and nucleotide sugar metabolism
Glycolysis / Gluconeogenesis
Phenylpropanoid biosynthesis
Sulfur metabolism
Starch and sucrose metabolism
Pentose phosphate pathway
Galactose metabolism
Proteasome
d
-log (P value)
P < 0.01
P < 0.05
Biosynthesis of amino acids
Carbon metabolism
Glycolysis / Gluconeogenesis
Biosynthesis of secondary metabolites
Ribosome
Pentose phosphate pathway
Cysteine and methionine metabolism
2-Oxocarboxylic acid metabolism
Carbon fixation in photosynthetic organisms
Monobactam biosynthesis
e
-log (P value)
P < 0.01
P < 0.05
Ribosome
Oxidative phosphorylation
Glutathione metabolism
Phagosome
Plant-pathogen interaction
Nitrogen metabolism
Phenylpropanoid biosynthesis
Sulfur metabolism
Phenylalanine metabolism
Ascorbate and aldarate metabolism
f
-log (P value)
P < 0.01
P < 0.05
Carbon metabolism
Biosynthesis of amino acids
Fructose and mannose metabolism
2-Oxocarboxylic acid metabolism
Ribosome
Pentose phosphate pathway
Glycolysis / Gluconeogenesis
Oxidative phosphorylation
Tyrosine metabolism
Tropane, piperidine and pyridine alkaloid biosynt
g
-log (P value)
P < 0.01
P < 0.05
Ribosome
Nitrogen metabolism
Glutathione metabolism
Phagosome
Ascorbate and aldarate metabolism
Carbon metabolism
Pentose phosphate pathway
Proteasome
Fatty acid metabolism
Carbon fixation in photosynthetic organisms
h
-log (P value)
P < 0.01
P < 0.05
Ribosome
Biosynthesis of amino acids
Glycolysis / Gluconeogenesis
Carbon fixation in photosynthetic organisms
Oxidative phosphorylation
Pentose phosphate pathway
Fructose and mannose metabolism
Proteasome
Phagosome
Carbon metabolism

Fig. S3 KEGG analysis of differentially expressed proteins in the PAHs, GO and PG groups. (a) KEGG analysis of downregulated proteins in the PAHs group compared with the control group; (b) KEGG analysis of upregulated proteins in the PAHs group compared with the control group; (c) KEGG analysis of downregulated proteins in the GO group compared with the control group; (d) KEGG analysis of upregulated proteins in the GO group compared with the control group; (e) KEGG analysis of downregulated proteins in the PG group compared with the control group; (f) KEGG analysis of upregulated proteins in the PG group compared with the control group; (g) KEGG analysis of downregulated proteins in the PG group compared with the PAHs group; (h) KEGG analysis of upregulated proteins in the PG group compared with the PAHs group.

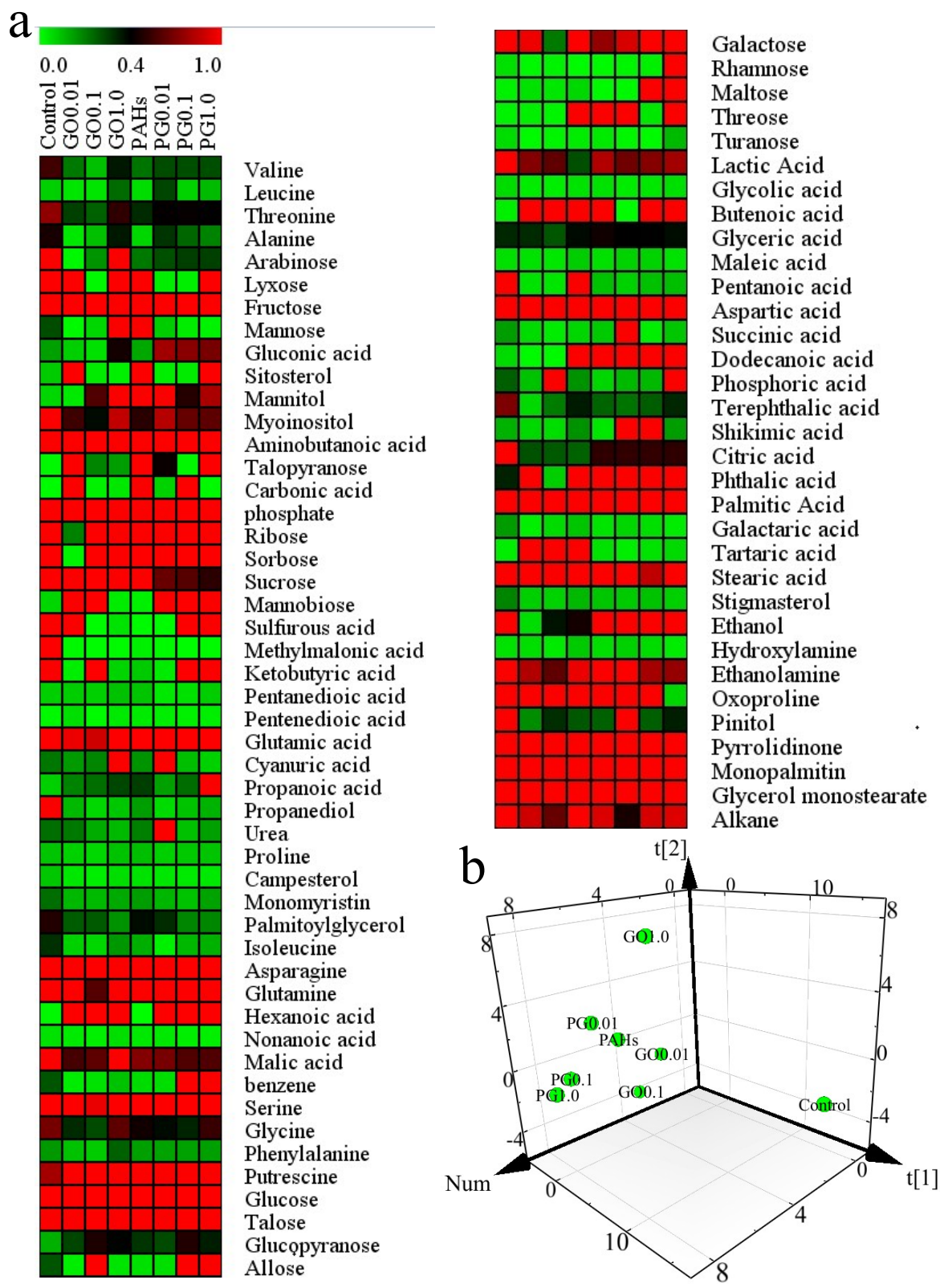

Fig. S4 Metabolic analysis of rice roots exposed to PAHs and PG. (a) Heat map represents the relative levels of identified metabolites; (b) principal component analysis.

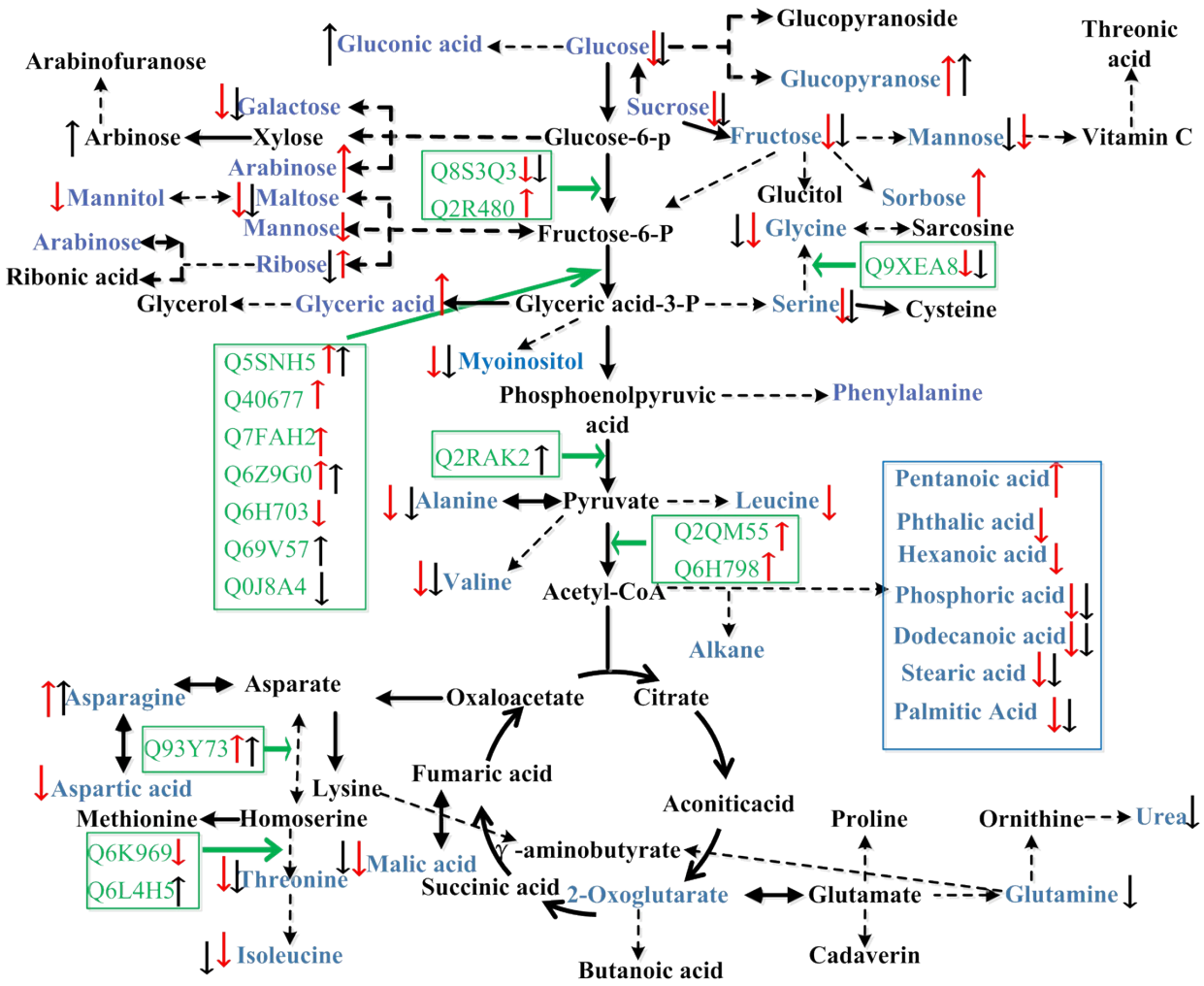

Fig. S5 Connections between protein alterations and metabolic pathway disturbance. The metabolic pathways are established based on KEGG database. The metabolites with blue text are the metabolites detected in the present work. Up- and down-directions of the arrows represent the upregulated or downregulated expression of proteins, respectively. The red arrows denote the proteins that are upregulated or downregulated by the PAHs at 10 μg/L. The black arrows denote the proteins that are upregulated or downregulated by the PG (10 μg/L PAHs with 1 mg/L GO). The details of the proteins are listed in Table S1 in the Supporting Information.

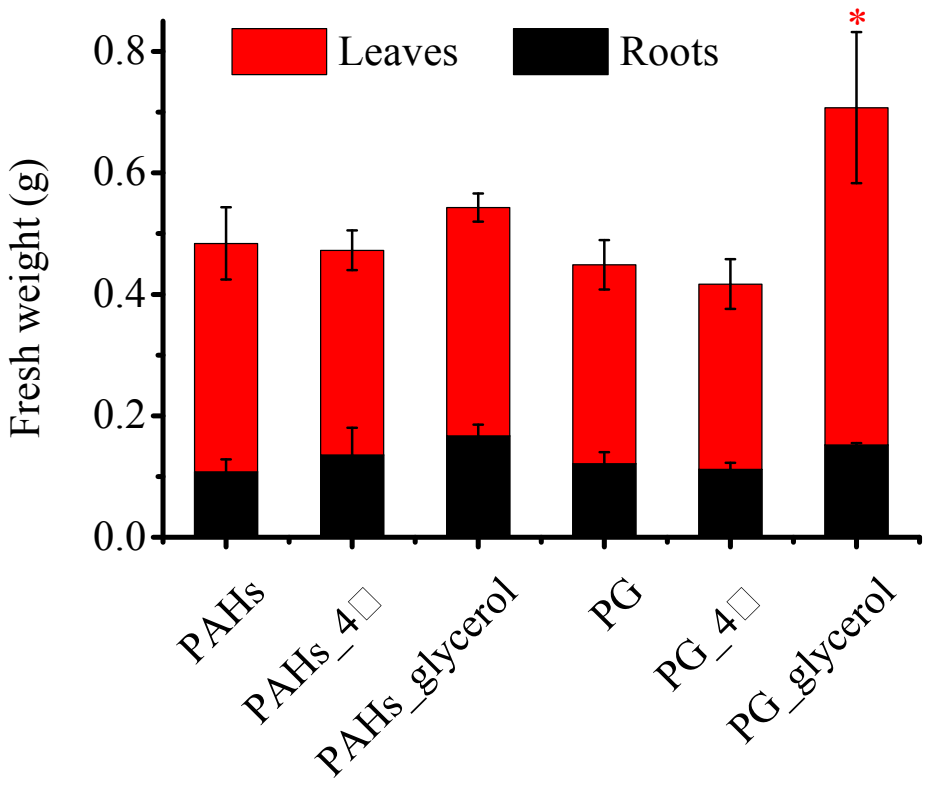

Fig. S6 Biomass of rice roots and leaves exposed to PAHs and PG at low temperature (4℃) and glycerol. PAHs at a concentration of 100 μg/L were tested for consistency with the experiment of PAH uptake. PG represents PAHs at 100 μg/L with GO at 1.0 mg/L. Red "*" represents significant differences between PG and PG with glycerol groups in rice leaves.

Table S1 Differentially expressed proteins related to metabolic pathway. UniProt and descriptions represent the name and function of the proteins in the UniProt database, respectively.

Uniprot	Descriptions	Experimental groups (* E+08)			Statistical analysis	
		Control	PAHs	PG	*P* (control v PAHs)	*P* (control v PG)
Q8S3Q3	apospory-associated protein C	10.1	0.9	0.0	0.048	0.027
Q2R480	6-phosphogluconate dehydrogenase, decarboxylating 2, chloroplastic	0.0	19.8	0.0	0.000	0.500
Q5SNH5	ATP-dependent 6-phosphofructokinase	0.0	3.0	4.8	0.000	0.000
Q40677	fructose-bisphosphate aldolase, chloroplastic	0.0	4.1	2.1	0.000	0.500
Q7FAH2	glyceraldehyde-3-phosphate dehydrogenase 2, cytosolic	0.0	332.1	0.0	0.000	0.500
Q6Z9G0	glyceraldehyde-3-phosphate dehydrogenase	1.1	9.9	13.2	0.000	0.000
Q6H703	glyceraldehyde-3-phosphate dehydrogenase	44.2	19.8	69.6	0.039	0.094
Q69V57	fructose-bisphosphate aldolase	0.0	0.0	97.3	0.500	0.000
Q0J8A4	glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic	552.3	340.5	0.0	0.131	0.002
Q9XEA8	cysteine synthase	20.1	0.0	0.0	0.036	0.041
Q2RAK2	pyruvate kinase 1, cytosolic	0.0	0.0	37.7	0.500	0.000
Q2QM55	pyruvate dehydrogenase E1 component subunit beta-3, chloroplastic	0.0	2.5	0.5	0.000	0.500
Q6H798	acetyl-coenzyme A synthetase	1.8	7.6	4.8	0.005	0.230
Q93Y73	aspartate-semialdehyde dehydrogenase family protein	1.7	8.0	8.5	0.000	0.001
Q6K969	putative homoserine kinase	35.3	4.3	30.7	0.007	0.322
Q6L4H5	putative threonine synthase	1.0	2.2	5.4	0.428	0.006

Table S2 Forty-eight differentially expressed proteins related to transmembrane transport. UniProt and descriptions represent the name and function of the proteins in the UniProt database, respectively. The abbreviations denote the function of the proteins in the interaction network.

Uniprot	Descriptions	Abbreviation	Experimental groups (* E+08)			Statistical analysis		
			Control	PAHs	PG	*P* (control v PAHs)	*P* (control v PG)	*P* (PAHs v PG)
Q10A14	alanine--tRNA ligase	alanine-tRNA ligase	10.2	11.8	39.5	0.429	0.000	0.000
Q6K215	probable aquaporin PIP2-2	aquaporin PIP2-2	0.0	0.0	16.7	0.500	0.000	0.000
Q01859	ATP synthase subunit beta, mitochondrial	ATP synthase-beta	183.0	175.0	0.0	0.465	0.002	0.000
Q8GTK7	ATP synthase subunit delta', mitochondrial	ATP synthase delta	8.0	3.3	0.0	0.112	0.040	0.078
Q6Z0Z9	V-type proton ATPase 16 kDa proteolipid subunit	ATPase 16 kDa proteolipid	0.0	0.0	6.3	0.500	0.000	0.000
Q10P12	V-type proton ATPase subunit a3	ATPase subunit a3	1.8	5.2	7.0	0.080	0.003	0.314
Q76FS3	tubulin beta-6 chain	Beta-6-tubulin	27.7	20.9	0.0	0.228	0.028	0.004
Q6F3B0	dnaJ protein homolog	DnaJ protein	0.0	2.7	2.7	0.500	0.000	0.496
Q7XPW1	K(+) efflux antiporter 2, chloroplastic	K(+) efflux antiporter	0.0	0.0	2.8	0.500	0.000	0.000
Q6ZK52	lysine histidine transporter 1	Lysine histidine transporter	3.9	5.1	16.4	0.399	0.006	0.001
Q8HCQ9	ORFB protein	ORFB protein	0.0	0.0	4.2	0.500	0.000	0.000
Q7XPV4	oxalate-CoA ligase	oxalate-CoA ligase	52.4	44.4	21.3	0.326	0.046	0.025

Q2QS11	proline-tRNA ligase, cytoplasmic	proline-tRNA ligase	4.0	3.0	11.3	0.382	0.041	0.000
Q2RAK2	pyruvate kinase 1, cytosolic	pyruvate kinase	0.0	0.0	37.7	0.500	0.000	0.000
Q6H798	acetyl-coenzyme A synthetase	acetyl-coenzyme A synthetase	1.8	7.7	4.8	0.005	0.230	0.158
Q65XH8	actin-97	actin	51.8	0.0	51.0	0.001	0.458	0.000
Q7XSQ9	probable aquaporin PIP1-2	aquaporin PIP1-2	25.3	68.0	36.4	0.000	0.179	0.035
Q9SXF8	aquaporin PIP 1-3	aquaporin PIP1-3	10.5	0.0	11.0	0.032	0.471	0.000
P50156	probable aquaporin TIP1-1	aquaporin TIP1-1	24.4	39.1	16.1	0.049	0.196	0.020
Q94CS9	probable aquaporin TIP1-2	aquaporin TIP1-2	9.4	22.0	13.8	0.010	0.337	0.099
Q5Z6F0	probable aquaporin TIP2-2	aquaporin TIP2-2	0.0	7.5	0.0	0.000	0.500	0.017
Q0JEQ2	probable L-ascorbate peroxidase 3	ascorbate peroxidase	1.5	13.7	1.7	0.000	0.480	0.015
P45960	tubulin beta-4 chain	beta-4-tubulin	26.8	0.0	21.9	0.018	0.303	0.000
Q0DV32	4-coumarate--CoA ligase-like 1	coumarate-CoA ligase	0.0	7.0	0.0	0.000	0.500	0.018
Q9FE02	cytochrome c oxidase subunit 6b-2	cytochrome c oxidase-6b	0.0	2.5	0.0	0.000	0.500	0.058
Q9SXG0	uncharacterized protein At2g27730, mitochondrial	F1F0-ATPase inhibitor	0.6	4.6	3.8	0.000	0.055	0.348
Q943K7	probable mediator of RNA polymerase II transcription subunit 37c	heat shock protein	36.9	0.0	32.4	0.007	0.330	0.000

Q9AQZ5	heat shock 70 kDa protein 15	heat shock protein 15	43.0	19.8	28.4	0.043	0.159	0.163
Q84TA1	heat shock cognate 70 kDa protein 2	heat shock protein 2	32.0	53.8	33.9	0.042	0.485	0.072
Q0JM17	DEAD-box ATP-dependent RNA helicase 56	helicase	12.0	0.0	10.3	0.018	0.420	0.000
Q0JF01	9-beta-pimara-7,15-diene oxidase	oxidase	18.4	2.9	3.0	0.045	0.073	0.497
Q6Z0N5	threonine--tRNA ligase, mitochondrial 1	threonine-tRNA ligase	1.2	5.3	0.8	0.005	0.387	0.033
Q60EY9	mitochondrial dicarboxylate/tricarboxylate transporter DTC	translocator	18.8	37.2	27.2	0.006	0.206	0.142
Q8GU86	ABC transporter G family member 43	ABC transporter	18.4	2.4	0.0	0.042	0.034	0.054
Q08479	adenylate kinase 3	adenylate kinase	12.2	27.3	28.1	0.006	0.041	0.494
Q0DJC0	ADP, ATP carrier protein 1, mitochondrial	ADP, ATP carrier protein 1	0.0	72.0	88.2	0.000	0.000	0.311
B7EKW3	probable aquaporin PIP2-1	aquaporin PIP2-1	26.0	0.0	0.0	0.018	0.032	0.500
Q6EUQ9	V-type proton ATPase catalytic subunit A	ATPase catalytic subunit A	30.5	0.0	0.0	0.015	0.015	0.500
Q40665	tubulin beta-3 chain	Beta-3-tubulin	0.0	22.0	22.1	0.000	0.000	0.469
P46265	tubulin beta-5 chain	Beta-5-tubulin	0.0	22.0	22.0	0.000	0.000	0.468

Q9SNQ3	putative cytochrome c oxidase subunit 5b-like	cytochrome c oxidase-5b	0.0	4.3	3.3	0.000	0.000	0.306
Q8H4X9	GMP synthase [glutamine-hydrolyzing]	GMP synthase	10.2	0.0	1.3	0.036	0.047	0.500
Q0J4P2	heat shock protein 81-1	heat shock protein 81-1	137.0	230.0	0.0	0.018	0.002	0.000
Q8RUP8	methionine--tRNA ligase, cytoplasmic	methionine-tRNA ligase	0.0	11.0	9.3	0.000	0.000	0.348
Q7JAI4	NADH dehydrogenase subunit 9	NADH dehydrogenase	0.0	8.4	6.9	0.000	0.000	0.300
Q5SNH5	ATP-dependent 6-phosphofructokinase 6	phosphofructokinase	0.0	3.0	4.8	0.000	0.000	0.227
Q8L6I1	plasma membrane ATPase 1	plasma membrane ATPase	0.0	40.2	41.5	0.000	0.000	0.496
Q6ZL94	probable succinyl-CoA ligase [ADP-forming] subunit alpha, mitochondrial	succinyl-CoA ligase	0.0	28.8	25.7	0.000	0.000	0.324

Table S3 Twenty-five differentially expressed proteins related to oxidative stress. UniProt and descriptions represent the name and functions of the proteins in the UniProt database, respectively. The abbreviations denote the function of the proteins in the interaction network.

Uniprot	Descriptions	Abbreviation	Experimental groups (* E+08)			Statistical analysis		
			Control	PAHs	PG	*P* (control v PAHs)	*P* (control v PG)	*P* (PAHs v PG)
B7E6Z4	L-ascorbate peroxidase 1, cytosolic	ascorbate peroxidase 1	0.0	389.0	422.0	0.000	0.000	0.442
Q06398	probable glutathione S-transferase GSTU6	GSTU6	0.0	33.0	0.0	0.000	0.500	0.001
Q0DWH1	alcohol dehydrogenase class-3	alcohol dehydrogenase	0.0	6.3	6.3	0.000	0.000	0.460
Q2R480	6-phosphogluconate dehydrogenase, decarboxylating 2, chloroplastic	phosphogluconate dehydrogenase	0.0	19.8	0.0	0.000	0.500	0.003
Q40677	fructose-bisphosphate aldolase, chloroplastic	aldolase	0.0	4.1	2.1	0.000	0.500	0.136
Q5SNH5	ATP-dependent 6-phosphofructokinase 6	phosphofructokinase	0.0	3.0	4.8	0.000	0.000	0.227
Q6ER94	2-Cys peroxiredoxin BAS1, chloroplastic	2-Cys peroxiredoxin	0.0	9.1	4.5	0.000	0.000	0.087
Q9XHX4	cytosolic isocitrate dehydrogenase [NADP]	isocitrate dehydrogenase	0.0	13.4	13.5	0.000	0.000	0.492

Q0JEQ2	probable L-ascorbate peroxidase 3	ascorbate peroxidase 3	1.5	13.7	1.7	0.000	0.480	0.015
P0C5D1	1-Cys peroxiredoxin B	1-Cys peroxiredoxin	70.8	0.0	130.0	0.001	0.027	0.000
Q852M1	probable aldehyde oxidase 2	aldehyde oxidase	4.4	15.5	11.9	0.007	0.092	0.253
Q93WY5	GST 23	GST	56.5	16.8	18.4	0.013	0.027	0.464
A2ZYR4	cysteine proteinase inhibitor 1	cysteine proteinase inhibitor	85.2	26.9	67.9	0.014	0.268	0.000
Q75IS1	peroxidase 5	peroxidase 5	25.5	42.6	28.0	0.033	0.420	0.092
Q0D3N0	peroxidase 2	peroxidase 2	18.6	0.0	0.0	0.037	0.034	0.500
Q60EA3	sulfite reductase [ferredoxin], chloroplastic	sulfite reductase	2.4	6.9	3.9	0.044	0.384	0.130
Q9FE01	L-ascorbate peroxidase 2, cytosolic	ascorbate peroxidase 2	289.0	197.0	0.0	0.188	0.002	0.000
Q6ZBH2	sorbitol dehydrogenase	sorbitol dehydrogenase	2.7	3.0	10.0	0.460	0.003	0.000
O04985	non-symbiotic hemoglobin 2	hemoglobin	0.0	0.0	7.0	0.500	0.000	0.000
Q0D9C4	catalase isozyme B	catalase	0.0	0.0	84.9	0.500	0.000	0.000
Q2RAK2	pyruvate kinase 1, cytosolic	pyruvate kinase	0.0	0.0	37.7	0.500	0.000	0.000
Q42997	ferredoxin--nitrite reductase, chloroplastic	ferredoxin--nitrite reductase	0.0	0.0	5.7	0.500	0.000	0.000
Q6ERW5	probable cinnamyl alcohol dehydrogenase 8D	cinnamyl alcohol dehydrogenase	0.0	0.0	7.7	0.500	0.000	0.000

Q7XJ02	probable L-ascorbate peroxidase 7, chloroplastic	ascorbate peroxidase 7	0.0	0.0	11.8	0.500	0.000	0.000
Q8H8U5	protein IN2-1 homolog B	IN2-1 homolog B	0.0	0.0	4.8	0.500	0.000	0.000

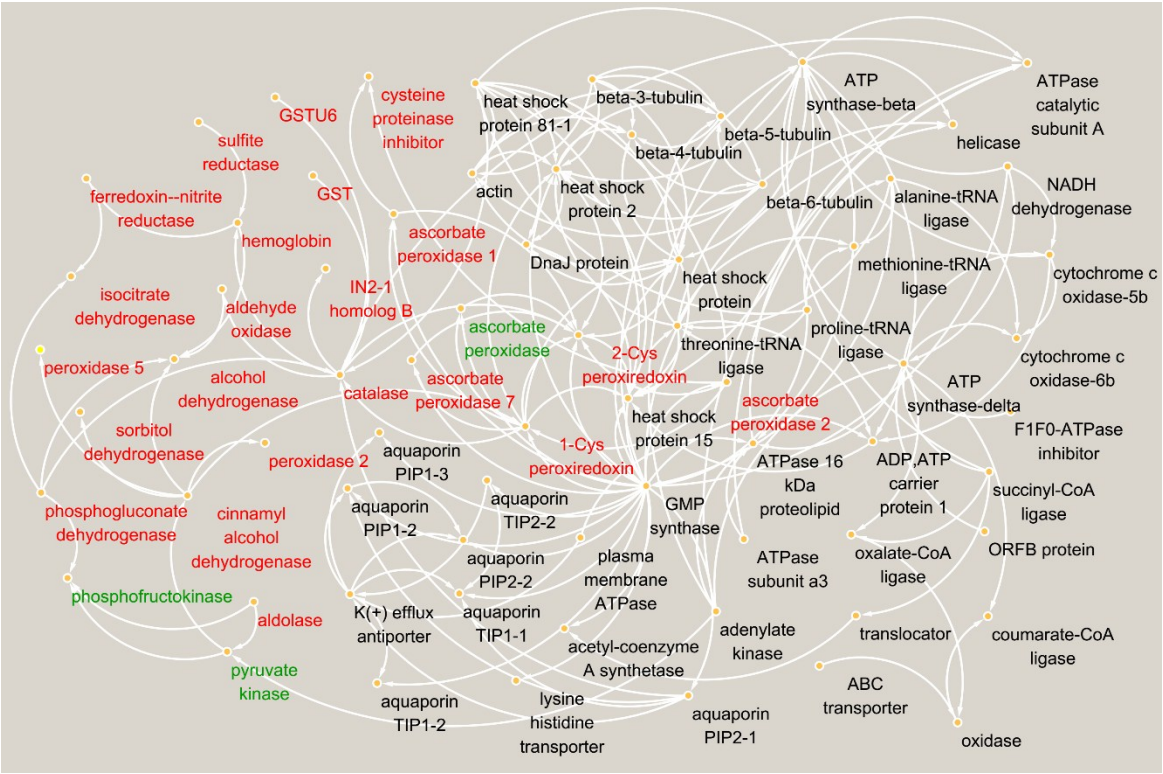

Fig. S7 The protein-protein interactions between oxidative stress and transmembrane transport. The red and black colored proteins are related to oxidative stress and transmembrane transport, respectively. The green colored proteins play dual roles in oxidative stress and transmembrane transport.

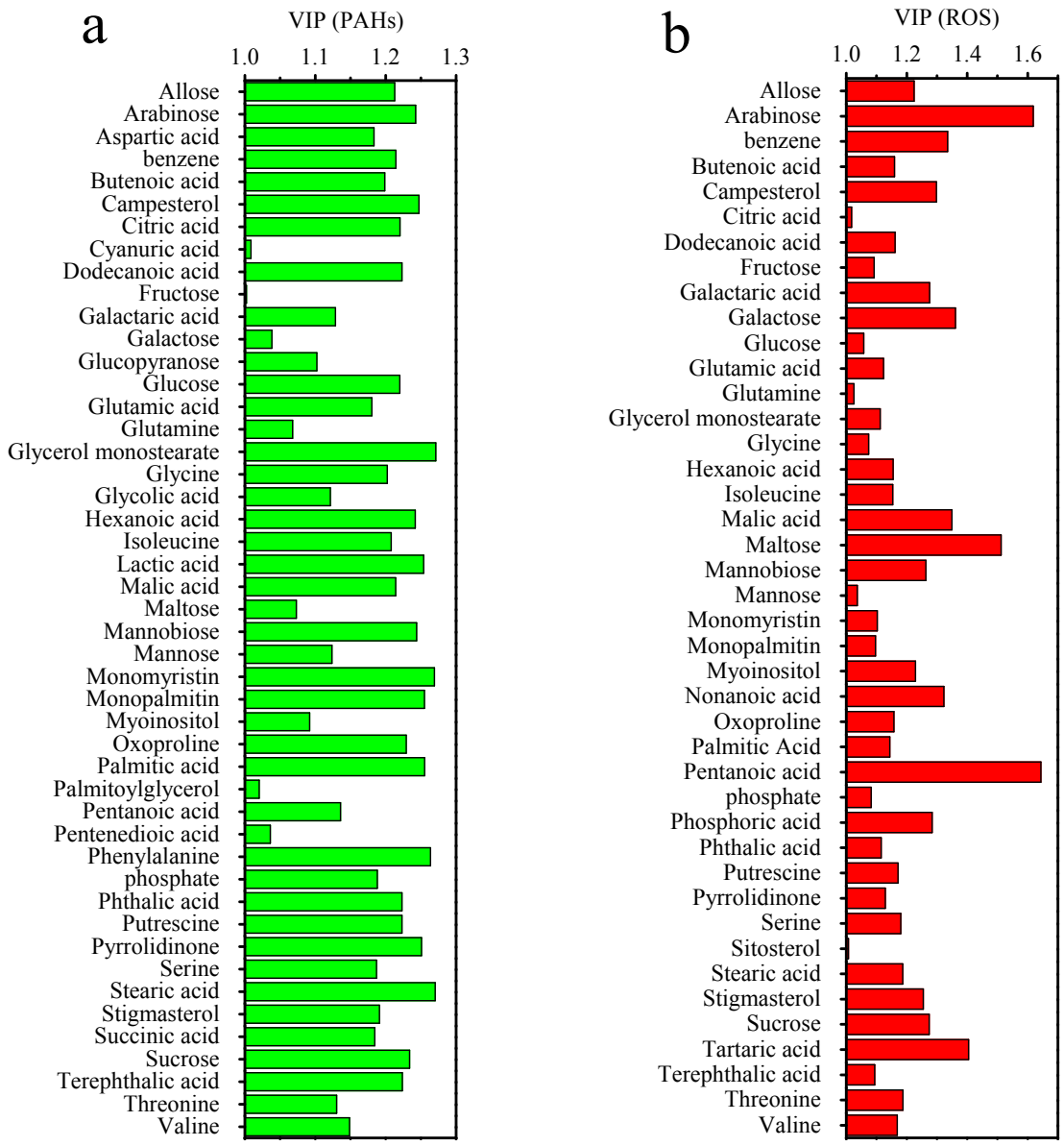

Fig. S8 Variable importance in the projection (VIP) of metabolites screened by orthogonal projections to latent structures discriminant analysis (OPLS-DA). (a) Uptake of PAHs as the biological endpoint (Y variable) and metabolic levels as X variables; (b) reactive oxygen species (ROS) as the biological endpoint (Y variable) and metabolic levels as X variables. The total concentrations of tested ten PAHs are used as the uptake of PAHs. Relative levels of ROS are represented by the fluorescence intensity using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) as

a fluorescence probe. Relative levels of metabolites are represented by the peak area of metabolites from GC-MS measurement.

Table S4 The components of the culture solution. The pH was adjusted to 5 with 1 mol/L hydrochloric acid.

Reagent	Preparation (g/L distilled water)	Usage
NH_4NO_3	11.42500	10 mL/L
$NaH_2PO_4 \cdot 2H_2O$	5.03800	
K_2SO_4	8.92500	
$CaCl_2$	11.07500	
$MgSO_4 \cdot 7H_2O$	40.50000	
$MnCl_2 \cdot 4H_2O$	0.18750	
$(NH_4)_6 \cdot MO7O_{24} \cdot 4H_2O$	0.00925	
H_3PO_4	0.11675	
$ZnSO_4 \cdot 7H_2O$	0.00438	
$CuSO_4 \cdot 5H_2O$	0.00388	
$FeCl_3 \cdot 6H_2O$	0.96250	
Citric acid (monohydrate)	1.48750	

Dissolve and then combine with 6.25 mL of concentrated H_2SO_4