Supporting Information

CdS Nanoparticles in Soil Induce Metabolic Reprogramming in Broad Bean (*Vicia faba* L.) Roots and Leaves

Liyan Tian[§], Huiling Zhang[§], Xiaopeng Zhao[§], Xueyuan Gu[§], Jason C. White[#], Lijuan Zhao[§], Rong Ji[§,*]

[§]State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, China

[#]Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station (CAES), New Haven, Connecticut 06504, United States

*Corresponding author. Tel: +86 025-8968 0581; fax: +86 025-8968 0581.

Email: ji@nju.edu.cn

Soil

The soil samples for the analysis of heavy metals (Cu, Zn, As, Cd and Pb), pH, cation exchange capacity (CEC), organic matter (OM), total nitrogen (TN) and total phosphorus (TP) were air-dried for 2 weeks and then sieved through a 2-mm sieve (10 mesh). The pH (solid: distilled water = 1:2.5) of the soil samples was measured using a pH meter; the OM content of the soil was determined using the Walkley-Black method,[1] and the CEC by the analysis of a 1 M KCl extract, following Fageria et al.[2] TN was determined by the Berthelot reaction method and TP by the molybdenum blue method, as described in the standard methods of the Soil Science Society of China (SSSC) (**Table S1**).[3]

GC-MS Analysis

The GC-MS analysis was performed on an Agilent 7890B gas chromatograph system coupled to an Agilent 5977A MSD mass detector (Agilent Technologies Inc., CA, USA). The derivatives were separated on a DB-5MS fused-silica capillary column (30 m × 0.25 mm × 0.25 μm, Agilent J & W Scientific, Folsom, CA, USA). Helium (> 99.999%) served as the carrier gas, with a constant flow rate of 1 mL min^{-1}. The injector temperature was maintained at 260°C. The injection volume was 1 μL. An aliquot of the quality control (QC) sample was injected every four samples throughout the analytical run, to provide a set of data from which repeatability could be assessed. The initial oven temperature was 60°C, ramped to 125°C at a rate of 8°C min^{-1}, to 210°C at a rate of 5°C min^{-1}, to 270°C at a rate of 10°C min^{-1}, to 305°C at a rate of 20°C min^{-1} and finally held at 305°C for 5 min. The temperature of the MS quadrupole and ion source (electron impact) was set to 150°C and 230°C, respectively. The collision energy was 70 eV. Mass spectrometric data were acquired using full-scan mode (*m/z* 50−500).

Table S1. Physicochemical properties and total metal contents of field soil.

pH	OM (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	CEC (cmol kg^{-1})	Clay (%)	Silt (%)	Sand (%)
6.91 ± 0.08	48.3 ± 0.4	2.37 ± 0.06	1.10 ± 0.15	18.5 ± 0.5	30.6 ± 0.2	33.4 ± 0.6	34.2 ± 0.3	32.4 ± 0.5
Cd (mg kg^{-1})	**Cu (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Pb (mg kg^{-1})**	**Cr (mg kg^{-1})**	**K (g kg^{-1})**	**Mg (g kg^{-1})**	**Ca (g kg^{-1})**	**Fe (g kg^{-1})**
0.15 ± 0.02	34.5 ± 0.7	88.4 ± 0.4	18.2 ± 0.9	50.0 ± 0.4	13.7 ± 0.3	2.13 ± 0.06	5.15 ± 0.14	27.5 ± 0.3

Table S2. Concentration of nutrient elements in the tissues (dry weight) of broad bean plants exposed for 28 days to 10 and 100 mg CdS-NPs kg^{-1}. Data are reported as the mean and standard deviation (n = 4). Different letters indicate significant ($p < 0.05$) differences among the values according to a one-way ANOVA followed by Tukey's test.

		Element concentration in tissues (mg g plant dry weight^{-1})						
Tissues	**Treatment**	**K**	**Ca**	**Mg**	**Fe**	**C**	**N**	**S**
Roots	Control	2.7 ± 0.5 a	6.2 ± 0.9 a	4.2 ± 0.8 a	2.5 ± 0.9 a	409.5 ± 2.9 a	52.5 ± 2.3 a	4.3 ± 0.2 a
	10 mg CdS-NPs kg^{-1}	2.7 ± 0.3 a	6.3 ± 0.6 a	4.8 ± 0.4 a	2.2 ± 0.6 a	405.1 ± 5.5 a	49.6 ± 2.6 a	4.6 ± 0.1 a
	100 mg CdS-NPs kg^{-1}	2.8 ± 0.4 a	7.0 ± 0.5a	5.0 ± 0.6 a	2.1 ± 1.0 a	407.5 ± 2.5 a	52.5 ± 1.8 a	4.4 ± 0.5 a
Stems	Control	3.3 ± 0.4 a	3.8 ± 0.6 a	1.4 ± 0.2 a	0.1 ± 0.0 a	396.6 ± 6.0 a	32.5 ± 2.7 a	4.7 ± 0.3 a
	10 mg CdS-NPs kg^{-1}	3.4 ± 0.4 a	3.4 ± 0.4 a	1.3 ± 0.1 a	0.1 ± 0.0 a	403.4 ± 3.2 a	33.7 ± 4.7 a	4.5 ± 0.2 a

	100 mg CdS-NPs kg^{-1}	3.3 ± 0.4 a	3.8 ± 0.4 a	1.4 ± 0.1 a	0.1 ± 0.0 a	397.1 ± 3.2 a	36.5 ± 6.6 a	4.3 ± 0.2 a
Leaves	Control	2.2 ± 0.3 a	7.1 ± 0.7 a	4.4 ± 0.5 a	0.2 ± 0.1 a	408.0 ± 2.5 a	86.8 ± 2.3 a	4.3 ± 0.2 a
	10 mg CdS-NPs kg^{-1}	2.2 ± 0.1 a	7.1 ± 0.7 a	4.3 ± 0.5 a	0.2 ± 0.0 a	412.3 ± 6.1 a	84.9 ± 2.4 a	4.4 ± 0.3 a
	100 mg CdS-NPs kg^{-1}	2.2 ± 0.1 a	7.1 ± 0.7 a	4.2 ± 0.3 a	0.2 ± 0.0 a	411.7 ± 2.1 a	83.3 ± 2.2 a	4.2 ± 0.2 a

Table S3. Concentration of water-extractable metals and nutrient elements after 28 days in soil containing 10 and 100 mg CdS-NPs kg^{-1}. Data are reported as the mean and standard deviation (n = 6). Different letters indicate significant ($p < 0.05$) differences among the values according to a one-way ANOVA followed by Tukey's test.

Treatments	**Cd (µg kg^{-1})**	**Zn (µg kg^{-1})**	**K (µg kg^{-1})**	**Ca (µg kg^{-1})**	**Mg (µg kg^{-1})**
Control	9.8±0.2 b	13.1 ± 1.4 a	14.8 ± 0.7 a	146.5 ± 15.3 a	35.9 ± 3.9 a
10 mg CdS-NPs kg^{-1}	10.3±0.7 b	9.6 ± 2.9 b	14.1 ± 0.1 a	144.6 ± 2.2 a	32.2 ± 0.4 a
100 mg CdS-NPs kg^{-1}	25.2±7.8 a	5.5 ± 0.9 b	14.2 ± 0.3 a	153.7 ± 4.9 a	33.8 ± 0.7 a

Table S4. Selected metabolites in the leaves of broad bean plants exposed for 28 days to 10 and 100 mg CdS-NPs kg^{-1}. Data are reported as the mean and standard deviation (n = 4).

Metabolite	VIP	CdS-NPs concentration (mg kg^{-1})			
		10	100	10	100
		p		Fold change[a]	
Amino acids and derivatives					
Alanine	1.15	0.631	0.028	1.0	1.2
D-Alanyl-D-alanine	2.29	0.105	0.011	4.7	4.2
N-Acetyl-5-hydroxytryptamine	2.71	0.497	0.101	NM[b]	NM
L-Allothreonine	0.37	0.485	0.012	1.5	0.7
N-Carbamylglutamate	2.43	0.020	0.015	1.4	1.6
Cycloleucine	0.55	0.023	0.008	1.5	2.0
Cysteinylglycine	3.1	0.448	0.083	1.1	1.1
N-Ethylglycine	1.90	0.519	0.618	0.9	0.9
Glutamic acid	0.46	0.009	0.012	1.5	1.8
N-Methyl-DL-alanine	0.12	0.991	0.035	1.0	1.2
N-Methyl-L-glutamic acid	0.24	0.046	0.259	1.6	1.5
Nicotinoylglycine	0.16	0.207	0.019	1.1	1.2
Norleucine	0.24	0.764	0.002	1.0	1.4
Oxoproline	0.32	0.008	0.005	1.2	1.5
Ornithine	1.23	0.086	0.008	4.2	6.6
Threo-β-hyrdoxyaspartate	3.17	0.552	0.104	1.0	1.4
N-Containing compounds					
Aminomalonic acid	0.19	0.118	0.005	1.2	1.3
3-Aminoisobutanoic acid	0.41	0.145	0.006	1.4	1.7
5-Aminovaleric acid	0.18	0.173	0.022	1.3	1.2
2,4-Diaminobutanoic acid	0.23	0.223	0.002	1.1	1.4
2-Amino-3-(4-hydroxyphenyl) propanoic acid	0.79	0.042	0.002	2.0	2.9
2'-Deoxycytidine 5'-triphosphate	0.38	0.071	0.015	1.3	1.6
Ethanolamine	0.09	0.878	0.038	1.0	1.1
Guanine	5.65	0.436	-	0.7	[c]ND
3-Hydroxypyridine	1.68	0.568	0.445	0.1	1.1
Isoxanthopterin	0.76	0.029	0.007	0.5	0.4
3-Methylamino-1,2-propanediol	0.38	0.392	0.000	1.1	1.6
1-Methylhydantoin	0.16	0.220	0.040	1.1	1.2
Noradrenaline	0.43	0.013	0.004	1.5	1.7
Putrescine	0.18	0.413	0.002	1.2	1.3
Thymine	1.85	0.049	0.145	41.8	54.2
Urea	2.00	0.561	0.081	1.1	1.2
Sugar and sugar alcohols					
Acetol	0.04	0.220	0.040	1.1	1.0

(-)-Dihydrocarveol	1.69	0.056	0.131	0.6	0.7
Dodecanol	0.57	0.013	0.001	1.4	2.1
Fucose	0.16	0.020	0.005	0.8	0.8
Galactinol	0.37	0.038	0.019	0.9	0.8
Glucose	1.56	0.606	0.684	1.0	1.1
Glycerol	0.12	0.171	0.044	0.9	0.9
1-Kestose	2.69	-	-	NM	NM
Maltose	2.21	0.519	0.618	0.9	0.7
Raffinose	1.78	0.446	0.212	1.1	2.6
Organic acids					
Adipic acid	3.97	-	-	NM	NM
Allantoic acid	3.06	-	0.134	ND	4.2
Benzoic acid	0.13	0.007	0.081	1.2	1.2
Benzoylformic acid	0.21	0.352	0.038	1.2	0.8
Cumic acid	0.32	0.341	0.017	1.2	1.5
Fumaric acid	0.22	0.016	0.006	1.5	1.3
Galactonic acid	0.12	0.078	0.047	1.2	1.2
D-Galacturonic acid	1.75	0.668	0.246	0.9	0.7
Glycolic acid	0.28	0.032	0.006	1.2	1.4
4-Hydroxybenzoic acid	2.81	0.258	0.007	1.5	2.0
4-Hydroxybutanoic acid	0.28	0.489	0.011	1.1	1.5
6-Hydroxy caproic acid dimer	1.47	0.510	0.068	1.0	0.5
6-Hydroxy caproic acid trimer	2.25	0.191	0.279	0.7	0.6
Isocitric acid	0.35	0.045	0.077	1.6	1.6
Itaconic acid	0.97	0.177	0.040	3.2	4.5
Maleic acid	0.61	0.027	0.020	2.3	2.5
Nicotinic acid	0.39	0.157	0.004	3.7	2.9
Pipecolinic acid	0.24	0.142	0.008	1.3	1.4
Pyruvic acid	0.18	0.247	0.046	1.2	0.8
Saccharic acid	0.07	0.045	0.212	1.1	1.1
Others					
N-Acetylisatin	0.13	0.037	0.310	1.4	1.2
Butyraldehyde	2.56	0.837	0.028	1.2	1.4
Carnitine	1.04	-	-	NM	NM
Citral	2.38	0.800	0.028	1.2	1.4
Creatine	0.2	0.350	0.028	1.1	1.3
Cortisone	1.61	0.077	0.007	1.6	2.6
Dihydroxyacetone	0.25	0.305	0.003	1.1	1.4
2-Ketovaleric acid	0.22	0.063	0.007	1.3	1.3
Flavone	2.88	0.135	0.000	13.0	39.2
Guaiacol	0.17	0.040	0.717	2.2	1.5
1-Hydroxyanthraquinone	1.31	0.787	0.008	1.4	2.1
4-Hydroxy-3-methoxycinnamaldehyde	4.88	0.209	-	0.6	ND

β-Hydroxypyruvic acid	0.26	0.045	0.005	1.2	1.4
Indolelactic acid	0.28	0.021	0.074	1.7	1.4
Monoolein	0.17	0.170	0.022	1.2	1.3
2-Monoolein	2.49	0.646	0.181	1.0	1.0
Mono(2-ethylhexyl) phthalate	0.7	0.569	0.049	2.4	4.2
Noradrenaline	0.43	0.013	0.004	1.5	1.7
Octanal	0.65	0.646	0.001	1.2	2.4
Phenylacetaldehyde	2.43	0.178	0.391	0.5	0.7
Prostaglandin E2	0.8	0.059	0.024	3.1	3.9
Synephrine	1.68	0.604	0.995	2.6	2.2
4-Vinylphenol dimer	0.19	0.038	0.019	1.2	1.3

[a]Relative abundance of metabolites in the roots of plants in the 10 and 100 mg CdS-NPs kg^{-1}treatments compared with the control.

[b]New metabolites that appeared in plants exposed to the two doses of CdS-NPs.

[c]Not detected.

Figures

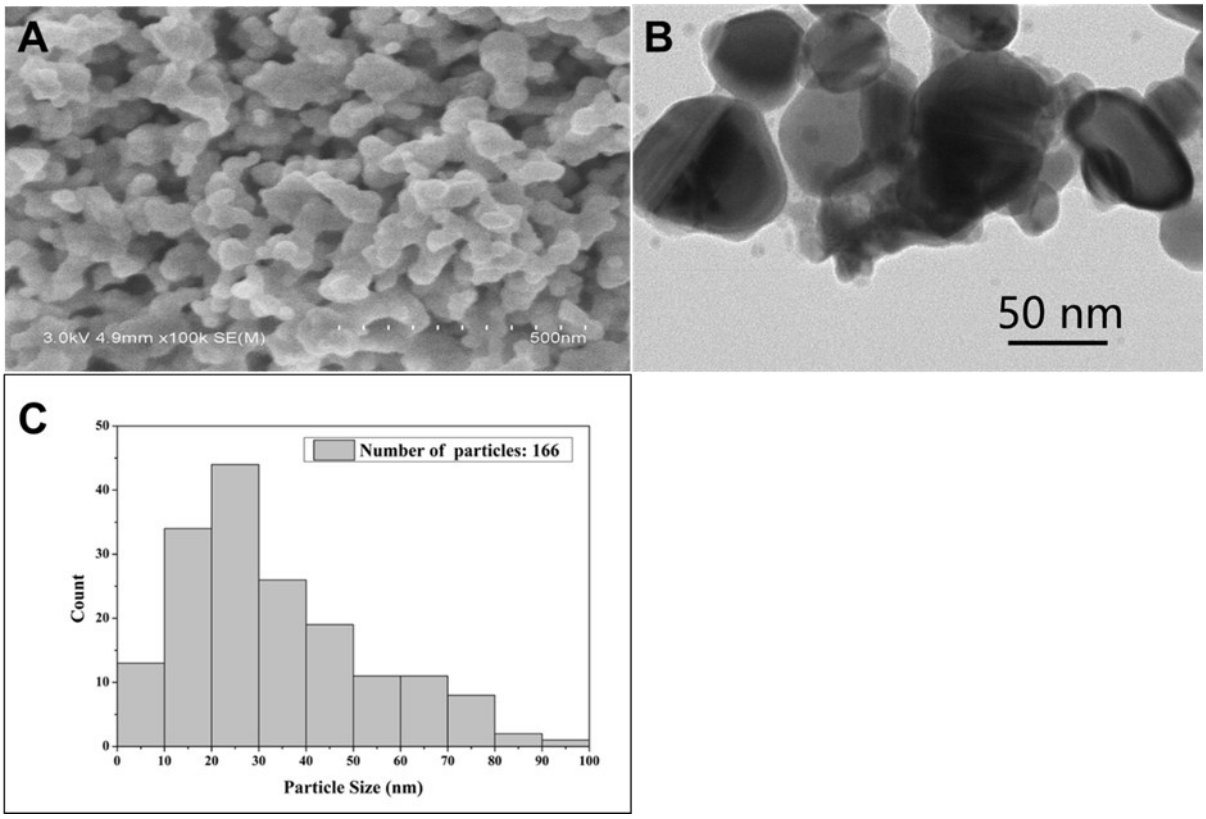

Figure S1. Characterization of CdS-NPs by scanning electron microscopy (SEM) (A) and transmission electron microscopy (TEM) (B). Particle size distribution (C).

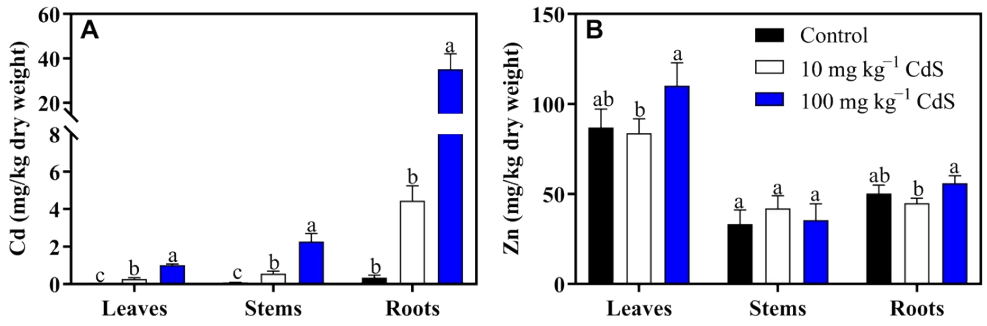

Figure S2. Concentration of Cd (A) and Zn (B) in the leaves, stems and roots of broad bean plants exposed to 0, 10 and 100 mg CdS-NPs kg^{-1}. Data are reported as the mean and standard deviation ($n = 4$).

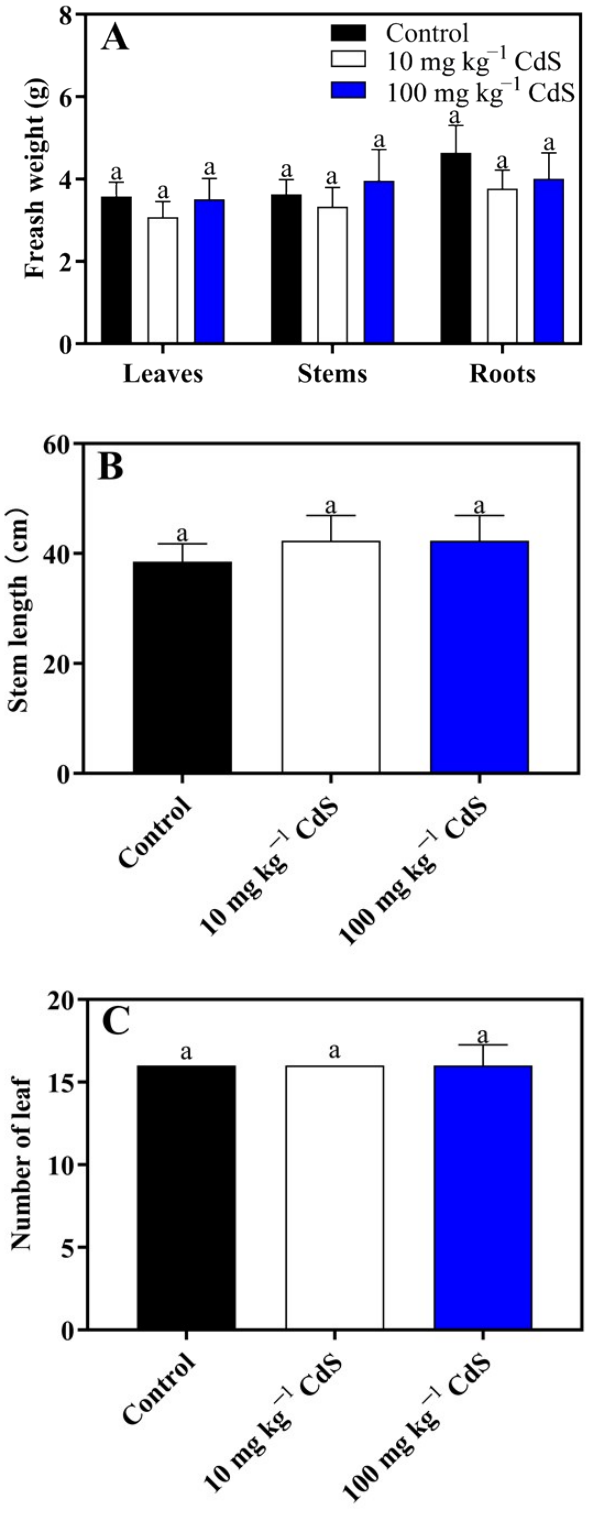

Figure S3. Fresh biomass (A) of broad bean tissues, stem length (B) and number of leaves (C) in plants exposed for 28 days to 0, 10 and 100 mg CdS-NPs kg^{-1}. Data are reported as the mean and standard deviation (n = 4).

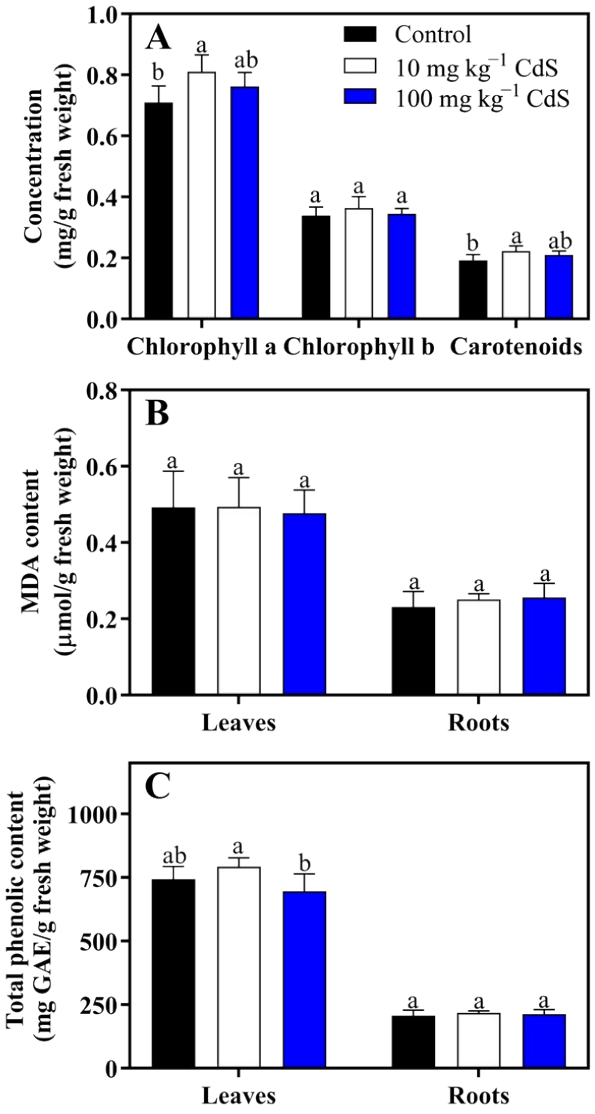

Figure S4. Photosynthetic pigments (A), lipid peroxidation (B), and total phenolic compounds (C) in broad bean tissues exposed to 0, 10 and 100 mg CdS-NPs kg^{-1}. Data are reported as the mean and standard deviation ($n = 4$).

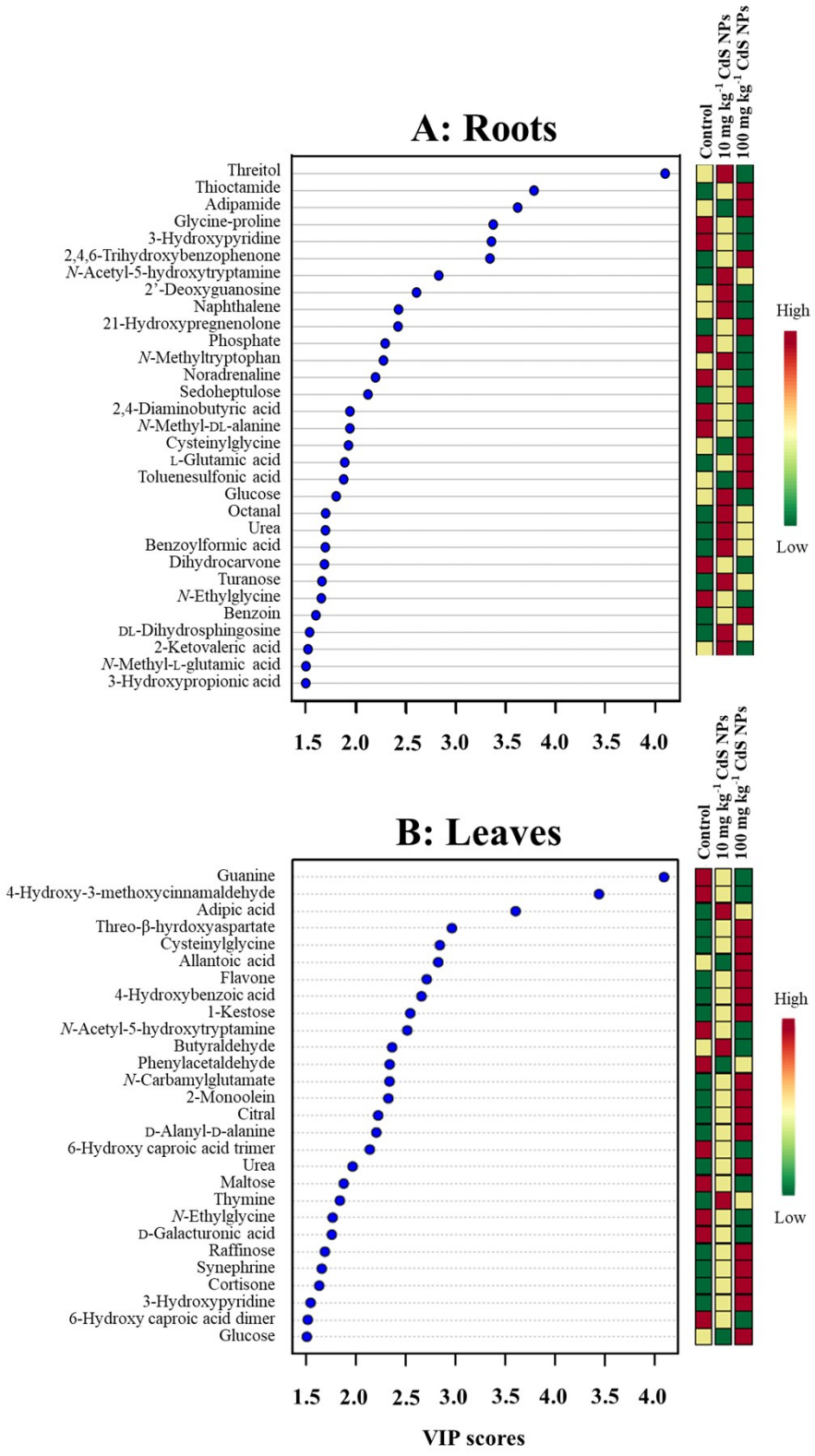

Figure S5. VIP scores from the PLS-DA analysis of metabolites in broad bean roots (A) and leaves (B). The discriminating metabolites account for the group separation.

References

1. D. W. Nelson and L. E. Sommers, Total carbon, organic carbon, and organic matter 1, *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 1982, 539-579.
2. N. Fageria and V. Baligar, Ameliorating soil acidity of tropical Oxisols by liming for sustainable crop production, *Advances in agronomy*, 2008, **99**, 345-399.
3. M. Abdulaha-Al Baquy, J.-y. Li, R.-y. Shi, M. A. Kamran and R.-k. Xu, Higher cation exchange capacity determined lower critical soil pH and higher Al concentration for soybean, *Environmental Science and Pollution Research*, 2018, **25**, 6980-6989.