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Supporting Information for

Metabolomics Reveal that Engineered Nanomaterial Exposure in Soil Alters Both Soil Rhizosphere Metabolite Profiles and Maize Metabolic Pathways

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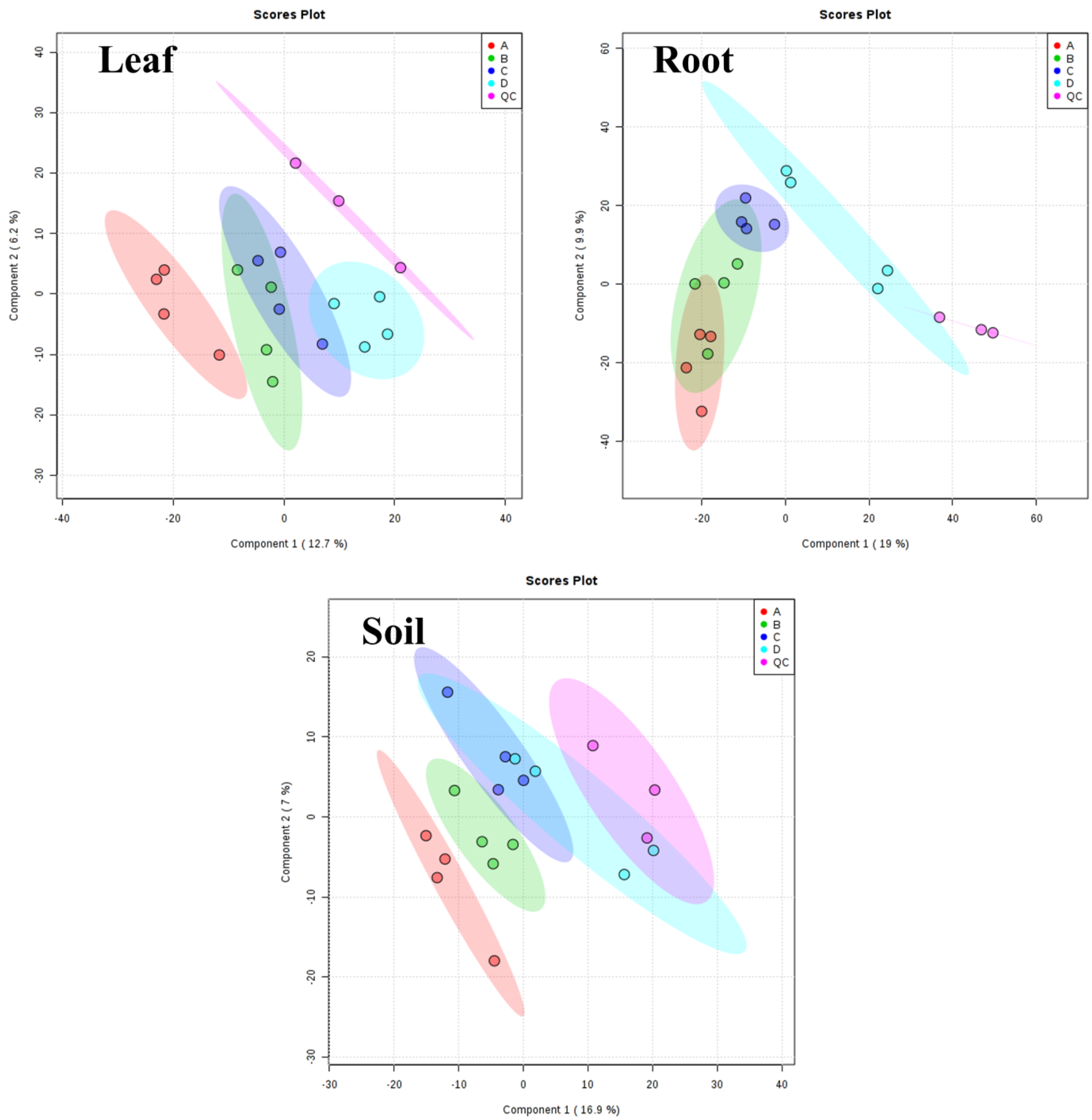
Supporting Information for Method

Soil pH and water soluble elements determination. Soil pH and water soluble elements were determined following the methods of Houba et al.[1] with some modification. Briefly, a mixture of 3 g soil and 30 mL water was shaken for two hours at 20°C and at 200 rpm. The pH of the suspension was then measured using a pH meter (Mettler-Toledo, Switzerland). After centrifugation at 10000 rpm for 10 min, the supernatant was collected and filtered through 0.22μm. The water soluble elements were determined by ICP-OES (Optima 8300, Perkin Elmer, U.S.A) and ICP-MS (NexION-300, PerkinElmer).

*Extraction of Metabolites from Plant Tissues***.** First, 60 mg of tissue was weighed and transferred to a 1.5-mL Eppendorf tube containing two small steel balls. Then 360 μL of cold methanol and 40 μL of 2-chloro-l-phenylalanine (0.3 mg/mL), dissolved in methanol as internal standard, were added to each sample, which were then placed at -80 °C for 2 min and were sonicated at 60 HZ for 2 min. An aliquot of 200 μL of chloroform was added to the samples, which were then vortexed and amended with 400 μL water. The samples were vortexed again and ultrasonicated at ambient temperature for 30 min. The samples were centrifuged at 13900 g for 10 min at 4 °C. A QC sample was prepared by mixing aliquots of all samples (a pooled sample). A 300 µL aliquot of supernatant was transferred to a glass sampling vial for vacuum-drying at room temperature; 80 μL of 15 mg/mL methoxylamine hydrochloride in pyridine was then added. The resultant mixture was vortexed vigorously for 2 min and incubated at 37 °C for 90 min. Eighty μL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (with 1% Trimethylchlorosilane) and 20 μL n-hexane was then added, and the sample was vortexed vigorously for 2 min prior to derivatization at 70 °C for 60 min. The samples were placed at ambient temperature for 30 min before GC-MS analysis.

*Extraction of Metabolites from Soil***.** One gram of soil was weighed and transferred to a 1.5-mL Eppendorf tube, followed by the addition of 1mL of methanol and water (1: 1 = v: v) and 20 μL of 2-chloro-l-phenylalanine (0.3 mg/mL, dissolved in methanol as internal standard). The mixture was sonicated at 60 HZ for 2 min. The extraction was repeated three times and was centrifuged at 12000 rpm for 10 min at 4 °C. The supernatant was transferred to a 5-ml Eppendorf tube and freeze dried. The dried powder was resuspended in 400uL methanol: water (v: v= 1:1), vortexed for 60s followed by sonication for 30s. The suspensions were centrifuged at 12000 rpm for 10 min at 4 °C.

A QC sample was prepared by mixing aliquots of the all samples to obtain a pooled sample. An aliquot of the 300 μL supernatant was transferred to a glass sampling vial for vacuum-drying at room temperature. Eighty μL of 15 mg/mL methoxylamine hydrochloride in pyridine was subsequently added. The resulting mixture was vortexed vigorously for 2 min and incubated at 37 °C for 90 min. Eighty μL of BSTFA (with 1% TMCS) and 20 μL n-hexane was added to the mixture, which was vortexed vigorously for 2 min and then derivatized at 70 °C for 60 min. The samples were placed at ambient temperature for 30 min before GC-MS analysis.



PLS-DA with QC samples (A, B, C and D represent control, SiO_2, TiO_2 and Fe_3O_4, respectively)

Reference

1. V. J. G. Houba, E. J. M. Temminghoff, G. A. Gaikhorst and W. van Vark, *Communications in Soil Science and Plant Analysis*, 2008, **31**, 1299-1396.

Table S1. Characteristics of engineered nanomaterials

Nanoparticles	Original size (nm)	Purity	Hydrodynamic size (nm)		Zeta potential (mV)	
			Ave	SD	Ave	SD
SiO_2 NPs	20	99.9%	876.40	41.77	-19.50	0.37
TiO_2 NPs	5-10	99.9%	591.50	6.93	-21.44	0.64
Fe_3O_4 NPs	30	99.9%	1230.67	55.54	11.62	0.64

Table S2. Significantly changed metabolites in maize tissues and soil exposure to different NPs.

Metabolites	Retention time	Classification	Control	SiO_2	TiO_2	Fe_3O_4
		Leaf				
L-glutamic acid	16.7	amino acid	15.4 ± 0.83	20.7 ± 2.42	23.0 ± 1.45	31.1 ± 3.88
glutamic acid	19.0	amino acid	34.6 ± 10.7	66.2 ± 13.0	79.0 ± 5.83	119 ± 15.1
oxoproline	8.0	amino acid	39.1 ± 1.23	40.6 ± 3.76	50.1 ± 4.50	56.8 ± 6.86
Isoleucine	11.0	amino acid	5.65 ± 1.26	7.54 ± 0.94	9.39 ± 0.90	10.5 ± 1.47
citrulline	9.8	amino acid	0.25 ± 0.04	0.17 ± 0.04	0.17 ± 0.05	0.38 ± 0.06
serine	10.2	amino acid	5.67 ± 0.64	8.58 ± 1.24	7.68 ± 0.97	9.20 ± 1.43
valine	9.4	amino acid	18.0 ± 3.19	23.0 ± 2.37	23.0 ± 1.21	27.2 ± 3.38
phenylalanine	7.4	amino acid	3.22 ± 0.42	3.82 ± 0.28	3.80 ± 0.38	4.78 ± 0.63
proline	11.1	amino acid	2.33 ± 0.23	1.85 ± 0.25	2.01 ± 0.38	1.57 ± 0.17
aspartic acid	16.4	amino acid	35.8 ± 12.1	31.5 ± 8.36	13.6 ± 5.21	15.3 ± 8.54
tyrosine	22.6	amino acid	77.7 ± 11.1	89.6 ± 5.11	95.0 ± 8.94	96.9 ± 4.84
glycine	7.8	amino acid	5.37 ± 1.65	5.80 ± 0.42	4.15 ± 0.50	3.56 ± 0.59
Benzoylformic acid	6.0	organic acid	1.56 ± 0.13	1.41 ± 0.08	0.97 ± 0.07	0.88 ± 0.09
4-aminobutyric acid	16.6	organic acid	4.96 ± 1.04	10.7 ± 1.47	15.5 ± 4.27	12.6 ± 0.70
Pyruvic acid	6.5	organic acid	14.6 ± 1.97	12.2 ± 2.38	14.3 ± 1.75	7.6 ± 0.56
2-ketobutyric acid	5.7	organic acid	2.35 ± 0.21	2.81 ± 0.38	3.08 ± 0.37	3.98 ± 0.56
alpha-ketoglutaric acid	17.2	organic acid	0.52 ± 0.04	0.59 ± 0.08	0.65 ± 0.10	0.89 ± 0.13
3-Hexenedioic acid	9.6	organic acid	0.29 ± 0.06	0.32 ± 0.06	0.45 ± 0.05	0.56 ± 0.10
lactic acid	6.7	organic acid	6.77 ± 0.57	4.46 ± 0.45	6.75 ± 0.81	3.97 ± 1.18
2,4-diaminobutyric acid	8.7	organic acid	8.26 ± 0.47	8.27 ± 0.44	8.42 ± 0.33	10.1 ± 0.93
Threonic acid	16.9	organic acid	3.13 ± 0.53	4.22 ± 0.87	3.64 ± 0.06	5.20 ± 0.79
3-hydroxypyruvate	37.3	organic acid	0.80 ± 0.42	1.45 ± 0.34	1.25 ± 0.57	2.69 ± 0.51
gluconic acid	28.2	organic acid	2.89 ± 0.26	3.13 ± 0.57	3.41 ± 0.71	2.06 ± 0.39
Saccharic acid	27.3	organic acid	178 ± 29.9	239 ± 30.3	229 ± 19.9	190 ± 15.4
citric acid	24.1	organic acid	8.32 ± 0.60	8.51 ± 0.61	8.69 ± 0.72	10.1 ± 0.83
succinic acid	11.5	fatty acid	5.32 ± 0.89	4.02 ± 0.36	3.87 ± 0.35	3.78 ± 0.54
xylose	19.9	sugar	27.1 ± 1.06	29.5 ± 0.65	29.1 ± 0.77	27.3 ± 0.31
fructose-6-phosphate	34.9	sugar	3.22 ± 0.16	2.88 ± 0.23	2.45 ± 0.12	3.09 ± 0.39
alpha-D-glucosamine 1-phosphate	32.7	sugar	0.84 ± 0.28	1.05 ± 0.10	0.57 ± 0.12	1.22 ± 0.22
maltotriose	41.4	sugar	4.09 ± 0.48	5.61 ± 1.38	4.64 ± 1.40	2.99 ± 0.56
palatinitol	40.6	sugar alchohol	8.61 ± 1.09	9.63 ± 1.01	10.7 ± 0.60	13.6 ± 1.72
Dodecanol	14.3	alchohol	11.6 ± 5.57	5.35 ± 0.44	8.07 ± 4.29	3.74 ± 0.50
N-methylaniline	12.8	others	0.00 ± 0.00	0.02 ± 0.04	0.00 ± 0.00	0.10 ± 0.02

Synephrine	26.7	others	1.10 ± 0.32	1.15 ± 0.16	0.95 ± 0.70	0.00 ± 0.00
octanal	7.1	others	0.89 ± 0.09	0.86 ± 0.03	0.92 ± 0.03	1.26 ± 0.20
uracil	11.9	others	8.57 ± 1.34	6.12 ± 0.31	7.27 ± 1.48	5.09 ± 0.61
creatine degr	5.5	others	202 ± 43.0	251 ± 39.1	280 ± 48.1	387 ± 72.7
Cytosin	16.4	others	1.19 ± 0.18	0.88 ± 0.10	0.86 ± 0.15	0.72 ± 0.08
5,6-dihydrouracil	7.0	others	0.85 ± 0.15	0.97 ± 0.18	1.35 ± 0.17	1.14 ± 0.19
Maleimide	6.5	others	0.75 ± 0.13	0.59 ± 0.04	0.59 ± 0.21	0.29 ± 0.12
2'-Deoxycytidine 5'-triphosphate degr prod	5.5	others	70.5 ± 44.9	31.8 ± 1.10	29.4 ± 5.48	21.8 ± 2.38
Nicotinoylglycine	6.2	others	5.35 ± 0.22	5.73 ± 0.50	6.38 ± 0.48	6.43 ± 0.72
Carnitine	9.3	others	0.37 ± 0.06	0.50 ± 0.06	0.67 ± 0.09	0.51 ± 0.14
putrescine	14.1	others	65.1 ± 5.85	70.2 ± 5.82	77.0 ± 5.40	81.8 ± 6.29
succinate semialdehyde	8.9	others	0.05 ± 0.05	0.23 ± 0.07	0.33 ± 0.04	0.41 ± 0.14
Hydantoin, 5-4-hydroxybutyl-	14.3	others	0.90 ± 0.48	0.40 ± 0.06	1.26 ± 0.76	0.53 ± 0.03
D-erythronolactone	12.8	others	0.91 ± 0.12	1.16 ± 0.20	1.00 ± 0.04	1.30 ± 0.19
N-Carbamylglutamate	23.0	others	1.37 ± 0.44	0.87 ± 0.09	0.79 ± 0.11	0.81 ± 0.04
N-Acetyl-beta-D-mannosamine	30.2	others	0.27 ± 0.05	0.35 ± 0.03	0.31 ± 0.03	0.27 ± 0.02
			Root			
ornithine	10.2	amino acid	24.1 ± 3.43	25.6 ± 4.89	20.0 ± 3.20	12.8 ± 1.38
homovanillic acid	31.3	organic acid	0.70 ± 0.34	0.38 ± 0.07	0.58 ± 0.10	1.10 ± 0.18
D-glyceric acid	11.8	organic acid	4.84 ± 0.42	4.94 ± 0.20	4.98 ± 0.61	6.37 ± 0.15
beta-hydroxypyruvate	5.3	organic acid	1.59 ± 0.11	1.64 ± 0.14	1.54 ± 0.12	1.15 ± 0.17
mannose	27.7	sugar	0.95 ± 0.10	0.68 ± 0.09	0.71 ± 0.06	0.68 ± 0.04
phosphate	8.9	inorganic acid	603 ± 32.6	552 ± 31.1	532 ± 21.8	631 ± 15.6
adenine	25.2	others	6.36 ± 0.45	6.13 ± 0.56	4.35 ± 0.50	5.76 ± 0.27
			Soil			
4-hydroxycinnamic acid	25.7	organic acid	6.84 ± 2.04	15.3 ± 6.27	18.0 ± 3.65	11.3 ± 1.70
gluconic acid	25.6	organic acid	0.93 ± 0.34	1.76 ± 0.72	1.61 ± 0.25	1.98 ± 0.47
linolenic acid	24.7	fatty acid	0.93 ± 0.24	1.58 ± 0.47	1.33 ± 0.18	1.46 ± 0.30
levoglucosan	19.1	sugar	4.96 ± 0.20	6.31 ± 0.78	6.74 ± 0.65	9.03 ± 0.99
allo-inositol	24.7	alchohol	3.96 ± 0.26	4.74 ± 0.73	5.47 ± 0.18	5.56 ± 0.95
methyl-beta-D-galactopyranoside	18.4	glycoside	6.00 ± 0.70	7.35 ± 0.91	8.38 ± 1.69	10.2 ± 1.15
beta-mannosylglycerate	26.2	others	37.7 ± 7.83	55.5 ± 4.43	48.8 ± 7.66	63.3 ± 14.5
methyl phosphate	8.8	others	2.02 ± 0.21	2.75 ± 0.64	2.76 ± 0.17	3.51 ± 0.31

Data are average of four replicates.

Table S3. Perturbed biological pathways in maize leaves exposed to different engineered nanomaterials at 100 mg/kg soil

Pathway Name	*p*	Impact	Involved metabolites
			Fe_3O_4
Arginine and proline metabolism	1.46E-05	0.4571	Gamma-Aminobutyric acid, L-Glutamic acid, L-Proline, L-Glutamine, Citrulline, Putrescine, L-Aspartic acid, Fumaric acid, Ornithine
Glycine, serine and threonine metabolism	0.013063	0.6112	Pyruvic acid, 2-ketobutyric acid, L-Serine, Hydroxypyruvic acid, L-Allothreonine, L-Homoserine, L-Threonine, Glycine, L-Aspartic acid
Valine, leucine and isoleucine biosynthesis	0.020143	0.1178	Pyruvic acid, 2-ketobutyric acid, L-Isoleucine, L-Valine, L-Leucine, L-Threonine, Citraconic acid
Tyrosine metabolism	0.028945	0.2727	L-Tyrosine, Succinic acid semialdehyde, Succinic acid, Fumaric acid
Methane metabolism	0.002066	0.1667	L-Serine, Glycine
Isoquinoline alkaloid biosynthesis	0.019843	0.5	L-Tyrosine
Pantothenate and CoA biosynthesis	1.82E-04	0.1081	Pyruvic acid, Uracil, L-Valine, Dihydrouracil,
Citrate cycle (TCA cycle)	3.46E-04	0.3806	Pyruvic acid, Oxoglutaric acid, Citric acid, Succinic acid, Fumaric acid, L-Malic acid, Cis-Aconitic acid, Isocitric acid
Glycolysis or Gluconeogenesis	3.62E-04	0.1211	Pyruvic acid, L-Lactic acid, Glucose 1-phosphate
Pyruvate metabolism	4.27E-04	0.1487	Pyruvic acid, L-Lactic acid, L-Malic acid
Pyrimidine metabolism	0.012617	0.1246	L-Glutamine, Uracil, Orotic acid, Dihydrouracil, 3-Aminoisobutanoic acid, Thymidine, Thymine
			TiO_2
Arginine and proline metabolism	1.76E-04	0.4571	Gamma-Aminobutyric acid, L-Glutamic acid, L-Aspartic acid, Putrescine, Citrulline, L-Proline, L-Glutamine, Fumaric acid, Ornithine
Pantothenate and CoA biosynthesis	0.005862	0.1081	Dihydrouracil, L-Valine, Uracil, Pyruvic acid
Methane metabolism	0.006689	0.1667	L-Serine, Glycine
Glycerophospholipid metabolism	0.012036	0.181	Ethanolamine, Glycerol 3-phosphate

Pyrimidine metabolism	0.016486	0.1246	Dihydrouracil, 3-Aminoisobutanoic acid, Thymidine, Orotic acid, Uracil, Thymine, L-Glutamine
Isoquinoline alkaloid biosynthesis	0.016531	0.5	L-Tyrosine
Citrate cycle (TCA cycle)	0.019723	0.3806	Oxoglutaric acid, Succinic acid, Isocitric acid, Cis-Aconitic acid, Citric acid, L-Malic acid, Fumaric acid, Pyruvic acid
Tyrosine metabolism	0.03208	0.2727	L-Tyrosine, Succinic acid semialdehyde, Succinic acid, Fumaric acid
Glyoxylate and dicarboxylate metabolism	0.036189	0.7042	Succinic acid, Isocitric acid, Cis-Aconitic acid, Citric acid, L-Malic acid, Glycolic acid
			SiO_2
Arginine and proline metabolism	0.001322	0.4571	Gamma-Aminobutyric acid, L-Glutamine, L-Glutamic acid, L-Proline, Citrulline, Fumaric acid, Putrescine, Ornithine, L-Aspartic acid
Glycolysis or Gluconeogenesis	0.004142	0.1211	L-Lactic acid, Pyruvic acid, Glucose 1-phosphate
Pyruvate metabolism	0.004753	0.1487	L-Lactic acid, Pyruvic acid, L-Malic acid
Methane metabolism	0.014631	0.1667	L-Serine, Glycine
Pantothenate and CoA biosynthesis	0.020762	0.1081	Uracil, L-Valine, Pyruvic acid, Dihydrouracil
Glycine, serine and threonine metabolism	0.022363	0.5348	L-Serine, Hydroxypyruvic acid, 2-ketobutyric acid, L-Allothreonine, L-Threonine, Pyruvic acid, Glycine, L-Aspartic acid

Table S4. Perturbed biological pathways in maize roots exposed to different engineered nanomaterials of 100 mg/kg soil

Pathway Name	p	Impact	Involved metabolites
Fe_3O_4			
Inositol phosphate metabolism	0.002597	0.245	D-Glucuronic acid, Myoinositol
Ascorbate and aldarate metabolism	0.01273	1	D-Glucuronic acid, Ascorbic acid, Myoinositol, D- Glutarate
Glycerolipid metabolism	0.01709	0.2222	Glyceric acid, Glycerol, Glycerol 3-phosphate
Citrate cycle (TCA cycle)	0.02991	0.3806	Pyruvic acid, Oxoglutaric acid, Citric acid, Succinic acid, Fumaric acid, L-Malic acid, Cis-Aconitic acid, Isocitric acid
TiO_2			
Inositol phosphate metabolism	0.022634	0.245	D-Glucuronic acid, Myoinositol
Ascorbate and aldarate metabolism	0.024315	1	D-Glucuronic acid, Ascorbic acid, Myoinositol, D-Glucarate
Methane metabolism	0.035033	0.1667	L-Serine, Glycine
Citrate cycle (TCA cycle)	0.043907	0.3806	Citric acid, Isocitric acid, L-Malic acid, Oxoglutaric acid, Succinic acid, Fumaric acid, Pyruvic acid, Cis-Aconitic acid,
Glyoxylate and dicarboxylate metabolism	0.049186	0.7042	Citric acid, Isocitric acid, L-Malic acid, Glycolic acid, Succinic acid, Cis-Aconitic acid,
SiO_2			
Inositol phosphate metabolism	0.009897	0.245	D-Glucuronic acid, Myoinositol
Citrate cycle (TCA cycle)	0.019425	0.3806	Isocitric acid, Oxoglutaric acid, L-Malic acid, Succinic acid, Fumaric acid, Citric acid, Pyruvic acid, Cis-Aconitic acid

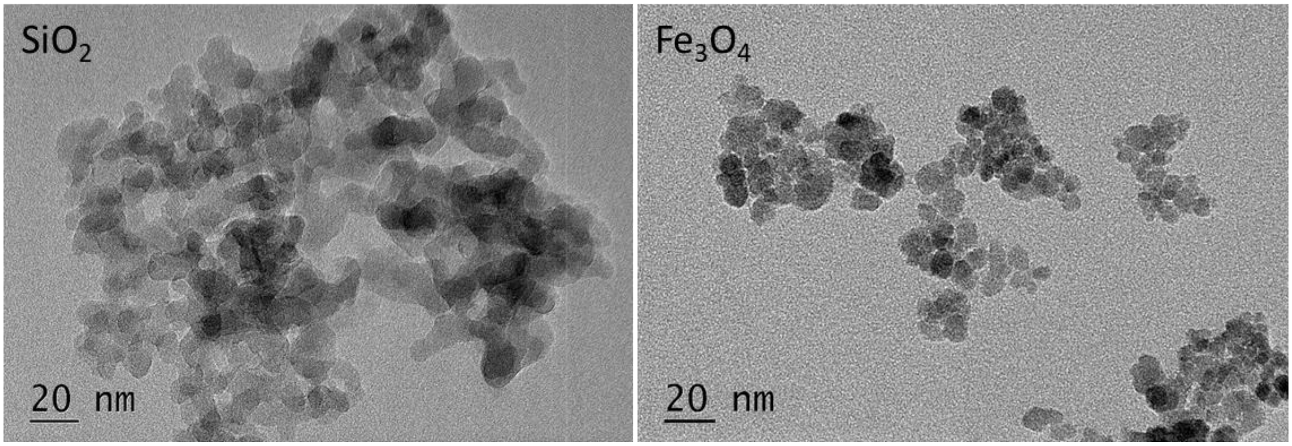


Figure S1. Representative TEM images of SiO_2 and Fe_3O_4 with a scale bar of 20 nm.

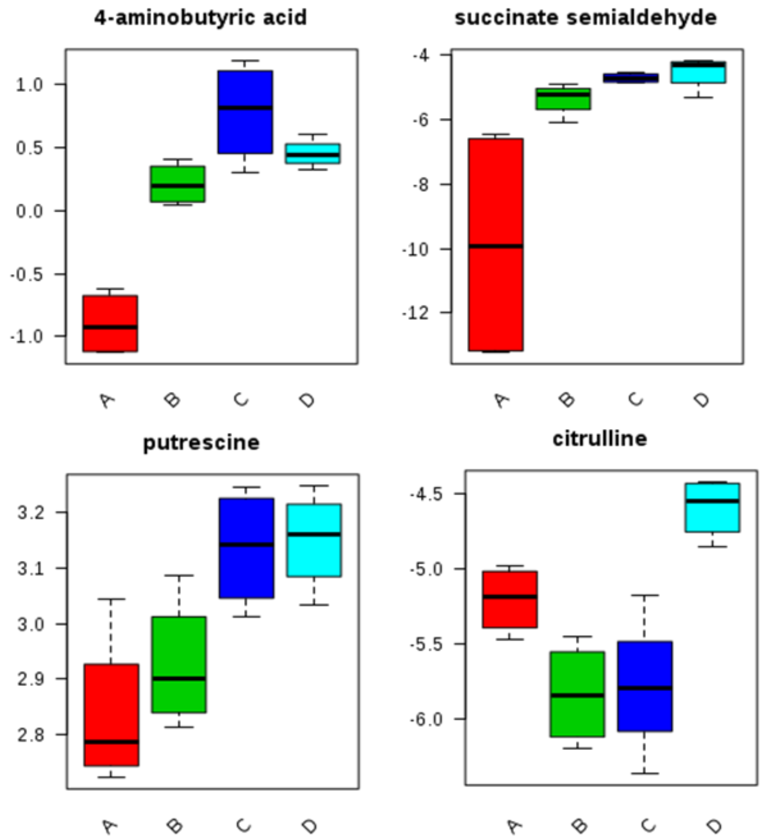


Figure S2. The relative abundance of four nitrogen-related compounds in maize leaves grown in soil without (control, A) or with ENMs (B, SiO_2; C, TiO_2, D, Fe_3O_4).

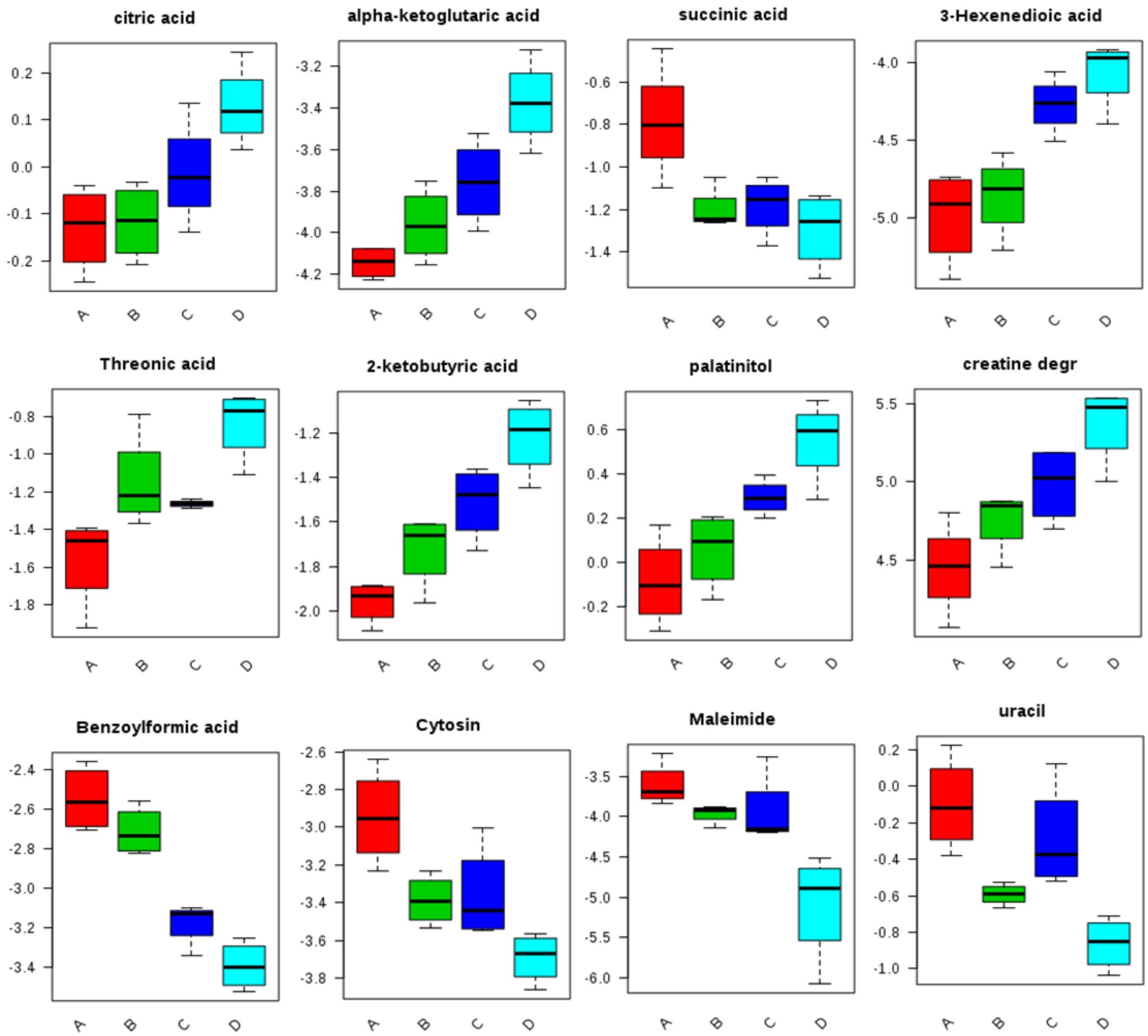


Figure S3. Up-regulated and down-regulated carbohydrates in maize leaves in response to ENMs exposure. A, B, C and D represent control, SiO_2, TiO_2 and Fe_3O_4 respectively.

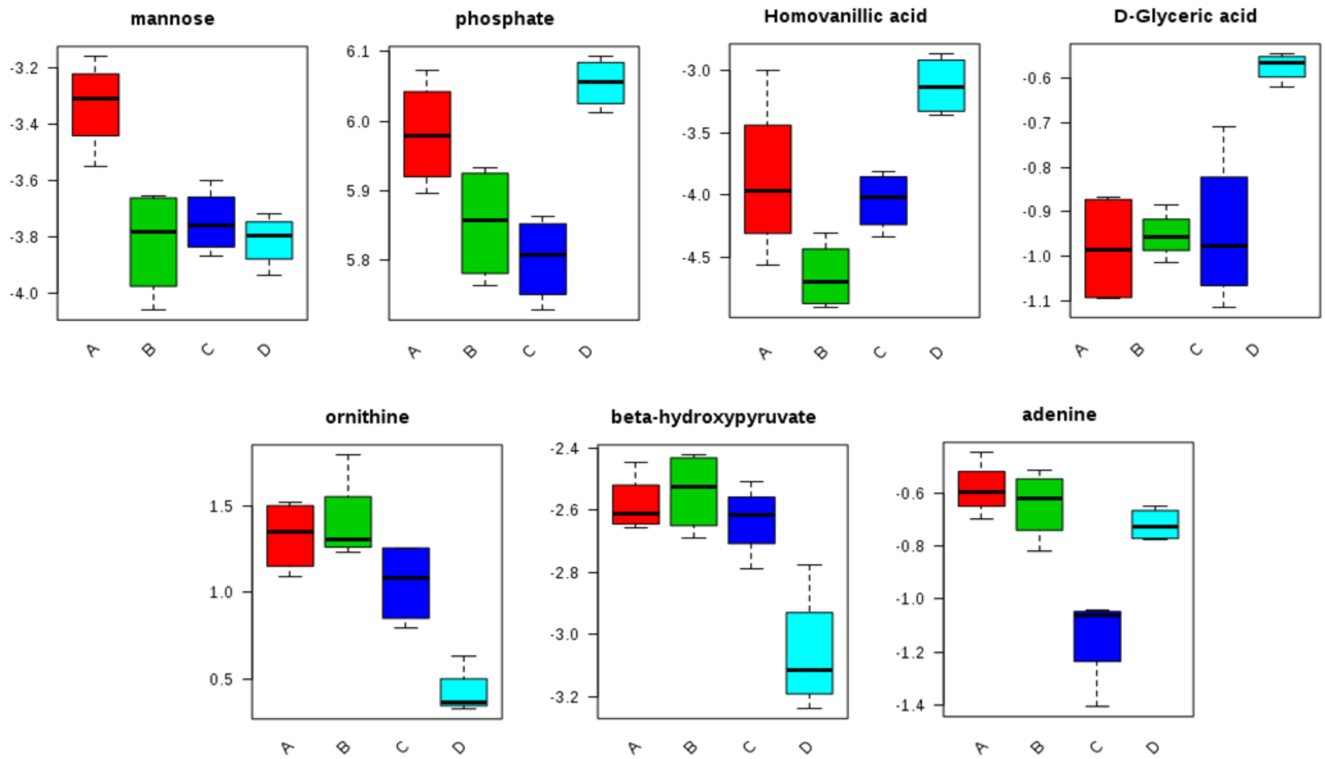


Figure S4. Significantly changed metabolites in maize roots as a function of ENMs exposure. A, B, C and D represent control, SiO_2, TiO_2 and Fe_3O_4 respectively.

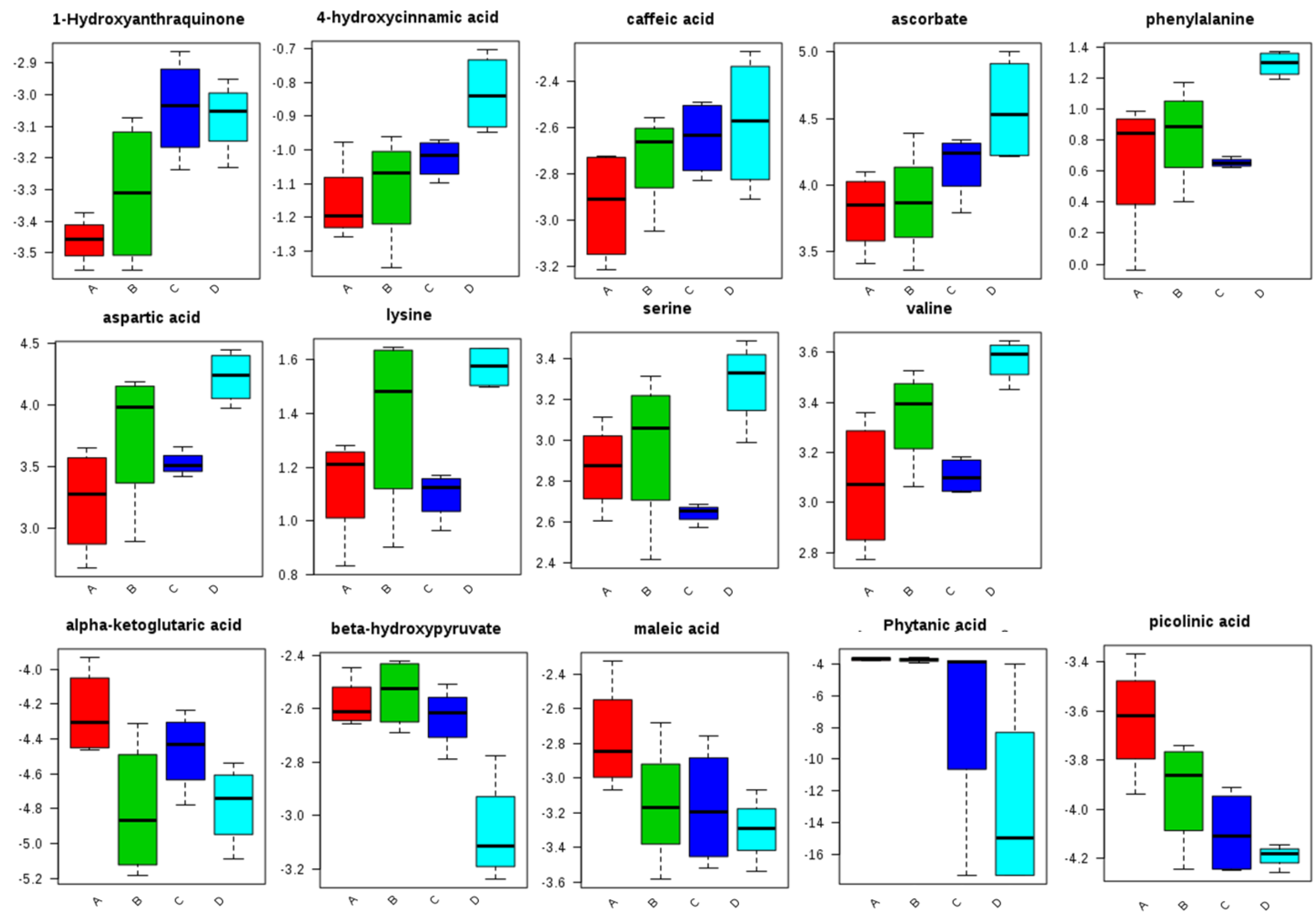


Figure S5. Metabolites in maize roots that only respond to Fe_3O_4. A, B, C and D represent control, SiO_2, TiO_2 and Fe_3O_4 respectively.

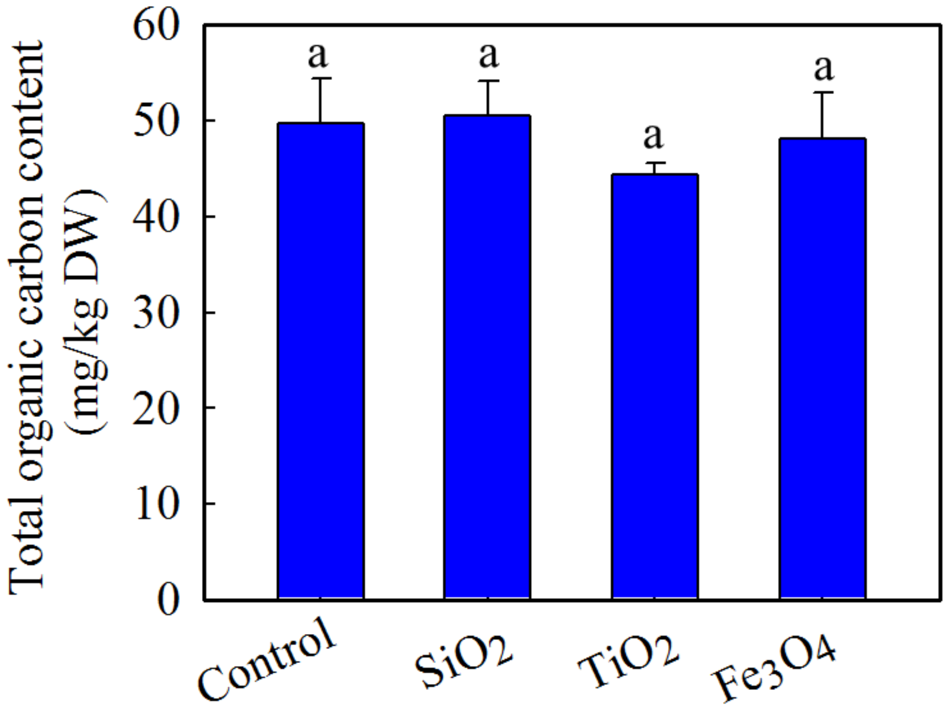


Figure S6. Total organic carbon content in soil spiked with different ENMs dosing at 100 mg/kg.