Supplementary Information (SI) for Environmental Science: Nano.

Electronic Supplementary Information (ESI)

Mechanistic understanding of Iron oxide nanobiotransformation in *Zea mays*: a combined synchrotron-based, physiological and molecular approach

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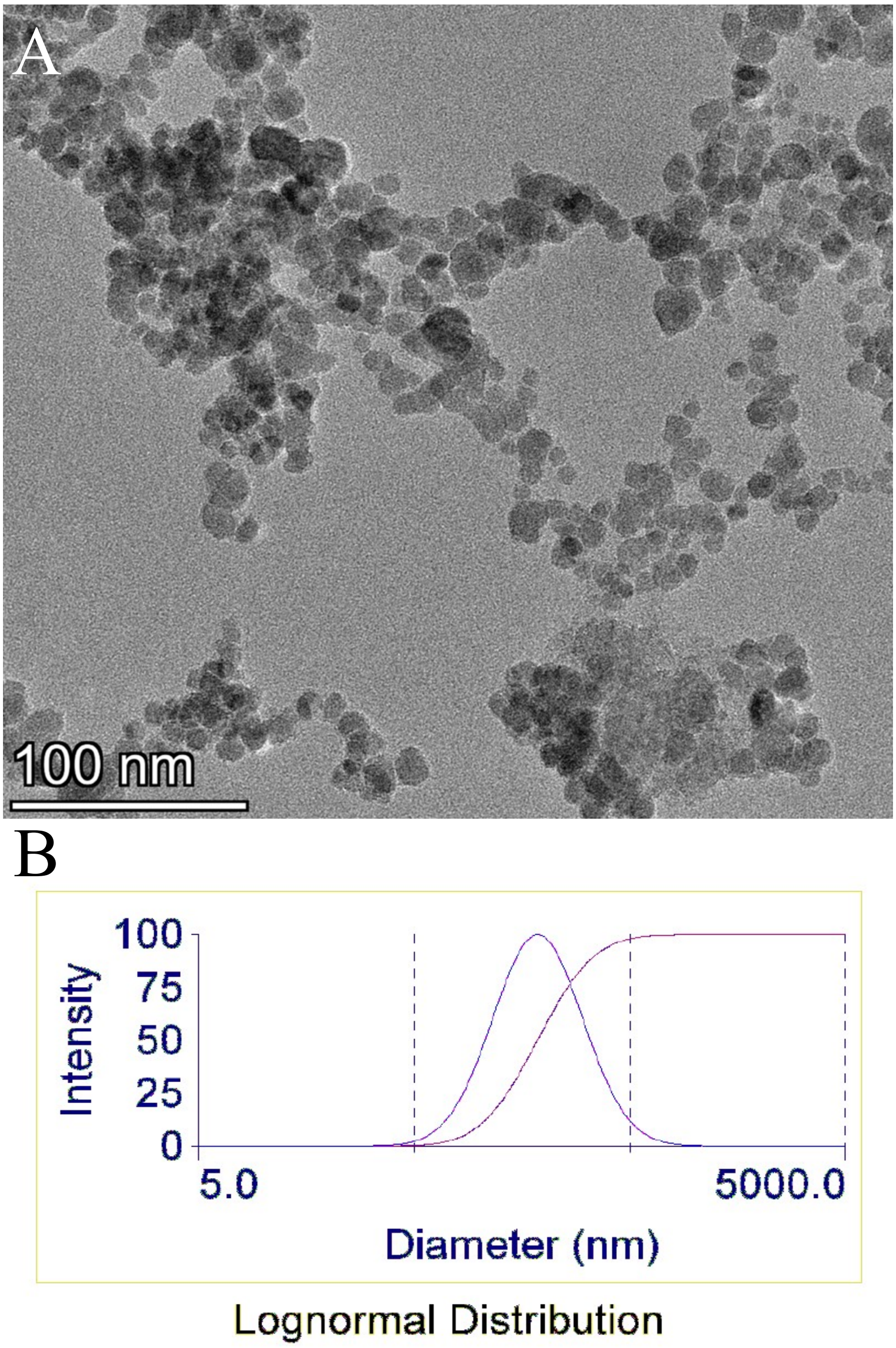

Figure S1. (a) TEM image of nFe_3O_4. Average particle size has been estimated in 10 nm. Detailed description of the characterization methods and results are reported in the experimental section and in Pagano et al, 2022.[20] Polydispersity (b) used as a measure of broadness of molecular weight distribution was calculated as 0.248 ± 0.012.

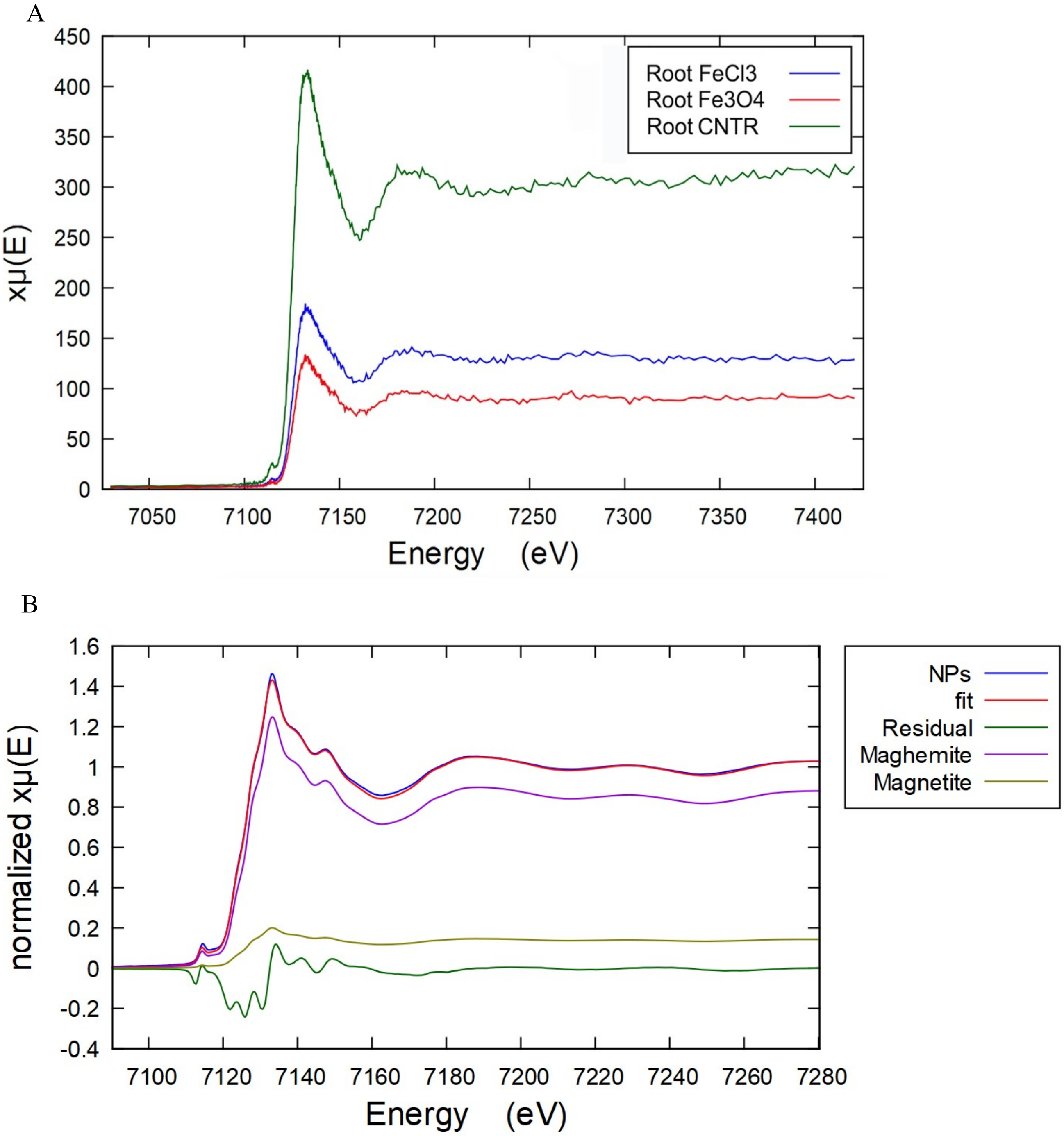

Figure S2. (A) Comparison of raw (not normalized) XANES spectra collected on roots of V_2 stage maize plants. (B) LCF performed using maghemite (violet line) and magnetite (greenish line) standard reference spectra on the spectrum of NPs (blue line) used to treat the samples. The residual curve is the green line, the fit is the red line.

Table S1. List of primers utilized for qPCR analyses, and genes' description.

Gene name	Gene ID	RefSeq	Primer	Primer Seq.
gsr1	Zm00001eb004790	NM_001305818.1	F	CAACCTGACACCAGTTGCAC
			R	GGGAGAACACAGCAGAAGGT
sod1	Zm00001eb008850	NM_001155647.1	F	TCCATTCTGGGAAGGGCAGT
			R	GCTCCTGCGTTTCCTGTTGA
nas3	Zm00001eb052890	XM_008666956.4	F	TGTCTACACCACATGCGTGA
			R	GCTCGGACTTCGACTTCTACC
fer1	Zm00001eb195010	NM_001112093.2	F	CAACTCTGGGTCGGTGGATT
			R	GACCGAACTGACACATCGCA
mate1	Zm00001eb261140	NM_001170581.1	F	TCCTTGTTGCCGTTGTCAGT
			R	AAACATCCGGAGGCTCATGT
pro1	Zm00001eb347680	NM_001319696.1	F	CCTTCCCTTACAATGAGGCTGT
			R	ACCATTCTATGCTCCTGCCTG
aas13	Zm00001eb358430	NM_001174342.1	F	CGTTTAGCAGCCGTGTCATC
			R	TCGAGTGAGTTCGCTGCTTC
***act2* (HK)**	Zm00001d012277	NM_001154731.2	F	ACGCCGAGAACACTTTGAGG
			R	AAACCCGCCTTGACCATTCC

gsr1 (glutathione reductase 1), encodes for the enzyme involved in ROS scavenging by converting oxidized glutathione back to its active form; *sod1* (superoxide dismutase 1), encodes for the enzyme involved in cellular defense against oxidative stress, catalyzing the dismutation of superoxide radicals into oxygen and hydrogen peroxide. *nas3*, (nicotianamine synthase3) encodes the enzyme (NAS) that synthesizes nicotinamine (NA), that is the precursor of mugineic acid (phytosiderophore). *fer1* (ferritin1), product synthesis in response to increased iron concentration. Also involved in the formation of ferrihydrite. *mate1* (multidrug and toxic compound extrusion 1), ortholog of *A. thaliana* FDR3 gene. Located in root pericycle, mediates citrate efflux into xylem to chelate and transport iron. It is part of response pathways to abiotic stimuli and stress. *pro1* (proline responding1), encodes for Δ1-pyrroline-5-carboxylate synthetase (P5CS), involved in proline biosynthesis for osmoregulation in plants. *aas13* (auxin amido synthetase13), also known as Jasmonic acid amido synthetase (JAR1), encodes for enzyme that catalyzes the biosynthesis of 7-iso-JA-Ile (represses jasmonate signaling pathway, activates abiotic stress response pathway). Act2 (actine2), involved in cytoskeleton formation, has been utilized ad housekeeping (HK) gene for the qPCR analyses.

Table S2. Iron and chloride content in soil and plant tissues.

Fe content (mg kg^{-1})								
sample	soil	st. dev.	roots	st. dev.	leaves	st. dev.	seeds	st. dev.
untreated	4.307	0.130	0.123	0.057	0.123	0.011	0.074	0.011
nFe_3O_4	5.667	0.245	0.155	0.057	0.083	0.114	0.091	0.011
$FeCl_3$	5.570	0.198	0.098	0.001	0.204	0.057	0.066	0.023
Cl^- content (mg kg^{-1})								
sample	soil	st. dev.	roots	st. dev.	leaves	st. dev.	seeds	st. dev.
untreated	158.20	27.72	50.90	5.37	42.00	4.24	48.00	18.38
nFe_3O_4	139.00	15.56	84.50	45.96	68.50	14.85	75.35	23.69
$FeCl_3$	144.00	56.57	58.25	15.20	75.00	25.46	89.00	22.63

One-way ANOVA has been applied; no significant differences have been observed.

Table S3. translocation factors indexes for untreated plants, nFe_3O_4 and $FeCl_3$, calculated as reported.

Translocation factor index	**untreated**	**nFe_3O_4**	**$FeCl_3$**
Soil-Root	0.028	0.026	0.018
Root-Shoot	1.000	0.553	2.082
Shoot-Seed	0.601	1.096	0.324
Soil-Seed	0.017	0.016	0.011

Translocation factors (TF) indexes calculation (from Table S2 mean values):

Formula 1. Soil-Root TF_{index}: $[Fe]_{root}/[Fe]_{soil}$

Formula 2. Root-Shoot TF_{index}: $[Fe]_{shoot}/[Fe]_{root}$

Formula 3. Shoot-Seed TF_{index}: $[Fe]_{seed}/[Fe]_{shoot}$

Formula 4. Soil-Seed TF_{index}: $[Fe]_{seed}/[Fe]_{soil}$