Supporting information

Dynamic microbial regulation of triiron tetrairon phosphate nanomaterials in the tomato rhizosphere

Liya Jiao[abc], Le Yue[abc], Xiehui Le[abc], Xuesong Cao[abc], Feiran Chen[abc], Chuanxi Wang[abc], Xiaoli Zhang [abc], Hua Zou*[c], Zhenyu Wang[abc]

[a] *Institute of Environmental Processes and Pollution Control, Jiangnan University, Wuxi 214122, China.*

[b] *Jiangsu Engineering Laboratory for Biomass Energy and Carbon Reduction Technology, Wuxi 214122, China.*

[c] *School of Environment and Ecology, Jiangnan University, Wuxi 214122, China.*

Text S1 Synthesis of $Fe_7(PO_4)_6$ NMs

First, 3.45 g $NH_4H_2PO_4$ was dissolved in 20 mL deionized water (DI) at 25 °C to produce 173 mg·L^{-1} $NH_4H_2PO_4$. To create nanoscale suspended particles, a solution of 0.5 g PVP-K30 (Polyvinylpyrrolidone K30) in 10 mL DI water was prepared and combined with the aforementioned $NH_4H_2PO_4$ solution. $FeCl_3 \cdot 6H_2O$ (2.025 g) was dissolved in 20 mL DI water, making a 101 mg·L^{-1} ferrous solution. Then, the ferrous solution was added dropwise for 6 s per drop into the PVP-K30 and $NH_4H_2PO_4$ mixture with a magnetic stirrer until all ferrous solution was consumed. Following this, the mixture was centrifuged for 10 min at 6000 rpm, and the supernatant was discarded. Then the precipitate was transferred into a 100 mL Teflon-lined stainless steel autoclave. The autoclave was then sealed and heated at 180 °C for 6 h. After cooling down, the products were separated by centrifugation at 5000 rpm for 5 min and washed with DI water for three times. $Fe_7(PO_4)_6$ NMs were obtained by drying in a vacuum oven for 2 h at 60 °C. The productivity of synthetic $Fe_7(PO_4)_6$ NMs was 0.92 g. The chemical reaction process is shown below:

$$Fe^{3+} + PO_4^{3-} \rightarrow FePO_4 \xrightarrow[180\ °C,6\ h]{PVP\ K30,hydrothermal} Fe_7(PO_4)_6$$

Text S2 Particle concentration analysis

The prepared surface solution was filtered through a 0.45 μm membrane. Tomato fruits (25 mg) was homogenized in 3 mL 20 mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer (pH = 5.0). After homogenization, 2 mL of 5% macerozyme R-10 was added into each homogenate, and the mixture was shaken at 37 °C for 24 h. After precipitation for 1 h, the supernatant was passed through a 0.45 μm filter membrane and diluted with DI water. After digestion, the surface solution and interior enzyme solution were diluted with DI water for SP-ICP-MS analysis.

Text S3 *HPLC-MS/MS operating parameters used in this study*

After the plant sample extraction, 10 μL samples were injected onto a Acquity UPLC HSS T3 (2.1×100 mm, 1.8μm) using an 18-min linear gradient at a flow rate of 0.35 $mL \cdot min^{-1}$. The eluents in negative/positive mode were eluent A (0.1% v/v formic acid in water) and eluent B (0.1% formic acid in acetonitrile). The solvent gradient was set as follows: 5% B, 0 min; 5% B, 1.5 min; 100% B, 14.0 min; 100% B, 15.5 min; 5% B, 16 min; 5% B, 18 min. Nitrogen was used as sheath gas (35 $L \cdot min^{-1}$) and aux gas (15 $L \cdot min^{-1}$). The spray voltage was set as 3 kV (-3 kV for negative mode and 3 kV for positive mode) and capillary temperature was 320 ℃. The resolution of full MS was set as 70000 and scan range was acquired between 70 to 1050 m/z in full MS-dd MS^2 mode. The following dd-MS^2 product ion spectra were collected at resolution of 17500, isolation window of 1.5 m/z, and collision energy of nce: 20, 40, 60. The raw data files were processed using Compound Discoverer 3.1 software coupled with the mzCloud and S-7 Chem Spider libraries. Principal components analysis (PCA), and supervised methods, such as partial least squares discriminant analysis (PLS-DA) were conducted on the LC–MS/MS data via MetaboAnalyst 5.0 (http://www.metaboanalyst.ca/). All data was log-transformed and normalized before statistical analysis. Significantly changed metabolites were determined in univariate statistical analyses (Variable importance in projection (VIP) score > 1; and Student's t-test, $p < 0.05$) [1].

Text S4 *16S rRNA gene sequencing of the rhizosphere soil microbial community*

The purity of the extracted DNA was further measured and quantified by spectrophotometry using a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). The 16S rRNA genes were amplified using the forward primers 314F (CCTACGGGNGGCWGCAG) and the reverse primer 806R (GGACTACHVGGGTATCTAAT) targeting the V3-V4 variable regions [2]. The amplified products of the bacterial 16S rRNA gene were checked by 2% agarose gels

and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U. S.) and quantified using ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (PE250) on an Illumina platform according to the standard protocols. The 16S rRNA genes were subsequently sequenced on an the PacBio Sequel platform. The paired-end reads were generated and assigned to each sample based on their barcodes and then were merged with FLASH (version 1.2.11) software. High-quality filtering of the raw tags was conducted to acquire clean tags using QIIME (version 1.7.0) [3]. The clean tags were clustered into operational taxonomic units (OTUs) of ≥ 97% similarity using UPARSE (version 9.2.64) pipeline. All chimeric tags were removed using UCHIME algorithm and finally obtained effective tags for further analysis. The representative OTU sequences were classified into organisms by a naive Bayesian model using Ribosomal Database Project (RDP) classifier (version 2.2) based on SILVA database (version 138.1), with the confidence threshold value of 0.8.

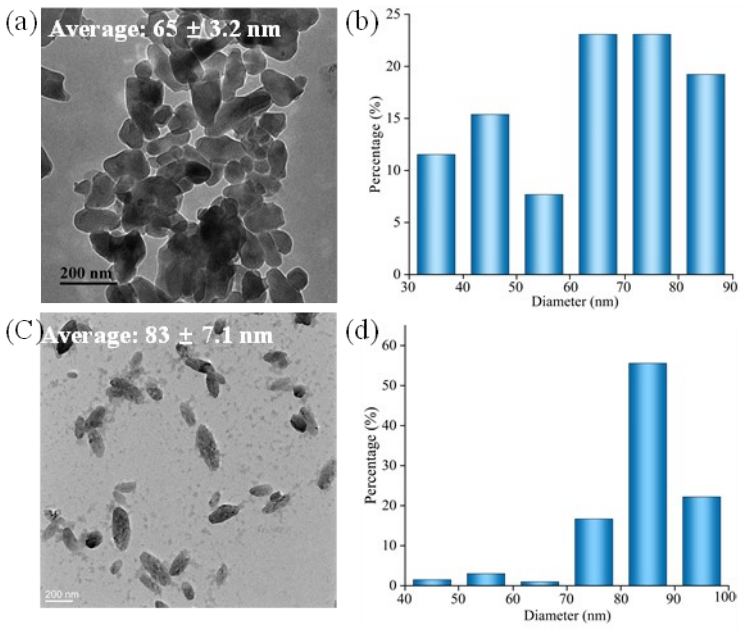

Fig. S1 Characterizations of synthesized $Fe_7(PO_4)_6$ NMs in deionized (DI) water and soil solution. (a) TEM image in DI water, (b) size distribution of $Fe_7(PO_4)_6$ NMs in DI water, (c) TEM image in soil solution and (d) size distribution of $Fe_7(PO_4)_6$ NMs in soil solution.

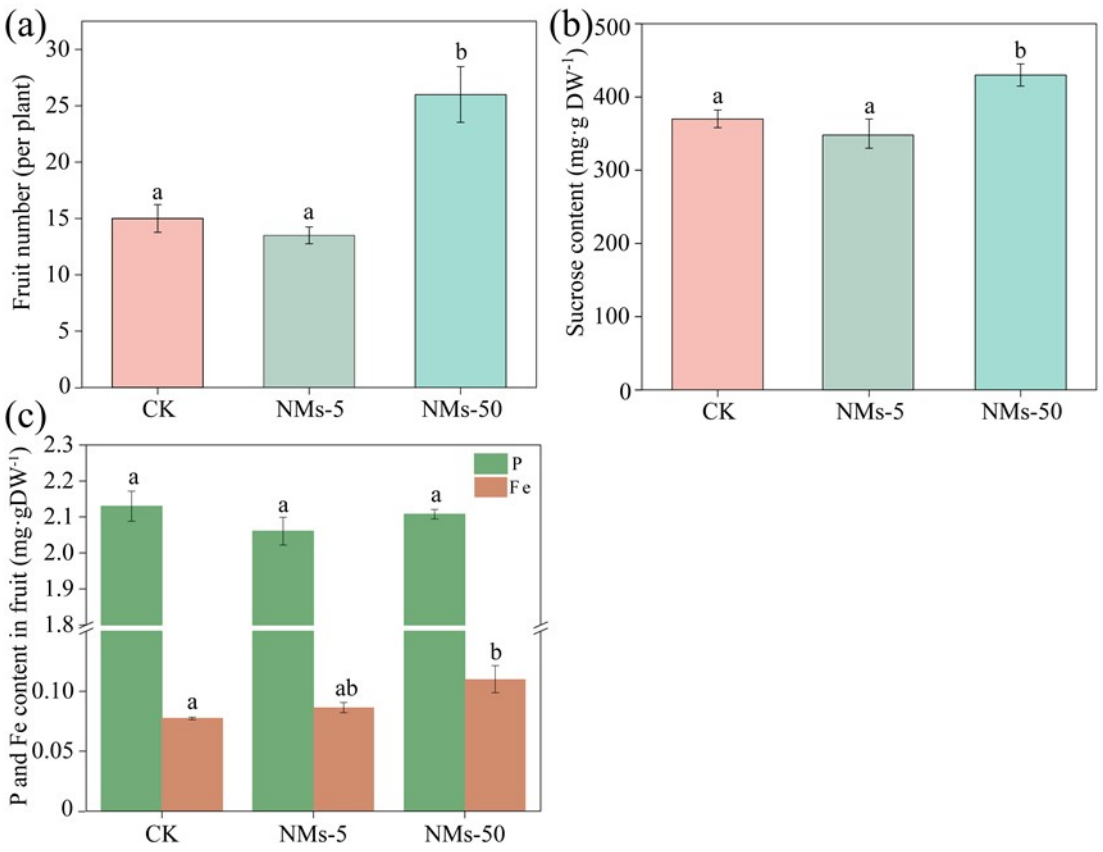

Fig. S2 (a) Fruit number, (b) sucrose content, and (c) P and Fe contents in fruits. in tomato fruit upon 5 mg·kg^{-1} (NMs-5) and 50 mg·kg^{-1} (NMs-50) NMs. Different letters represent significant difference; same letter represents no significant difference.

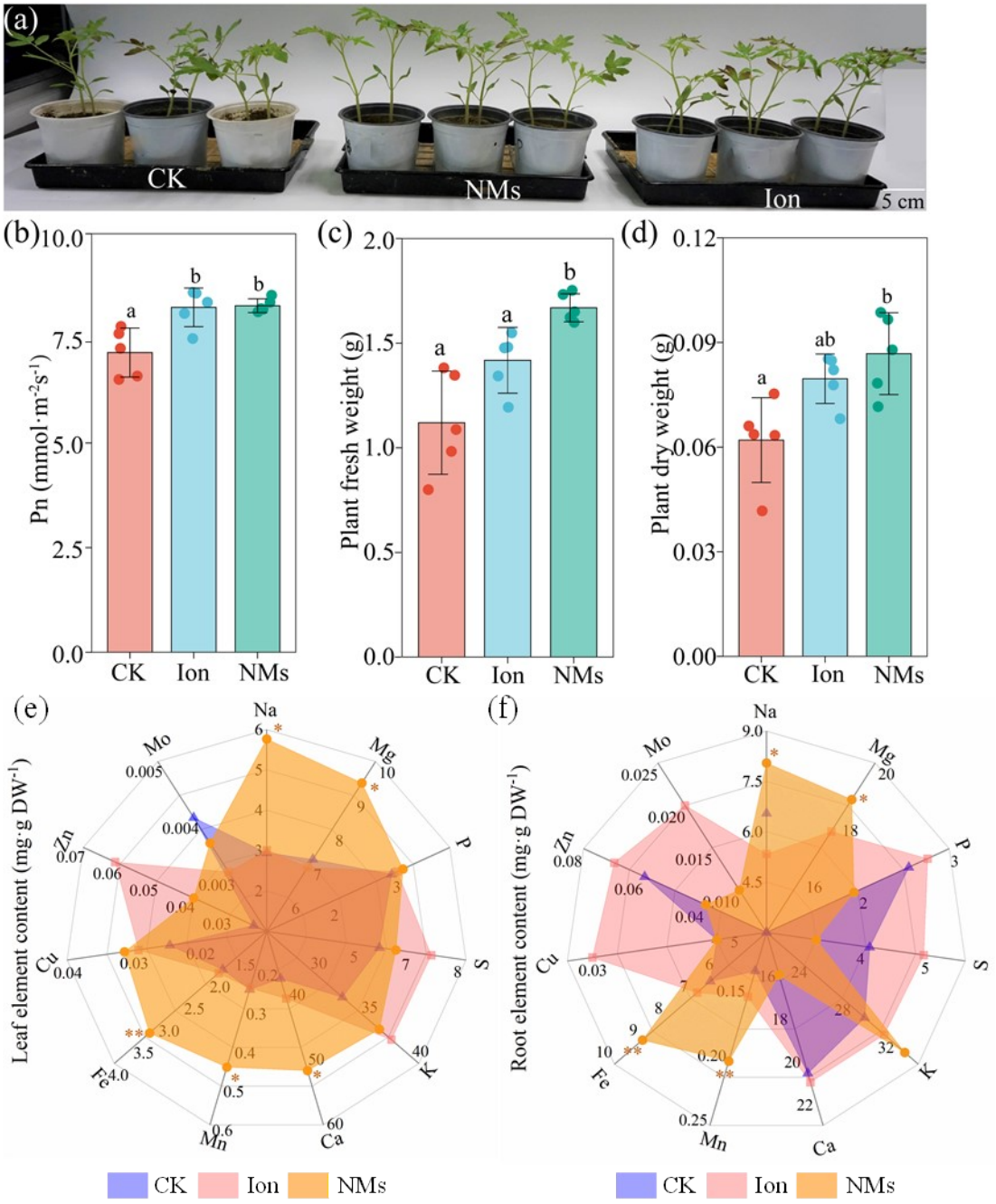

Fig. S3 Growth parameters, Pn and element content of tomato seedlings as affected by soil application with $Fe_7(PO_4)_6$ NMs and Ion. (a) The growth phenotypes of CK, NMs and Ion treatments, (b) Pn, (c) plant fresh weight, (d) plant dry weight, (e) leaf element content and (f) root element content. Different letters or with asterisk represent significant difference; same letter represents no significant difference.

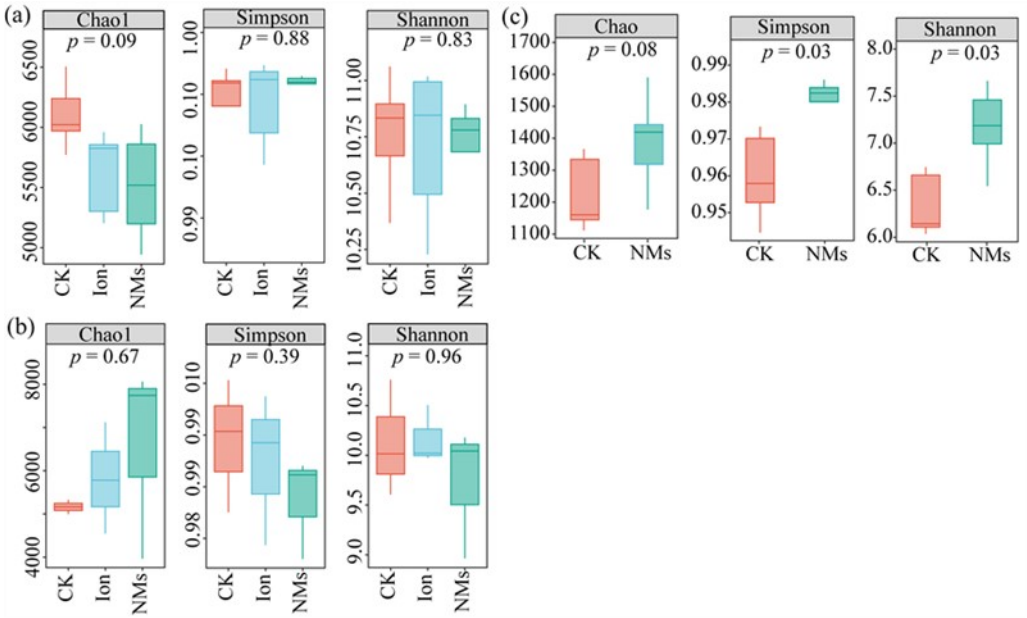

Fig. S4 The α-diversity indexes (including Chao1, Simpson and Shannon) of rhizosphere microbes during the tomato developmental stages. The α-diversity indexes at (a) seedling stage, (b) flowering stage and (c) mature stage.

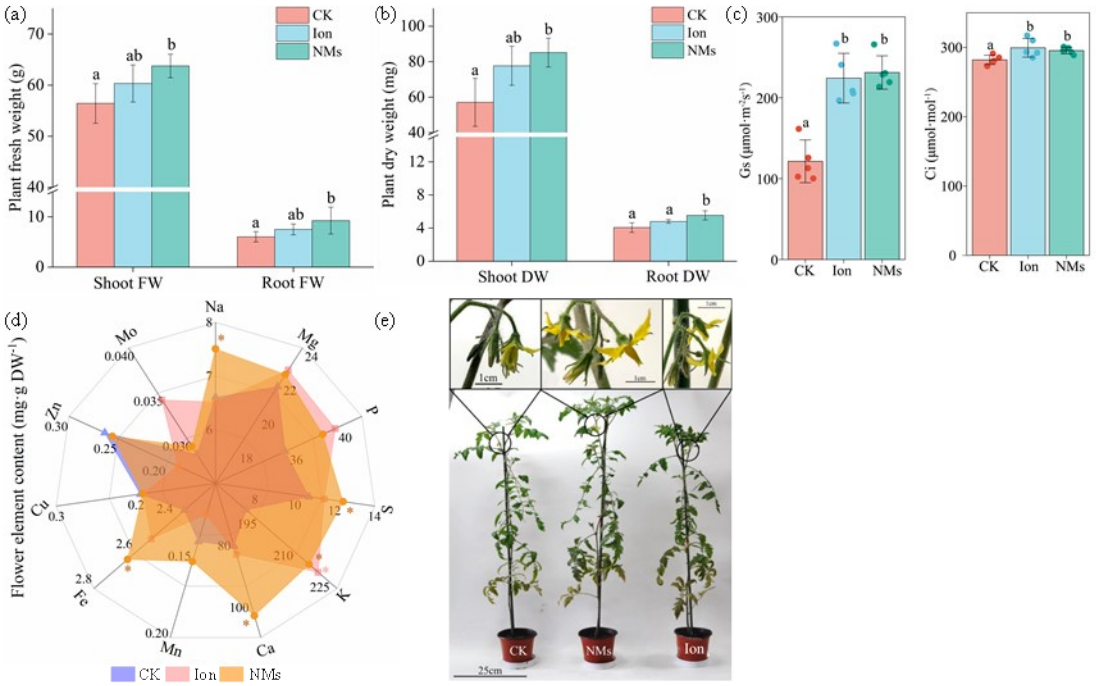

Fig. S5 Promotion of plant growth and flower development with soil amendment $Fe_7(PO_4)_6$ NMs. (a) Plant fresh weight, (b) plant dry weight, (c) G_s and C_i, (d) flower element content and (e) phenotypic image of flowering development at the flowering stage. Different letters or with asterisk represent significant difference; same letter represents no significant difference.

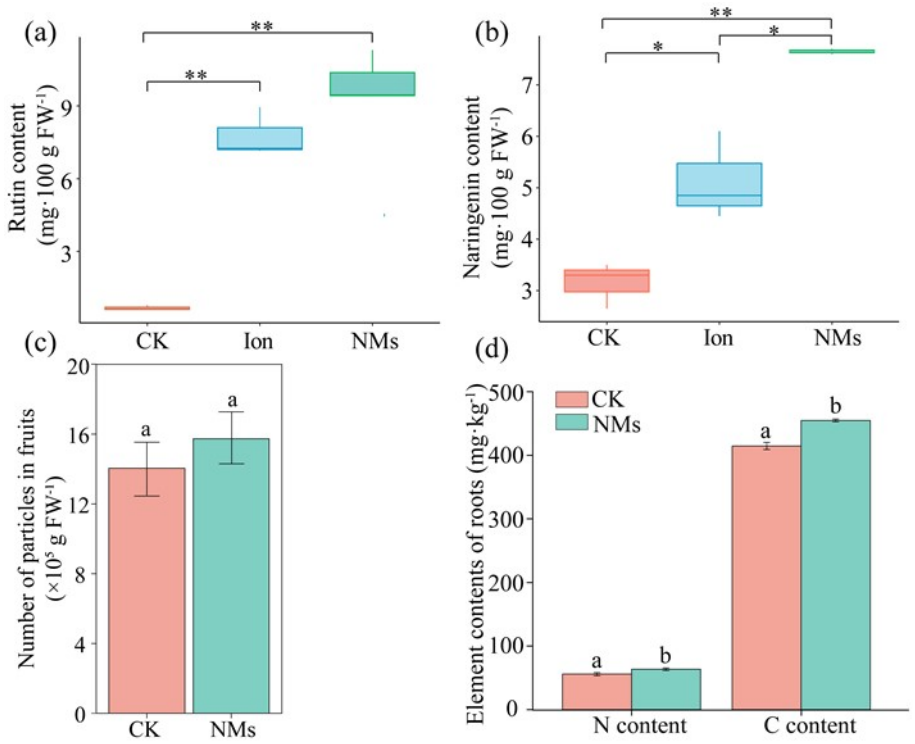

Fig. S6 The contents of flavonoid single particles in tomato fruits and C and N contents in roots upon $Fe_7(PO_4)_6$ NMs application. (a) Content of rutin, (b) naringenin (c) single particles in tomato fruits and (d) element contents of tomato roots. Asterisks represent significant differences with * $p < 0.05$ and ** $p < 0.01$ and different letters represent significant difference at $p < 0.05$.

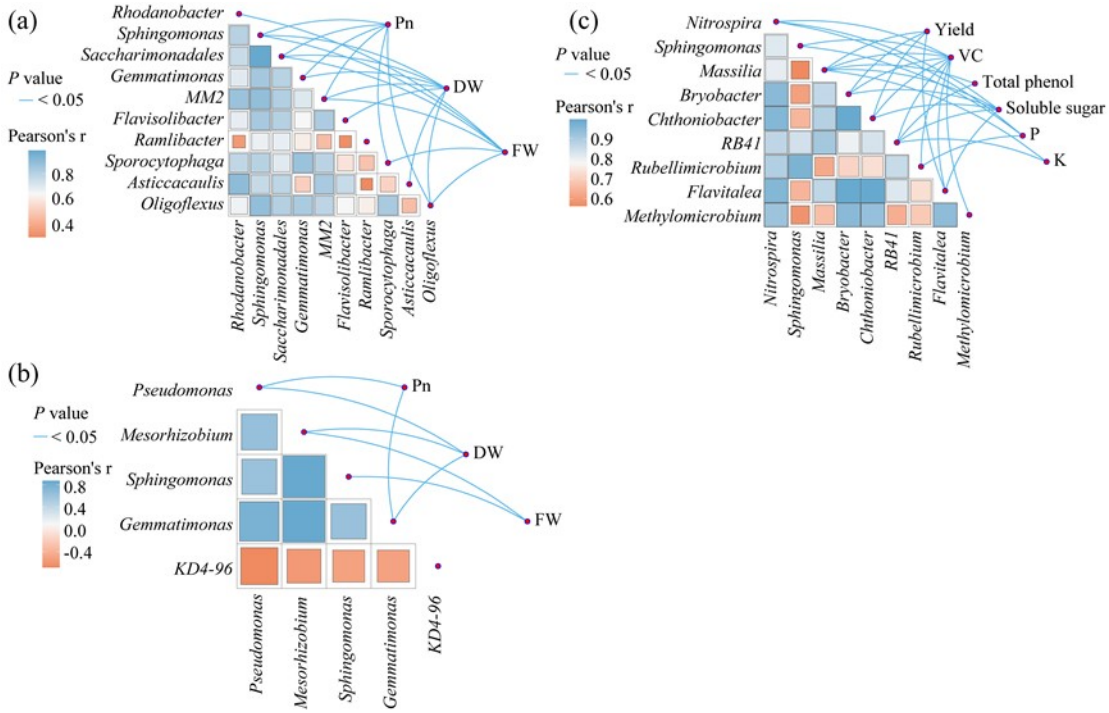

Fig. S7 Correlation analyses of the major variables between the control and NMs at different stages. (a) seedling stage (b) flowering stage and (c) mature stage. The blue line indicates a significant positive correlation (Pearson correlation analysis, $p < 0.05$).

Table S1. The hydrodynamic diameter and zeta potential of 50 $mg \cdot kg^{-1}$ $Fe_7(PO_4)_6$ NMs in deionized (DI) water and soil solution.

Medium	Hydrodynamic diameter (nm)	Zeta potential (mV)
DI water	469.2 ± 44.8	-18.4 ± 1.3
Soil solution	846.2 ± 13.9	-10.3 ± 0.1

Table S2. Typical sensitivity, detection limits, and R^2 for element detection by ICP-MS.

Elements	Linearity range (mg/L)/ R^2	Linearity equation	Detection limit (μg/L)	Bush branches and leaves (GBW07602) Standard value (μg/g)	Bush branches and leaves (GBW07602) ICP-MS measured value (ug/g), n=3
Na	0-10/0.996	y = 675.11 x + 12961.11	0.261	11000 ± 130	10982 ± 120
Mg	0-10/0.997	y = 274.74 x + 877.45	0.449	2870 ± 180	2932 ± 65
K	0-10/0.999	y = 154.69 x + 3658.67	1.295	8500 ± 500	8602 ± 131
Ca	0-10/0.999	y = 11.02 x + 850.48	6.214	2200 ± 130	2311 ± 122
Fe	0-10/0.998	y = 220.25 x + 117.02	0.141	1020 ± 67	1076 ± 25
Mn	0-0.1/0.999	y = 5.58 x + 120.51	0.004	58 ± 6	62 ± 3
Zn	0-0.1/0.996	y = 2346.79 x + 16110.99	0.064	20.3 ± 2.2	22.0 ±1.04
Cu	0-0.1/0.998	y = 18597.00 x + 21068.42	0.095	5.2 ± 0.5	5.9 ± 0.1

Table S3. Sequences of primer pairs used to measure the transcription of several genes by real-time PCR.

Gene name	Primer
SUT1	forward: 5'-GAACTCCCGGAGAAAGAGCTAGA-3'
	reverse: 5'-TCGCATCACCGACTTGTCCACC-3'
SUT2	forward: 5'-TCTGAAGCAGGAAGTGGA-3'
	reverse: 5'-CGGTGGAGGAAGAGGTAGATTAG -3'

Table S4. Physicochemical properties of soil samples under $Fe_7(PO_4)_6$ NM and ion treatments.

Soil	Treatment	pH	EC (us·cm^{-1})	Na	Mg	P	S	K	Ca	Mn	Fe
							(mg·kg^{-1})				
Rhizosphere	CK	7.9±0.1b	0.2±0.05a	9.8±0.8a	7.4±0.8a	0.9±0.3a	0.4±0.1a	104±16a	9.4±1.1a	0.9±0.4a	35.2±4.5a
	Ion	7.6±0.1c	0.2±0.07a	9.4±0.4a	7.4±1.1a	0.7±0.1a	0.4±0.1a	120±6a	9.4±1.4a	0.7±0.2a	31.6±2.4a
	NMs	7.5±0.2ac	0.6±0.2a	9.3±0.4a	7.2±0.3a	0.6±0.1a	0.3±0.1a	119±8a	9.5±0.9a	0.6±0.1a	30.4±1.4a
Non-rhizosphere	CK	7.9±0.1a	0.1±0.05a	9.7±0.7a	7.2±0.2a	1.6±0.5a	0.2±0.07a	133.5±6.8a	9.7±0.9a	0.7±0.08a	32.3±1.5a
	Ion	7.7±0.1a	0.1±0.01a	9.5±0.4a	7.1±0.2a	1.7±0.2a	0.2±0.01a	133.2±75a	9.5±0.4a	0.6±0.04a	31.7±1.4a
	NMs	7.8±0.1a	0.3±0.01a	9.6±0.3a	7.1±0.1a	2.6±1.7a	0.6±0.06a	128.6±5.0a	9.6±0.4a	0.7±0.2a	33.9±4.5a

Reference

1. L. Jiao, X. Cao, C. Wang, F. Chen, H. Zou, L. Yue and Z. Wang, Crosstalk between *in situ* root exudates and rhizobacteria to promote rice growth by selenium nanomaterials, *Sci. Total. Environ.*, 2023, **878**, 163175.
2. X. Zhou, J. Liu and F. Wu, Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth, *Plant Soil*, 2017, **415**, 507-520.
3. C. J. Zhang, J. Pan, Y. Liu, C. H. Duan and M. Li, Genomic and transcriptomic insights into methanogenesis potential of novel methanogens from mangrove sediments, *Microbiome*, 2020, **8**, 94.