Mechanisms of growth-promotion and Se-enrichment in *Brassica chinensis* L. by selenium nanomaterials: beneficial rhizosphere microorganisms, nutrient availability, and photosynthesis

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Supplementary Text S1: determination of Se ENMs hydrodynamic diameter and Zeta potential.

The hydrodynamic parameters and Zeta potential were determined by dynamic light scattering with a Zatasizer Nano (ZEN3600, Malvern, UK). Nanoparticle distilled water solution of 1.5 mL (10 mg·L^{-1}) was put into a polystyrene latex cell and measured at detector angle of 173°, wavelength of 633 nm, refractive index of 0.30, real refractive index of 1.59, and temperature of 25 °C. The distilled water solution was sonicated for 30 min (35 Hz, SCIENTZ–28C017, China). 20 measurements were operated for each sample.

Supplementary Text S2: Measurement of chlorophyll content in *B. chinensis* leaves.

The content of photosynthetic pigment of *B. chinensis* leaves were measured and calculated according to previous study.[1] In briefly, 40 mg fresh rice leaves were ground into small piece under liquid nitrogen and extracted with 80% acetone. Then water bath heated for 15 min at 68 ℃ and 4000 g centrifugation for 15 min. liquid supernatant were measured at 663、646、470 nm by a multifunctional microplate reader (Varioskan Lux, Thermo Scientific, Finland). The calculation method of chlorophyll content is as follows:

- Chl a = $12.21A_{663}$-$2.81A_{646}$ (Chloroplast a)
- Chl b = $20.13A_{646}$-$5.03A_{663}$ (Chloroplast a)
- Chl x= ($1000A_{470}$-3.27Chl a- 104Chl b)/229 (Carotenoid)

Supplementary Text S3: carbohydrate content determination in *B. chinensis* shoot.

Carbohydrates content of *B. chinensis* shoot was measured by phenol method.[2] Carbohydrates can be oxidized by sulfuric acid, and transformed into to furfural or its derivatives. The furfural or hydroxymethyl furfural can react with phenol to form an orange compound. The absorption at 490 nm is proportional to the carbohydrates content.

The specific steps are the following: 0.2 g *B. chinensis* shoot were cut and grinded. Small amount of ultrapure water was added and transferred into 10 mL test tube. After heating in boiling water bath for 15 min, these solutions were filtrated, and collected in

100 mL volumetric flask.

Glucose standard curve: various concentrations (0, 20, 40, 60, 80, 100, and 120 μg/mL) of glucose were put into 7 test tubes with stopper. Then phenol and H_2SO_4 were added into every tube. After shaking, these tubes were heated in a boiling bath. The absorbance was determined at 490 nm when these solutions were cooled to room temperature.

The calculation method of carbohydrate content is as follows:

$y = 0.6995x + 0.0899$ (R^2=0.9939), x is the absorbance at 490 nm; y is the Carbohydrates content (μg).

Supplementary Text S4: The defines of yield and selenobacteria.

Yield refers to the shoot fresh weight of *B. chinensis*.

Selenobacteria contains *Stenotrophomonas* sp. B19, *Enterobacter* sp. B16, *Bacillus* sp. R12 and *Pseudomonas* sp. R8.

Pn is net photosynthesis rate.

Supplementary Text S5. The dissolution experiment of resultant Se ENMs in soil

The 20 mL soil suspension (water/soil ratio 1:1) and 2 mL (20 $mg \cdot L^{-1}$) of Se ENMs suspension were mixed. Before sampling at 0, 6, 12, 48, 72 h, 6 mg of polyaluminum chloride were added to precipitated Se ENMs in each tube. After centrifugating at 8000 rpm for 20 minutes, the supernatant was passed through a 0.22 μm membrane, diluted 10 times with ultrapure water, and tested by ICP-MS.

Supplementary Text S6: low molecular weight compounds analysis

Soil extracts were obtained by adding 1 mL of solvent (Methanol: acetonitrile: water = 4: 4: 2, v/v/v) to 100 mg homogenized rhizosphere soil and shaking in 2 mL capped glass vials for two hours on an Eppendorf Thermomixer (4 ℃). Samples were removed from the shaker and soil extracts were collected by suspension filtered with 0.22 μm membrane. Quality control (QC) sample was prepared by mixing aliquots of all samples to obtain a pooled sample. Then, soil extracts (5 μL, full loop injection) were separated at 35 ℃ on a Thermo Scientific UPLC Vanquish equipped with a HSS T3 column (100×2.1 mm, particle size 1.8 μm, Waters) applying the following gradient at a flow rate of 0.35 μL/min: 0 min 95% A (Water/0.1% formic acid), 5% B (Acetonitrile /0.1%

formic acid); 1.5 min 5% B; 14 min 100% B; 15.5 min 100% B; 16 min 5% B; 22 min 5% B.

Eluting compounds were detected from m/z 70 to 1050 using a Thermo Scientific UPLC Vanquish coupled to the Q Exactive Orbitrap (UPLC-ESI-QE) in positive and negative ion mode using the following instrument settings: User role, Standard; Use lock mass, off; Chrom. Peak wi, 5 s; Runtime, 0 to 22 min; Polarity, Positive/negative; Exclusion, on; Default charge, 1; Resolution, 70,000; AGC target, 1e6; Maximum IT, 100ms; Scan range: 70 to 1050 m/z; dd-MS2, Resolution, 17500; AGC target, 5e4; Maximum IT, 50 ms; Loop count, 8; Top N, 8; Isolation window, 1.5 m/z; (N) CE/Stepped nce, 20, 40, 60; dd setting, Minimum AGC, 8.00e3; Intensity thresh: 1.6e5; Dynamic exclus 10.0 s. Data analysis accomplished by using Compound Discoverer software 3.1, and details of Compound Discoverer software 3.1 workflows and parameters are available in Figure S12 and Table S4.

Table S1. Detailed soil parameters before seed sowing and after the plant harvest.

Soil element content		Unplanted	Planted	
		CK	CK	Se ENMs
	C (g/kg)	61.03 ± 1.9	55.2 ± 1.4	52.5 ± 1.1
	Mg (g/kg)	5.6 ± 0.34	2.1 ± 0.04	1.6 ± 0.06
	N (g/kg)	23.6 ± 1.3	20.3 ± 1.1	18.3 ± 0.86
	P (g/kg)	0.38 ± 0.06	1.9± 0.03	1.7 ± 0.08
Macro-element	K (g/kg)	12.9 ± 0.50	4.7 ± 0.61	4.5 ± 0.84
	S (g/kg)	7.8 ± 0.81	0.36 ± 0.04	0.44 ± 0.03
	Ca (g/kg)	9.9 ± 0.62	3.0 ± 0.06	3.0 ± 0.02
	Na (g/kg)	6.1 ± 0.35	3.3 ± 0.23	3.4 ± 0.41
	Fe (g/kg)	24.3 ± 1.4	11.3 ± 1.2	10.8 ± 0.4
	Mn (g/kg)	0.58 ± 0.04	0.34 ± 0.04	0.26 ± 0.09
	Zn (g/kg)	0.21 ± 0.02	0.10 ± 0.02	0.11 ± 0.03
Micro-element	Cu (mg/kg)	126.7 ± 2.6	64.4 ± 3.7	119.9 ± 5.3
	Mo(mg/kg)	6.4 ± 0.5	3.6 ± 0.96	3.2 ± 0.56
	Se (mg/kg)	0.19 ± 0.03	0.12± 0.05	0.32 ± 0.03

Table S2. Primers of selected *BnSultr, BnSUCs* and *BnSWEETs* genes for qRT-PCR analysis

Gene	Forward Primer	Reverse Primer
BnSultr1,1	5' -AGATATTGCGATCGGACCAG-3'	5'-GAAAACGCCAGCAAAGAAAG-3'
BnSUC1,1	5' -TCCTCTCCGTAAAATAATCTCCGTC- 3'	5' –AAGGTGTGAGGAGAGAGTGCTGTAG- 3'
BnSUC1,4	5' -GGTCCTCTCTCATCTGGCTCTG- 3'	5' -CGAGTCTGTCTCCCATTGTGTG- 3'
BnSWEET10,2	5' -ACCTTATCGGCAGTTATGTGGCT- 3'	5' -TTATGCGGTTATGGTGTCTTCAATG- 3'

Table S3. The hydrodynamic diameter and Zeta potential of Se ENMs

Name	Value	Unit
Hydrodynamic diameter	648.9 ± 24.2	nm
Zeta potential	-34.4 ± 1.4	mV

Table S4. Detailed Compound Discoverer parameters for low molecular weight compounds data processing.

Select spectra	
Precursor Selection	Use MS(n-1) Precursor
Provide Profile Spectra	Automatic
Lower RT Limit	0
Upper RT Limit	0
First Scan	0
Last Scan	0
Ignore Specified Scans	
Lowest Charge State	0
Highest Charge State	0
Min Precursor Mass	0 Da
Max Precursor Mass	5000 Da
Minimum Peak Count	1
MS Order	Any
Polarity mode	Not specified
S/N Threshold	1.5

Align Retention Time	
Alignment Model	Adaptive Curve
Minimum Shift [min]	0.2
Mass Tolerance	5 ppm
Detect Compounds	
Mass Tolerance [ppm]	5 ppm
Intensity Tolerance [%]	30
S/N Threshold	3
Min. Peak Intensity	100000
Ions	[2M+ACN+H]+1; [2M+ACN+Na]+1; [2M+FA-H]-1; [2M+H]+1; [2M+K]+1; [2M+Na]+1; [2M+NH4]+1; [2M-H]-1; [2M-H+HAc]-1; [M+2H]+2; [M+ACN+2H]+2; [M+ACN+H]+1; [M+ACN+Na]+1; [M+Cl]-1; [M+DMSO+H]+1; [M+FA-H]-1; [M+H]+1; [M+H+K]+2; [M+H+MeOH]+1; [M+H+Na]+2; [M+H+NH4]+2; [M+H-H2O]+1; [M+H-NH3]+1; [M+K]+1; [M+Na]+1; [M+NH4]+1; [M-2H]-2; [M-2H+K]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H-H2O]-1
Min. Element Counts	C H
Max. Element Counts	C90 H190 Br3 Cl4 K2 N10 Na2 O15 P3 S5
Group Compounds	
Mass Tolerance	5 ppm
RT Tolerance	0.2
Preferred Ions	[M+H]+1; [M-H]-1
Fill Gaps	
Mass Tolerance	5 ppm
S/N Threshold	1.5
Normalize Aeras	
Min. QC Coverage [%]	50
Min. QC Aera RSD [%]	30
Normalization Type	[None]
Exclude Blanks	True
Mark Background Compounds	
Max. Sample/Blank	5
Max. Blank/Sample	0
Hide background	True

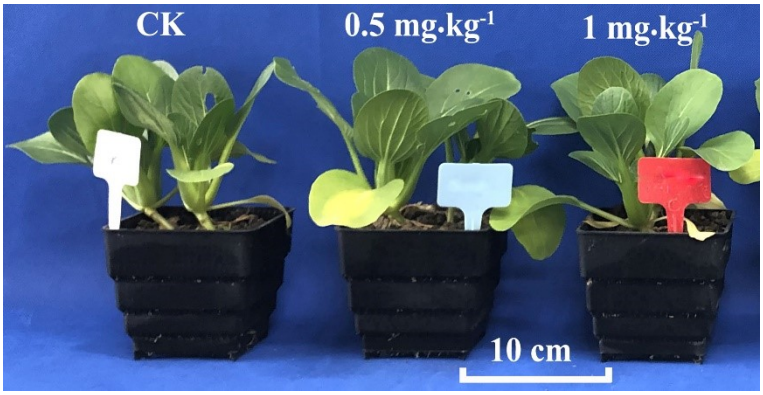

Figure S1. Growth of *B. chinensis* promoted by the soil application of Se ENMs (0, 0.5, and 1.0 mg·kg^{-1}).

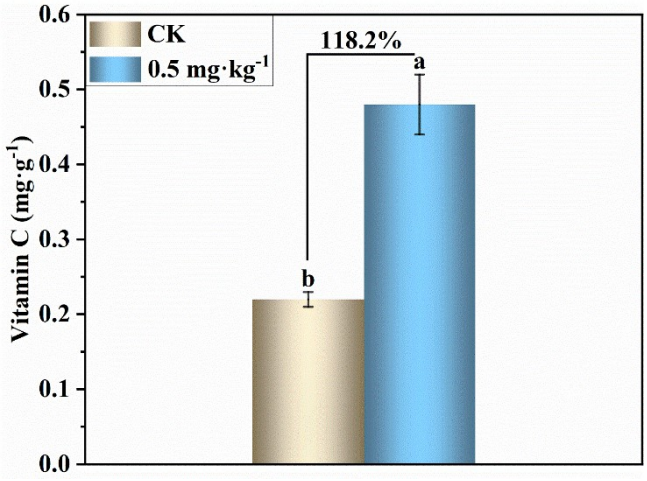

Figure S2. Vitamin C content in leaves of *B. chinensis* exposed to Se ENMs (0.5 mg·kg^{-1}). The content of vitamin C was increased by 118.2%.

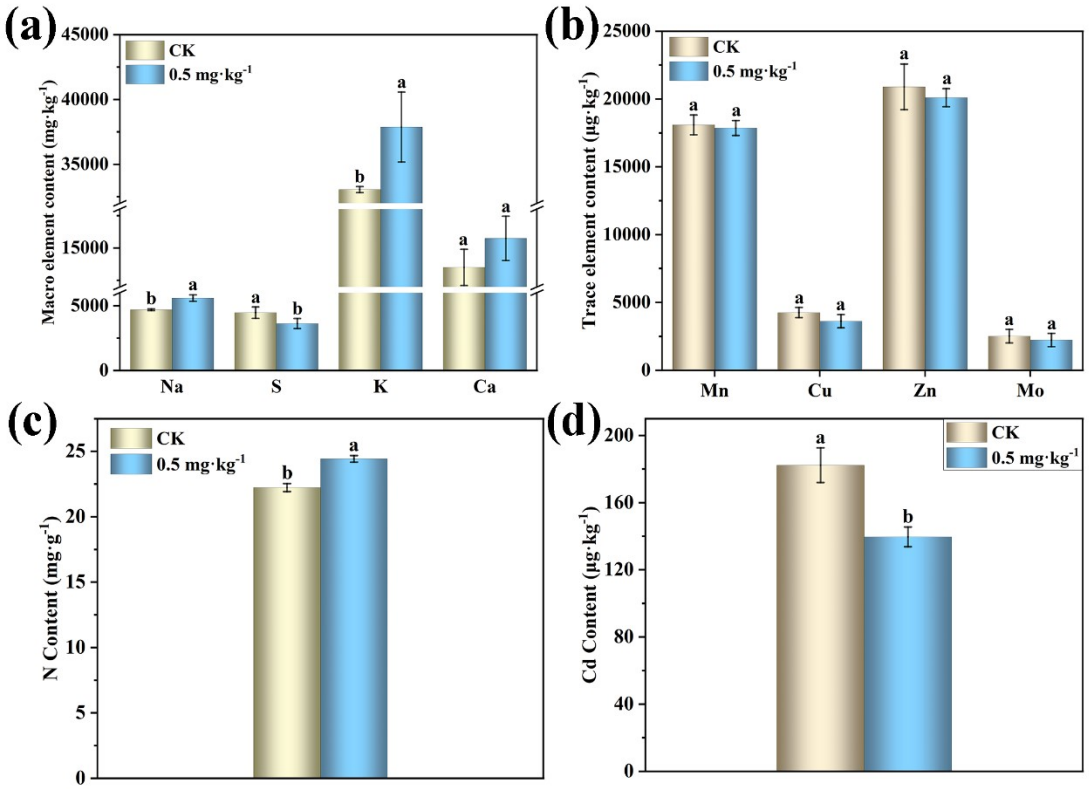

Figure S3. The content of mineral nutrients in *B. chinensis* leaves. a, Na, S, K, Ca; b, Mn, Cu, Zn, Mo; c, N and d, Cd. A reduction of Cd content in edible parts was observed upon Se ENMs (0.5 mg·kg^{-1}).

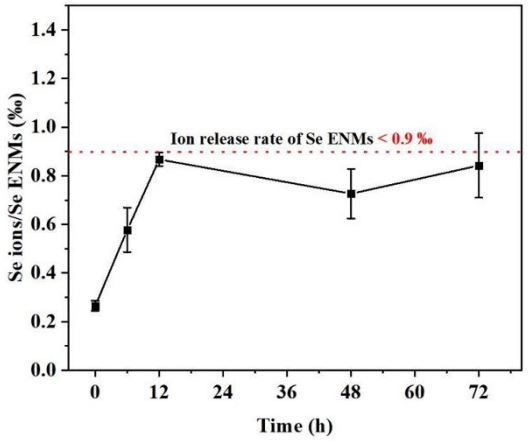

Figure S4. The dissolution experiment of resultant Se ENMs in soil. The results demonstrated about only 0.9% of Se could be released from Se ENMs.

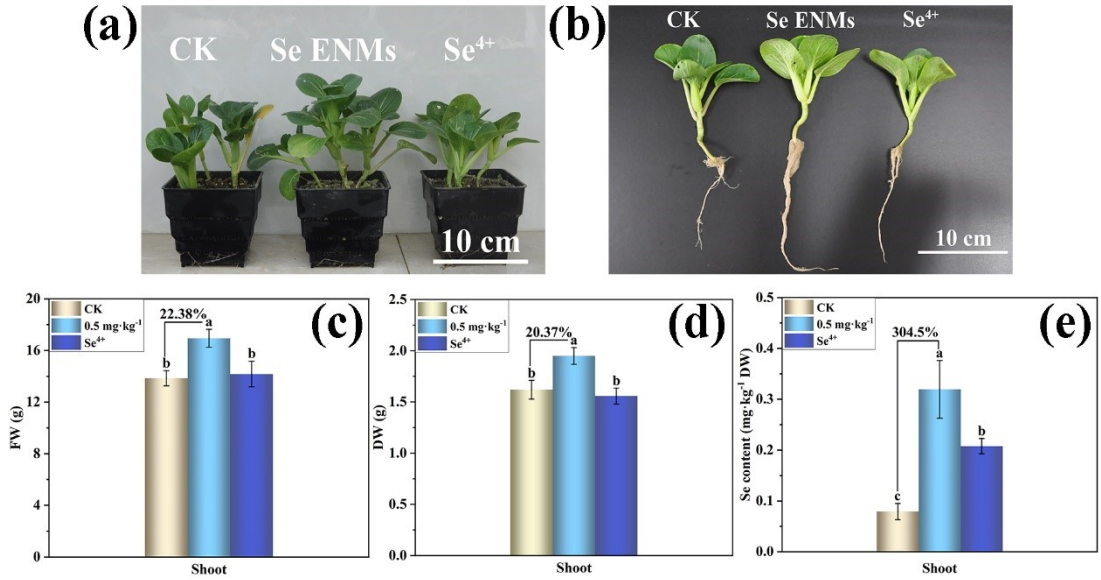

Figure S5. The growth of *B. chinensis* after exposing Se ENMs and Na_2SeO_3 at a same concentration (0.5 mg·kg^{-1}). a and b, photos of harvested *B. chinensis*; c, shoot FW and d, shoot DW of *B. chinensis*; e, Se content in *B. chinensis*. It is obvious that Se ENMs showed better promoted growth than Na_2SeO_3.

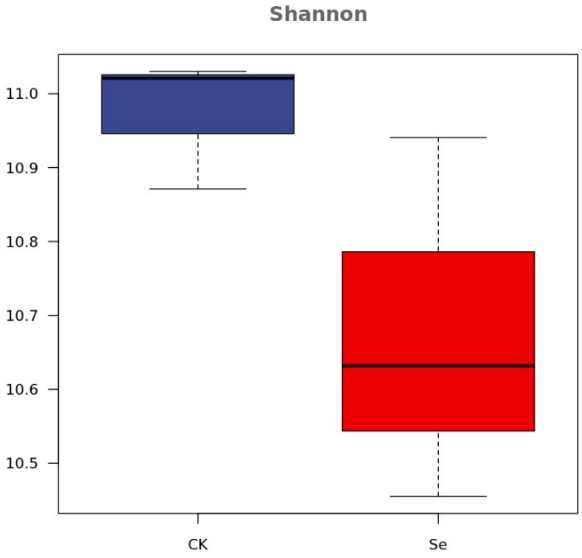

Figure S6. Alpha diversity index (Shannon) of rhizosphere microbiomes.

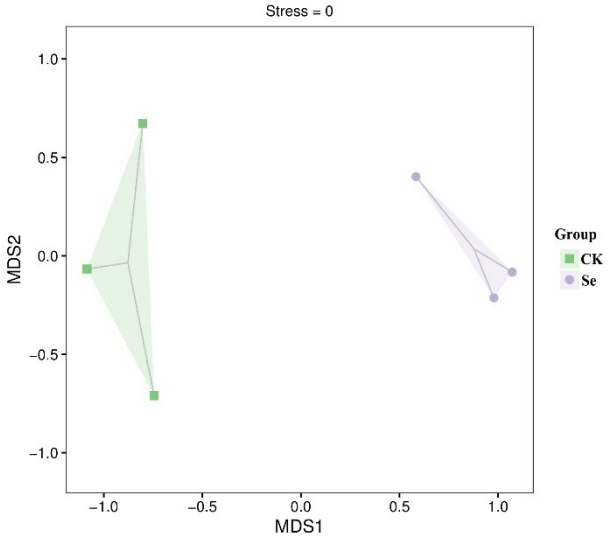

Figure S7. Non-metric multidimensional scaling analysis (NMDs) ($p < 0.05$) of rhizosphere microbiomes.

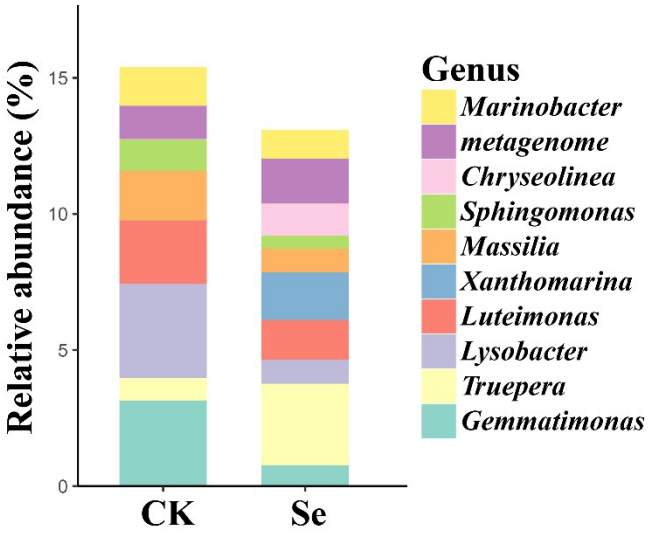

Figure S8. Relative abundances of rhizosphere microbiomes at genus level. The relative abundance of *Truepera*, *Chryseolinea* and *Xanthomarina* was elevated by 1.6%, 0.3% and 0.7%, respectively, upon exposure to Se ENMs (0.5 mg·kg^{-1}).

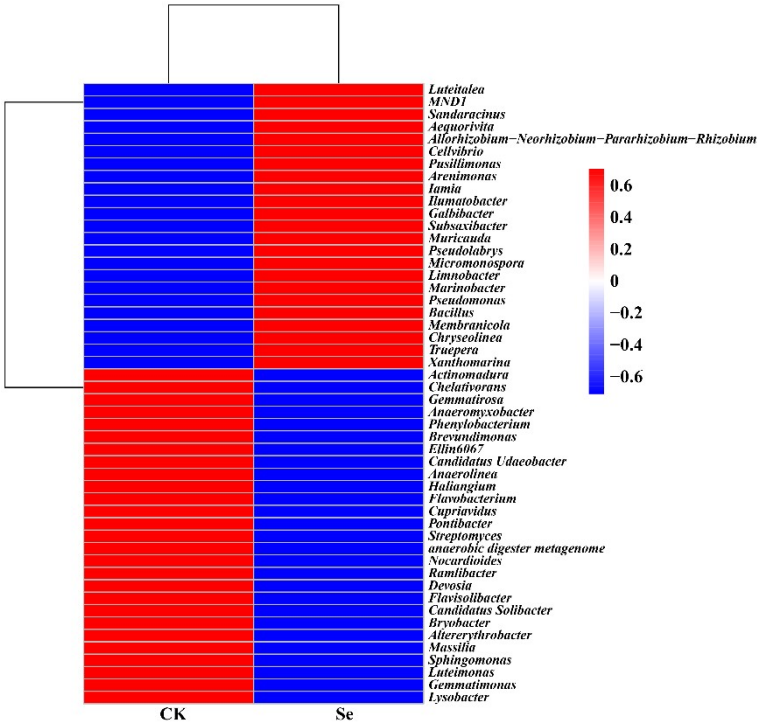

Figure S9. A heat map of the genus horizontal species of rhizosphere microbiomes.

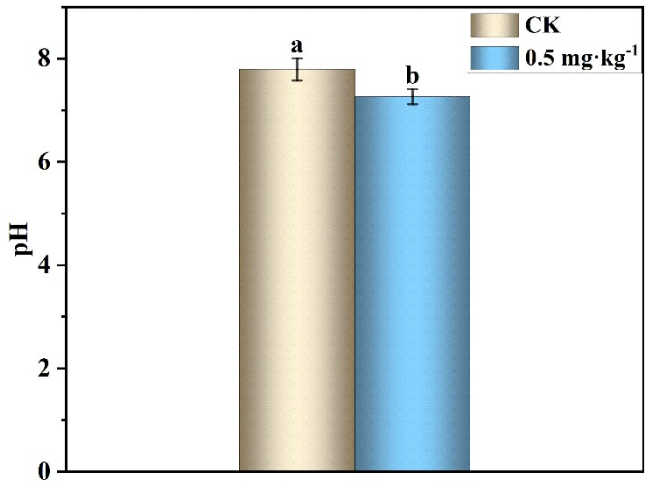

Figure S10. The rhizosphere soil pH was reduced from 7.8 to 7.3 after exposure to Se ENMs (0.5 mg·kg^{-1}).

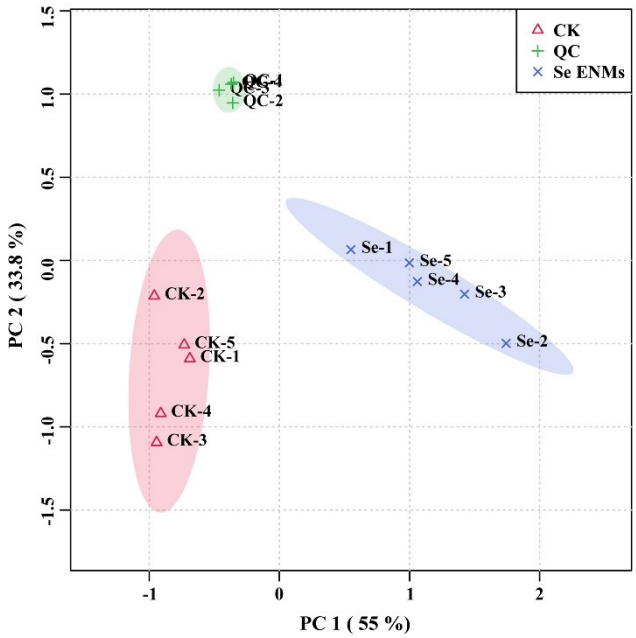

Figure S11. PCA analysis of significantly increased low molecular weight compounds in rhizosphere soil after exposure to Se ENMs (0.5 mg·kg^{-1}).

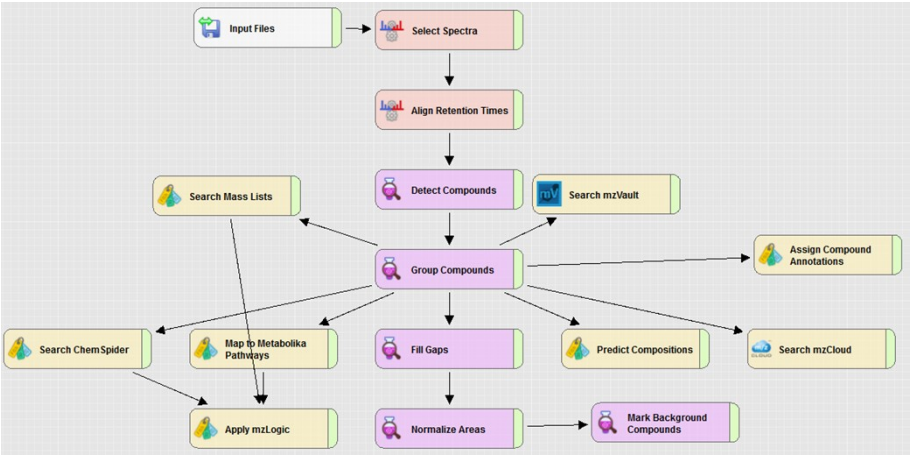

Figure S12. Exemplary data processing workflow for low molecular weight compounds on Compound Discoverer software 3.1.

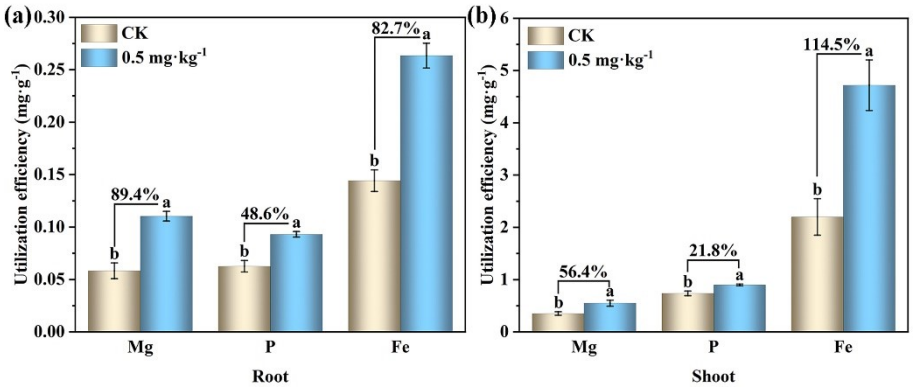

Figure S13. The utilization efficiencies of Fe, Mg and P in root (a) and shoot (b). The utilization efficiencies of these elements had been elevated in both root and shoot upon exposure to Se ENMs (0.5 mg·kg^{-1}). The utilization efficiency was calculated according to previous reports.[3,4] Briefly, that was the ratio of produced dry weight (DW) to nutrient accumulation (g). The DW was obtained by deactivating enzymes of fresh plant samples at 105 ℃ for 30 min, and following dried to constant weight (70 ℃).

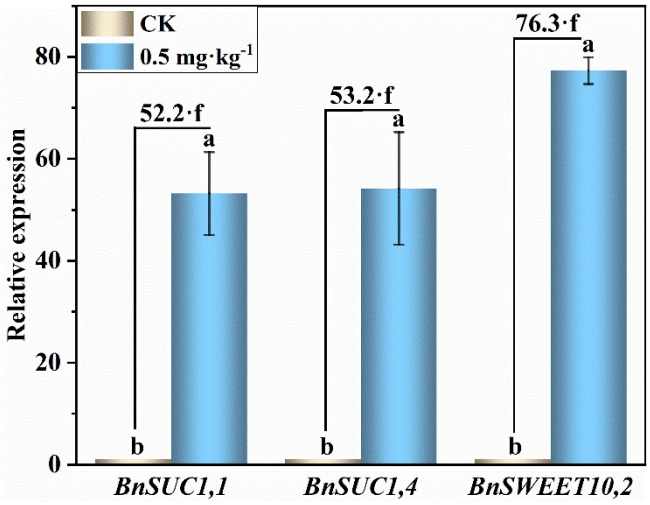

Figure S14. Relative expression of carbohydrates transport genes (*BnSUC1,1*, *BnSUC1,4* and *BnSWEET10,2*) in *B. chinensis* shoot, which was increased by 52.2, 53.2 and 76.3-fold, respectively, upon exposure to Se ENMs (0.5 mg·kg^{-1}).

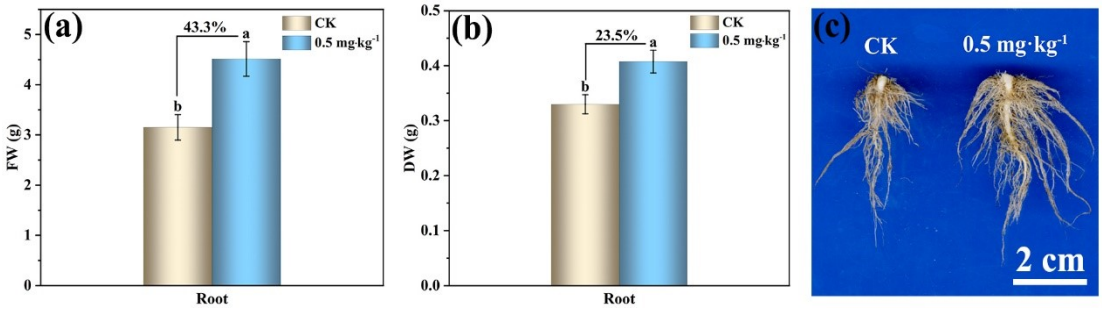

Figure S15. Biomass of *B. chinensis* root. a, FW and b, DW; c, root image at Se ENMs (0.5 mg·kg^{-1}) treatment relative to control.

References:

(1) H. K. Lichtenthaler, and A. R. Wellburn, Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents, 1983, 591-592.

(2) H. Wang, M. Zhang, Y. Song, H. Li, H. Huang, M. Shao, Y. Liu and Z. Kang, Carbon dots promote the growth and photosynthesis of mung bean sprouts. *Carbon*, 2018, **136**, 94-102.

(3) Z. Y. Wang, J. M. Kelly, and J. L. Kovar, Depletion of macro-nutrients from rhizosphere soil solution by juvenile corn, cottonwood, and switchgrass plants. *Plant and Soil*, 2007, **270** (1), 213-221.

(4) N. K. Fageria, Maximizing crop yields. CRC Press. 1992.