Environmental Science Nano

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

Journal:	*Environmental Science: Nano*
Manuscript ID	EN-ART-08-2021-000740.R2
Article Type:	Paper

Environmental significance

Nano-enabled agriculture is providing solutions for sustainable agriculture. Our results showed that Se engineered nanomaterials, through soil application, could regulate the rhizosphere microbiomes and nutrients availability to improve photosynthesis, yield and Se content of crops. Thus, Se engineered nanomaterials as nanofertilizers have the potential in replacing/reducing the use of traditional fertilizers in agriculture, but still could ensure crop yield and quality. These findings indicated Se ENMs-enabled nanotechnology was beneficial to human health and shows promising sustainable application in crop production.

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

Chuanxi Wang[ab], Le Yue[ab], Bingxu Cheng[ab], Feiran Chen[ab], Xiaoli Zhao[d], Zhenyu Wang*[ab], and Baoshan Xing[c]

[a] Institute of Environmental Processes and Pollution Control, and School of Environment and Civil Engineering, Jiangnan University, Wuxi, Jiangsu, 214122, China

[b] Jiangsu Key Laboratory of Anaerobic Biotechnology, Jiangnan University, Wuxi, Jiangsu, 214122, China

[c] Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA 01003, USA

[d] Chinese Research Academy of Environmental Sciences, Beijing, 100012, China

*Corresponding author: wang0628@jiangnan.edu.cn (Dr. Zhenyu Wang)

ABSTRACT

Maintaining a proper content of selenium (Se) in food is particularly important for human health. However, the mechanisms of uptake and enrichment of Se ENMs in crops are still unclear. Herein, Se engineered nanomaterials (Se ENMs) (size 62.3 ± 14.6 nm and surface charge −34.4 ± 1.4 mV) were synthesized and used as nanofertilizers for Se-fortified vegetables. The results demonstrated the Se content and yield were increased by 338.0% and 19.8% respectively in *Brassica chinensis* L. through soil application of Se ENMs (0.5 mg·kg^{-1}). The content of Se in vegetables increased up to 32.8 μg/100 g (7.5 μg/100 g for the control), which could provide the daily recommended Se intake (55–400 μg/day) for human. Amendments of the slightly alkaline soil with the Se ENMs improved beneficial rhizosphere microbiomes (*Pseudomonas* and *Bacillus*), and resulted in the plants accumulating more low molecular weight compounds (betaine, proline, glycine, norleucine, urocanic acid and indole-3-acrylic acid) with increases in the Se content of the plant by 264.9%. Moreover, the nutrient accumulation in leaves promoted the photosynthesis (16.7%) and increased carbohydrate content (6.5%). Also, the expression of carbohydrate transport-related genes (*BnSUC1,1*, *BnSUC1,4*, and *BnSWEET10,2*) were up-regulated by 52.2, 53.2 and 76.3-fold, respectively, promoting root growth, improving rhizosphere microbiome and nutrient availability. Therefore, such mutual benefits between leaves and roots using ENMs could provide an alternative model for cultivating Se-enriched crops.

KEYWORDS

Nano-enbles agriculture; Selenium nanomaterials; Rhizosphere microorganisms;

Nutrient availability; Photosynthesis

1. INTRODUCTION

Selenium (Se) contributes to the synthesis of selenoproteins for improving human antioxidant and immune system.[1] Until now, 25 selenoproteins have been identified, and their nutritional functions are achieved by the active center of selenocysteine in mammals.[1, 2] Se deficiency can lead to a variety of diseases including heart disease, weak immune system, and reproductive defects.[3] Therefore, supplying adequate Se is important for human health. The World Health Organization (WHO) has established the lowest limit of acceptable adult daily intake at 50 μg, and the Recommended Daily Allowance (RDA) in China is 55 μg for adults. Human intake of Se mainly comes from the diet, and the content of Se in foods widely depends on the crop growth conditions.[4] The concentration of Se in soil ranges from 0.01 to 2.0 $mg{\cdot}kg^{-1}$, with an average of ~0.4 $mg{\cdot}kg^{-1}$.[5] However, the bioavailability of natural-occuring Se is very low for plants, limiting the Se required for mammals through vegetable consumption. Globally, around 800 million people are deficient in Se.[6] Therefore, enhancing the Se content in foods is urgently needed for the human health.[7]

At present, application of inorganic Se salts for producing Se-enriched food is an effective way to solve Se deficiency in dietary.[6] It was shown that sodium selenate (Na_2SeO_4, 40 $mg{\cdot}L^{-1}$) can stimulate wheat growth, boost grain yield, increase Se content (5.99 $\mu g{\cdot}kg^{-1}$), and enhance plant resistance under drought stress.[8] Sodium selenite (Na_2SeO_3, 50 $mg{\cdot}kg^{-1}$) can increase the content of chlorogenic acids (an important active component of anti-oxidation) in *Lycium chinense* leaves, and elevate

the level of chlorophyll and carotenoids in photosynthesis system.[9] However, Se inorganic salts as fertilizers are adsorbed easily by soil minerals, resulting in low recovery efficiency (~14%).[10] So far, emerging studies reported that zero-valent Se engineered nanomaterials (Se ENMs) with higher mobility and solubility, prominent bioactivity and biosafety showed promising application in agriculture.[11–13] It has been reported that Se ENMs (50–100 mg·kg^{-1}) could promote callus organ formation and root growth in tobacco.[11] Under low-temperature stress (10 ℃), Se ENMs increased the chlorophyll content of tomato leaves by 27.5%, which outperformed the Na_2SeO_4 (19.2%).[14] Zahedi *et al*. reported that foliar spray of Se ENMs could stimulate the growth and increase the yield of strawberry under salinity stress.[15] These reports demonstrated that Se ENMs could promote crop growth and yield, but the key factors for improving Se availability remained to be identified. Moreover, the mechanism of enhanced photosynthesis and yield promoted by Se ENMs is unclear.

The rhizosphere microbiome plays a critical role in increasing nutrient availability and promoting plant growth.[16–18] For instance, symbiotic associations between plants and nitrogen (N)-fixing bacteria converted atmospheric N into ammonium-N which can be easily taken up by plants.[17] Phosphate (P)-solubilizing bacteria excrete organic acids and increase the dissolution of precipitated P, thereby improving the P availability.[18] Studies demonstrated that nutrients, such as iron (Fe), magnesium (Mg), and P could improve photosystem and promote photosynthesis,[19] and the resulting formation of carbohydrates could provide 11–40% carbon sources for microbes through root exudates.[20] Recent researches found that Ag (100 mg·kg^{-1}), SiO_2 (100 mg·kg^{-1}), TiO_2

(100 mg·kg^{-1}), and Fe_3O_4 ENMs (100 mg·kg^{-1}) could alter soil microbial community composition and metabolite profiles.[21,22] Therefore, we hypothesized that soil application of Se ENMs could increase the abundance of beneficial rhizosphere microorganisms, improve nutrient availability, and increase Se content in crop. The resulting increased nutrient accumulation in leaves could promote photosysthesis and even yield. The carbohydrates from photosynthesis transported from shoot to root, which may further promote root growth, improve rhizosphere microbiome and increase nutrient availability.

Brassica chinensis L. (*B. chinensis*) was selected as an experimental crop because it is an important vegetable and contains various vitamins for human health.[23] The following three aspects were specifically explored: (1) rhizosphere microbiomes in response to Se ENMs; (2) the mechanisms of Se ENMs uptake and Se-enrichment in *B. chinensis*; and (3) the change in photosynthesis and yield of *B. chinensis* under Se ENMs exposure. This is the first investigation on the relationship among beneficial rhizosphere microorganisms, nutrient availability and photosynthesis of *B. chinensis* upon Se ENMs application, which could provide an alternative model for cultivating Se-enriched crops.

2. MATERIALS AND METHODS

2.1 Synthesis and characterization of Se ENMs

The synthesis of Se ENMs was similar to that in a previous study.[24] Briefly, the mixed solution of 4×10^{-5} M selenic acid (H_2SeO_3, ≥95%, Sinopsin group chemical reagent., LTD) and raisin extracts were heated under refluxed condition (pH, 5.9). Then Se

ENMs were purified and obtained through centrifuging at 17280 g for 20 mins. The shape and size of Se ENMs were observed by a transmission electron microscope (TEM, JEM–2100, Nippon electronics co, JPN, operating at an acceleration voltage of 200 kV). The hydrodynamic diameter and Zeta potential were measured by Zatasizer Nano (ZEN3600, Malvern, UK), and the details are shown in Supplementary Text S1.

2.2 Plant cultivation and ENMs exposure

The soil was collected from Jiangsu province, China, and the seeds of *B. chinensis* (No. 9, Suzhouqing) were obtained from Jiangsu Academy of Agricultural Sciences. The soil was natural and their properties were pH of 7.8, redox potential of 318.5 mV, electrical conductivity of 0.36 mS/cm, total nitrogen of 23.6 g/kg, total phosphorus of 0.38 g/kg, total potassium of 12.5 g/kg, total carbon of 61.1 g/kg, organic matter of 22.5 $g \cdot kg^{-1}$.

Soil was homogenized, filtered through 5.0 mm sieve to remove large plant residues and fragments. Then the soil was blended vigorously with Se ENMs at different concentrations (0, 0.5, and 1 $mg \cdot kg^{-1}$) or with Na_2SeO_3 (0.5 $mg \cdot kg^{-1}$) to achieve a homogeneous mixture. Each pot was filled with 1 kg prepared soil without aging, and each treatment had five replicates. *B. chinensis* seeds were sown at 1 cm depth in soil. The seedlings were grown in a greenhouse for 60 days under conditions of 20/15 °C at day/night and 18/6 h light/dark cycle. During the growth stage, no additional fertilizers were applied. Detailed soil parameters before seed sowing and after the plant harvest are shown in Table S1. Photosynthesis parameters of *B. chinensis* leaves were determined before sampling by CIRAS-3 portable gas exchange system, Hansatech,

USA. Root parameters (root length, surface, average diameter, and number of tips) were analyzed by WinRHIZO Pro 2017b, Canada. The measurements of chlorophyll and carbohydrate content are shown in Supplementary Text S2 and S3, respectively. The vitamin C content of *B. chinensis* leaves were determined by the titration method with 2,6-dichlorophenolindophenol, using 1 g of fresh tissue and HCl (2%).[25]

2.3 Mineral nutrients analysis in plants

The contents of mineral nutrients and a heavy metal (cadmium, Cd) in plant tissues from different treatments were analyzed by inductively coupled plasma mass spectrometry (ICP–MS, iCAP–TQ, Thermo-Fisher, USA).[26,27] Briefly, dried tissues (25 mg) were digested (190 ℃, 30 min) in a microwave accelerated reaction system (CEM corp, Matthews. NC) with a mixture volume of HNO_3 (GR, 65–68%) and ultrapure water (v/v=4:3). The digested solution was filtered with microporous membrane (0.22 μm) and diluted to 50 mL by using ultrapure water (Mili–Q) for ICP–MS analysis. For quality control (QC) and quality assurance (QA), the standard reference material (GBW 07602, Bush twigs and leaves purchased from Nanjing Alida Biotechnology Co., LTD, China) were digested and measured using the same procedures. The recoveries of all elements were between 84.9 and 102.9%.

2.4 Soil microbial community composition analysis

To characterize the soil microbial diversity and composition, high-throughput sequencing of soil bacterial 16S rRNA genes was performed on an Illumina MiSeq platform (Personal Biotechnology Co., Ltd. Shanghai, China). Briefly, total DNA of soil microorganisms was extracted by using the Fast DNA SPIN extraction kits (MP

Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocols. The concentration and quality of total DNA were measured by Nanodrop (Thermo Scientific, NC2000, USA), and 1.2% agarose gel electrophoresis (Invitrogen, AM9870, USA). The V3-V4 region of microbial 16S rRNA gene was amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by a quantitative PCR system (ABI, 2729, USA). The amplification system including: 5×reaction buffer 5 μL, 5×GC buffer 5 μL, dNTP (2.5 mM) 2 μL, Forward primer (10 μM) 1 μL, Reverse primer (10 μM) 1μL, DNA Template 2 μL, ddH_2O 8.75 μL, Q5 DNA Polymerase 0.25 μL. The qPCR reaction program parameters were 98 ℃ 2 min, denaturation 98 ℃ 15 s, annealing 55 ℃ 30 s, extension 72 ℃ 30 s, final extension 72 ℃ 5 min, 10 ℃ hold. 25–30 cycles. After the amplification, Illumina's TruSeq Nano DNA LT Library Prep Kit was used to prepare the sequencing library, and the library was selected and purified by 2% agarose gel electrophoresis. After completion of the amplification step, amplicons were pooled in equal amounts, and pair-end 2×300 bp sequencing was performed using the Illlumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

2.5 Quantitative real-time PCR (qRT-PCR) analysis

qRT-PCR was used to investigate the regulation of key genes involved in Se translocation and carbohydrate transport. Total RNA of *B. chinensis* leaves (60 days) was extracted using a TaKaRa MiniBEST Plant RNA Extraction Kit according to the protocol of manufacturer. The concentration of RNA was measured by a ultramicro-

spectrophotometer (UltraM-QB200, Gallop tech Co., Ltd. Shanghai, China). cDNA was prepared from RNase-treated total RNA using a cDNA synthesis Kit (CW Biotech Co., Ltd. Jiangsu, China) according to the manufacturer's protocols. Each PCR reaction system (50 μL final volume) contained 2 μL cDNA template, 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM) (Table S2), and 25 μL 2×Ultra SYBR Mixture. qRT-PCR was performed by CFX96^{TM} real-time system (BIO-RAD, USA). The reaction program was 10 min at 95 ℃, followed by 40 cycles of 95 ℃ for 15 s and 60 ℃ for 1 min. Technical triplicate was done for each biological sample. The relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

2.6 Statistical Analysis

Biomass, photosynthesis, and root parameters were presented as the mean ± standard deviation. One-way ANOVA with the Fisher LSD test was used to analyze the variation among different treatments using Origin Statistics 2019b at $p < 0.05$. Sequence data analyses were mainly performed using QIIME and R packages (v3.5.0). All treatments were conducted at least in three triplicates.

3. RESULTS AND DISCUSSION

3.1 Characterization and promoted crop growth of Se ENMs

As-prepared Se ENMs showed a size distribution (Figure 1a) in 20–90 nm with an average size of 62.3 ± 14.6 nm (Figure 1b). The hydrodynamic diameter and Zeta potential of Se ENMs in ultrapure water were 648.9 ± 24.2 nm and −34.4 ± 1.4 mV (Table S3), respectively. Previous studies demonstrated that the size and surface charge had a profound impact on the root uptake and translocation of ENMs.[27-31] Although

some inconsistent results about size remained, it is certain that nanometer-sized materials can be taken by plants.[32] Previous study also demonstrated negatively charged CeO_2 ENMs were more efficiently translocated than that of positively charged ones in roots due to electrostatic repulsion.[30] Therefore, the nano-sized and negatively charged Se ENMs may have potential applications in agricultural production. The growth-promoting effect of Se ENMs after soil application was observed in *B. chinensis* (Figure S1 and 1c). Both fresh and dry weight (FW and DW) of *B. chinensis* were increased (19.8% and 24.8%) upon exposure of 0.5 mg·kg^{-1} Se ENMs as compared to the control (CK) (Figure 1d and 1e). Particularly, the yield (FW, defined in Supplementary Text S4) of *B. chinensis* was promoted by 19.8% upon 0.5 mg·kg^{-1} Se ENMs exposure (Figure 1d). Although no significant difference in FW of shoots was observed between 1 mg·kg^{-1} and 0.5 mg·kg^{-1} treatments, DW of shoots differed (Figure 1d and 1e). Moreover, the Se content in root and shoot of *B. chinensis* increased 257.1% and 338%, respectively (Figure 1f). Additionally, the content of vitamin C in *B. chinensis* was increased by 118.2% (Figure S2). Many other mineral nutrients had no statistical difference, while significant reduction of Cd content (23.41%) in edible parts was observed upon Se ENMs (0.5 mg·kg^{-1}) exposure (Figure S3). The dissolution experiment demonstrated Se ENMs showed stability in soil because only 0.9% of Se could be released from Se ENMs (Supplementary Text S5 and Figure S4). Moreover, the plant promotion effects of Se ENMs (0.5 mg·kg^{-1}) were better than that of Na_2SeO_3 (0.5 mg·kg^{-1}) (Figure S5). Therefore, we argued Se ENMs could be taken up by plants in its nanoparticle form, consistent with some previous researches.[28,33-34] For example,

Wang et al. reported that CuO ENMs were taken up by maize and transported from root to shoot.[28] CeO_2 ENMs were detected in root, nodule, stem, leaf, and pod of Soybeans.[33] In addition, previous studies showed that Se could be taken up by roots and transported to shoots, then accumulated in plant leaf, and metabolized in chloroplast.[35-37] Therefore, most of the increased Se content may be accumulated in chloroplast. It has been noted that the sublethal toxic concentrations of Se in food was 1-30 $\mu g \cdot g^{-1}$ dry weight,[38] and the content of Se was only up to 0.33 $\mu g \cdot g^{-1}$ in this study. Therefore, Se ENMs (0.5 $mg \cdot kg^{-1}$) applied in *B. chinensis* production in the soil that was studied were safe and beneficial. These results suggested the as-prepared negatively-charged Se ENMs have a promising potential for producing Se-enriched crops. Moreover, given that the bioavailability of Se ENMs at 1 $mg \cdot kg^{-1}$ was not superior than that of Se ENMs at 0.5 $mg \cdot kg^{-1}$, further experiments were focused on the effects of Se ENMs at 0.5 $mg \cdot kg^{-1}$.

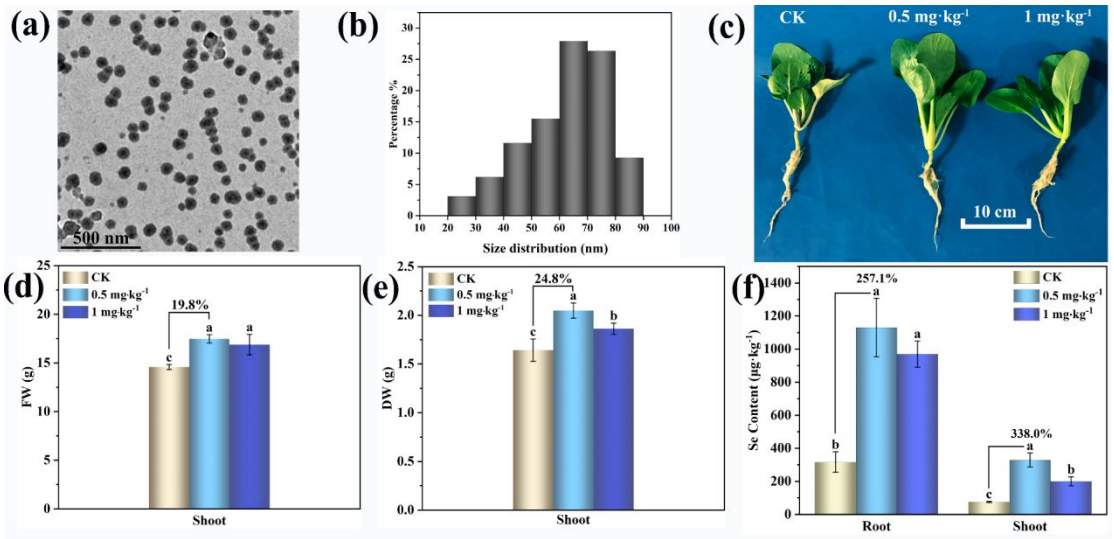

Figure 1. Characterization and promoted crop growth of Se ENMs. a, TEM image of Se ENMs; b, size distribution of Se ENMs; c, photos of harvested *B. chinensis* exposed to Se ENMs at 0, 0.5, and 1.0 $mg \cdot kg^{-1}$, respectively; d, shoot FW and e, shoot DW of *B. chinensis*; f, Se content in *B. chinensis*. (n=3, $p<0.05$)

3.2 Alteration in rhizosphere microbiome of *B. chinensis* in response to Se ENMs

The rhizosphere microbiome could improve the bioavailability of ENMs through

mediating the dissolution and anti-aggregation,[39] and also adsorb metal ions by electrostatic attraction.[40] In this study, the change of rhizosphere microbial community composition was observed after Se ENMs (0.5 mg·kg^{-1}) exposure. A total of 1012536 quality sequences of six soil samples were obtained from the analysis of rhizosphere bacterial community composition. The read lengths varied from 50 to 442 bp with an average of 382 bp at a 97% similarity level. No significant difference was observed in Shannon's H and Simpson indexes of Se ENMs-exposed treatments and CK (Figure 2a and S6, $p< 0.05$), suggesting Se ENMs at 0.5 mg·kg^{-1} did not alter the rhizosphere microbial diversity. While the beta-diversity of rhizosphere bacteria was changed distinctly according to the principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDs) based on the weighted UniFrac distance (Figure 2b and S7). The PCoA showed 78.2% and 8.6% of the total variation for the bacterial data, demonstrating that the bacterial species of CK was obviously different from that of the Se ENMs-treated soil. Meanwhile, the stress value of NMDs smaller than 0.2 further demonstated the significant composition difference between CK and Se ENMs-exposed groups. These results suggested that Se ENMs (0.5 mg·kg^{-1}) significantly affected the rhizosphere bacterial community composition. Other research indicated that metal and metal oxide ENMs in soil could alter rhizosphere microbial community compositions.[21,22] He *et al.* reported that FeO ENMs (1 mg·kg^{-1}) could promote the microbial growth via providing essential nutrients (C and N).[41] However, another study demonstrated that CuO ENMs could reduce the microbial activities.[42] Therefore, the interaction between ENMs and rhizosphere microbiome warrents further detailed

research.

In our studies with Se ENMs at the phylum level, the *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes*, *Deinococcus−Thermus*, *Patescibacteria*, *Firmicutes*, and *Verrucomicrobia* were the dominant phyla in both CK and Se ENMs (0.5 $mg \cdot kg^{-1}$) treated rhizosphere soils (Figure 2c). Notably, Se ENMs increased the abundance of *Proteobacteria* (3.3%), *Bacteroidetes* (3.3%), and *Deinococcus−Thermus* (1.7%). It has been demonstrated that *Proteobacteria* can act as plant growth promoting rhizobacteria for improving plant growth.[43] *Bacteroidetes* are known to degrade high molecular weight compounds including proteins and the polysaccharides, which might positively impact sugar and organic acid metabolism in soil.[21] It had been reported that the amount of *Deinococcus−Thermus* was increased under hydrocarbon contamination,[44] and had the ability of adsorbing heavy metals, such as lead (Pb), and Cd.[40] This could be the reason for the reduction in Cd content in harvested *B. chinensis* in this study (Figure S4d).

At the genus, the relative abundance of *Truepera*, *Chryseolinea* and *Xanthomarina* were elevated by 1.6%, 0.3% and 0.7%, respectively (Figure S8). *Chryseolinea* is responsible for the oxidization and utilization of diverse mono- and disaccharides as well as polysaccharides and organic acids.[45] The increased abundance of *Chryseolinea* might enhance the recycle of carbon in rhizosphere. *Xanthomarina* involves in phosphate solubilization, indole-3-acetic acid and siderophore production.[46] *Truepera* affiliated to the *Deinococcus−Thermus* phylum, which had potential to absorb heavy metals,[40] may reduce the adverse effect for plant growth by heavy metals.

Furthermore, the species composition heat map (Figure 2d) indicated Se ENMs (0.5 mg·kg^{-1}) changed the rhizosphere microbiomes, compared to CK. This result confirmed the change of rhizosphere bacterial composition, which was identified with PCoA and NMDs analysis (Figure 2b and S7). Se ENMs significantly increased the abundance of rhizosphere PGPR (Figure S9), such as *Cellvibrio*, *Pseudomonas*, *Bacillus*, and *Micromonospora*. The abundance of *Pseudomonas* and *Bacillus* were increased upon Se ENMs exposure, implying an increased colonization in rhizosphere which could stimulate plant growth. It is reported that *Pseudomonas* had the potential for enhancing growth of lettuce,[47] and root length of the tomato seedlings can be significantly improved in the presence of *Pseudomonas*.[48] Chowdhury *et al*.[49] reported that *Bacillus* was able to effectively reduce the disease severity (DS) of bottom rot on lettuce. A similar study found that *Bacillus* obviously promoted height of pepper by 27.2–54.5% under the stress of *Fusarium wilt*.[50]

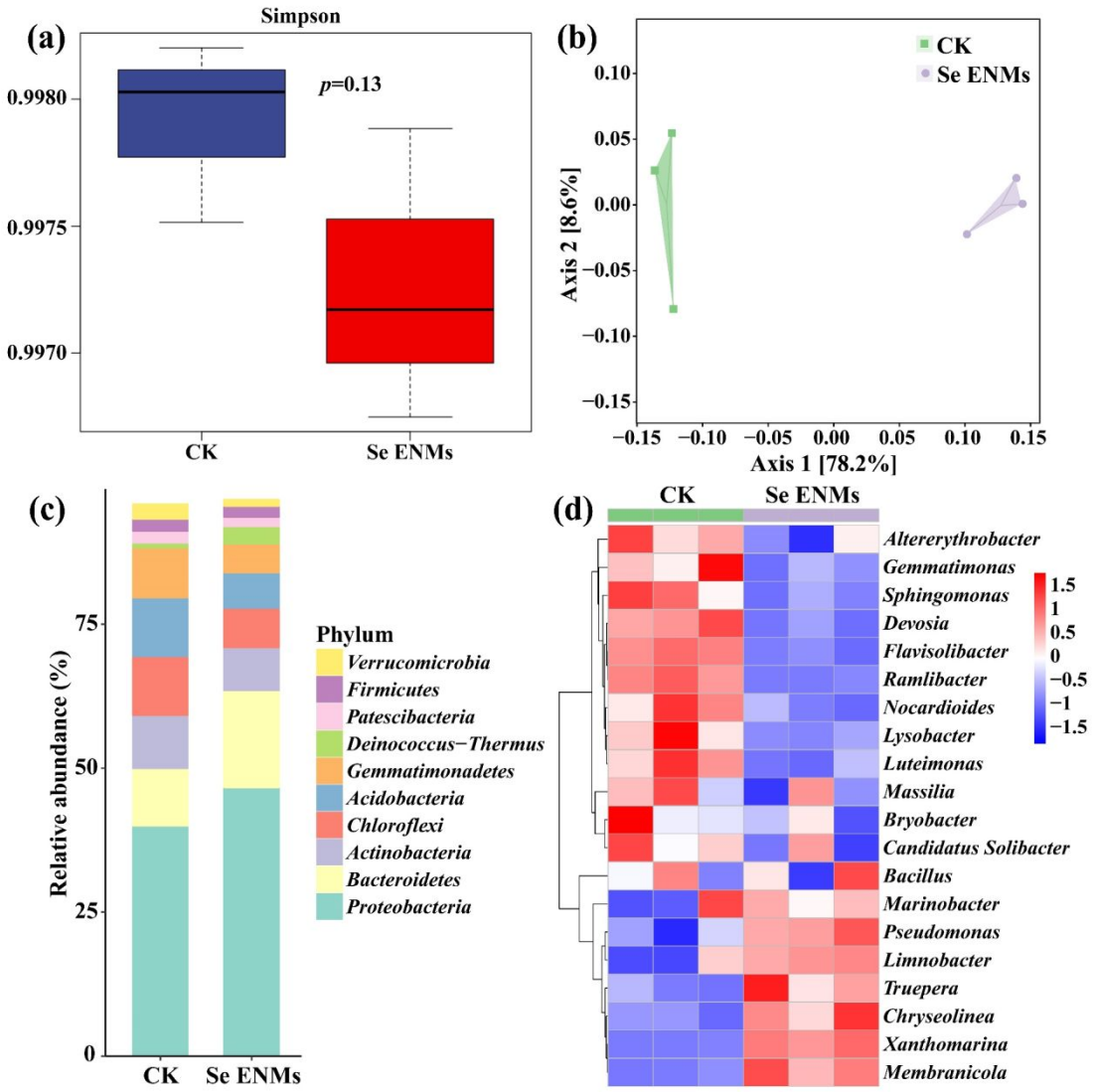

Figure 2. Rhizosphere microbiome of *B. chinensis* in response to Se ENMs. a, alpha diversity index (Simpson); b, principal coordinates analysis (PCoA); c, relative abundances of phylum; d, heat map clustering of microbial species. (n=3, $p<0.05$)

3.3 Increased uptake and accumulation of Se in *B. chinensis*.

As discussed above, the abundance of beneficial rhizosphere bacteria (*Pseudomonas* and *Bacillus*) was increased in *B. chinensis* upon Se ENMs exposure. Rhizosphere microorganisms could produce low molecular weight compounds (e.g., oxalic acid, malic acid, and citric acid),[51] which can increase the bioavailability of soil nutrients.[18] Martina *et al*. found that *Pseudomonas* promoted the growth of Se hyper-accumulators (*Brassicaceae*, *Fabaceae*).[52] *Bacillus* can produce antimicrobial metabolites to inhibit

phytopathogens, along with promoting plant growth and Se bio-fortification in plants.[53] Durán *et al*.[54] demonstrated that *Bacillus* enhanced Se content in wheat grain after co-inoculation of selenobacteria and arbuscular mycorrhizal fungi. In this study, after exposure to Se ENMs (0.5 mg·kg^{-1}), the dissolved organic carbon (DOC) in rhizosphere increased by 29.2% (Figure 3a) and the rhizosphere soil pH decreased from 7.8 to 7.3 (Figure S10). Figure 3b and S11 demonstrated the contents of low molecular weight compounds (betaine, proline, glycine, norleucine, urocanic acid and indole-3-acrylic acid) increased significantly in the rhizosphere of *B. chinensis* (details for low molecular weight compounds analysis are described in Supplementary Text S6, Table S4 and Figure S12), which could improve the availabilities of nutrients via promoting their solubility in soil.[17,18,39,55] In addition, low-molecular weight organic acids could reduce homo-aggregation and/or hetero-aggregation of ENMs in the rhizosphere,[56,57] resulting in increasing their bioavailability. Our results confirmed that the uptake efficiency (taking Se amount by per unit length of root) of Se was elevated by 264.9% at 0.5 mg·kg^{-1} Se ENMs compared to control (Figure 3c). Previous studies have shown that Se could be taken up and translocated by high-affinity sulfate transporter due to the similarity in chemical properties of Se and sulfate.[4] In our study, the gene expression of high-affinity sulfur transporter (*BnSULTR1,1*) was up-regulated by 149.0% (Figure 3d), accompanied by 338.0% increase in the Se content in *B. chinensis* (Figure 3e). Therefore, Se ENMs could increase the abundance of beneficial rhizosphere microorganism, and the total organic carbon along with improving Se-uptake efficiency, contributing to the enrichement of Se in *B. chinensis* (Figure 3f).

Se, an essential element, has a great importance in anti-oxidation, detoxification and prevention of diabetes for humans.[2,3] The appropriate content of Se in edible food is beneficial for human health. The content of Se significantly increased to 32.8 μg/100 g in *B. chinensis* upon Se ENMs (0.5 mg·kg^{-1}) exposure, while this value was only 7.5 μg/100 g in CK (Figure 3e). According to China's Dietary Guidelines, a daily consumption of vegetables should reach 300–500 g which accounts for 24.6–27.2% of the total food intake. Therefore, dietary of this Se-enriched *B. chinensis* could meet the the recommended daily intake of Se (55–400 μg/day) for human. Moreover, the Se ENMs may be methylated and integrated into cysteine and methionine in plants,[58,59] both which were beneficial for human health. However, it should be noted that high Se intake could cause selenosis, and resulted in some diseases, such as brittle hair and abnormal hair loss, broken nail walls, and motor and sensory abnormalities.[60]

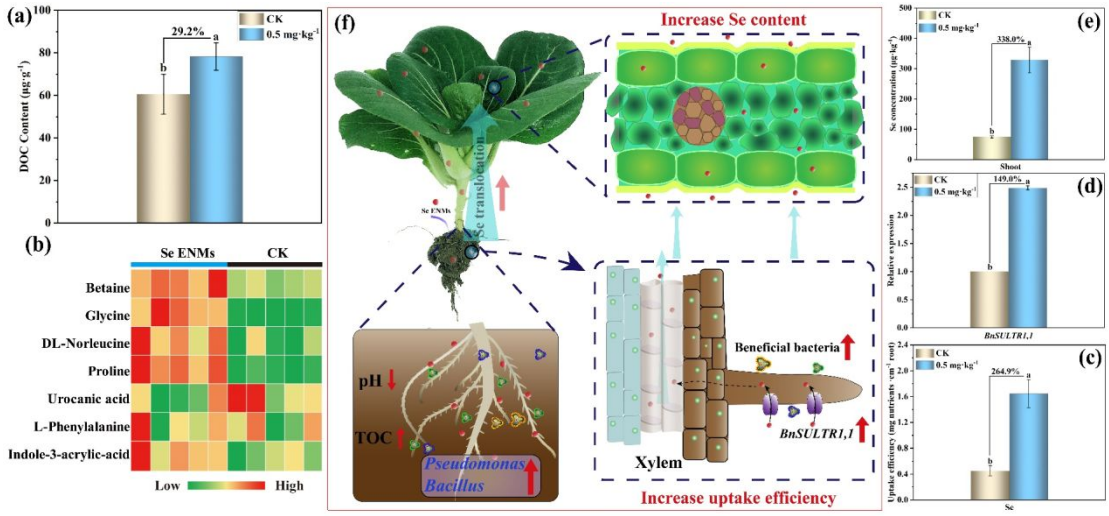

Figure 3. The mechanism of uptake and enrichment of Se ENMs. a, the DOC content in rhizosphere; b, low molecular weight compounds increased significantly in rhizosphere soil after exposure to Se ENMs (0.5 mg·kg^{-1}) (n=5, p<0.05); c-e, the uptake efficiency (c), transporter activity (d) and Se enrichment (e) in *B. chinensis*; f, schematic diagram of uptake and enrichment of Se ENMs in *B. chinensis*. (Figure 3a, c-e, n=3, p<0.05)

3.4 Promoted photosynthesis and transport of carbohydrates by Se ENMs

As stated previously, Se ENMs in soil changed the rhizosphere microbiomes and increased DOC in rhizosphere soil, indicating that the nutrient availability could be improved. It is reported that nutrients such as Mg, P, and Fe have a key role in enhancing photosystem.[19] In this study, these photosynthesis beneficial elements of Mg, P, and Fe in *B. chinensis* leaves were increased by 24.2%, 22.3%, and 2.5% under 0.5 mg·kg^{-1} Se ENM exposure compared to CK, respectively (Figure 4a). Meanwhile, their higher utilization efficiencies in root and shoot were observed (Figure S13), thus, these nutrients showed the major role in plant growth. Mg and Fe are the component of chlorophyll and are involved in photosynthesis.[61, 62] P, the component of chloroplast bilayer membrane, granule and adenosine triphosphate (ATP), plays a significant role in energy conversion.[63] After exposure to Se ENMs (0.5 mg·kg^{-1}) (Figure 4a), chlorophyll a and b, part of light harvesting complex,[6,64] were increased by 16.7% and 18.9%, which may be induced by the increased content of Mg and P in *B. chinensis* leaves. Thus, Se ENMs (0.5 mg·kg^{-1}) had a huge potential in improving light harvesting of *B. chinensis*. As shown in Figure 4a, the electron transfer rate (ETR) was increased by 27.4% in the photosystem. Consequently, the photosynthesis would be enhanced, since the increase of Mg, P, and Fe by rhizosphere microbiomes possibly elevated the content of chlorophyll. Studies showed that ENMs can increase yield through improving photosynthesis.[19] For example, CeO_2 ENMs (100 mg·kg^{-1}) stimulated plant growth by enhancing the photosynthesis rate (54%);[65] nanochitin (6 mg·L^{-1}) ENMs can raise the grain yield by 25% through enhanceing the photosynthesis (13.8%) (winter wheat), respectively.[66] Herein, the net photosynthesis rate (Pn) of *B. chinensis* upon soil

application of 0.5 mg·kg^{-1} Se ENMs was promoted significantly by 16.7% (Figure 4a), which could contribute to the increase in yield (Figure 1d).

Carbohydrate is the product of photosynthesis.[67] As shown in Figure 4b, the carbohydrate content was increased by 6.5% upon 0.5 mg·kg^{-1} Se ENMs). Sucrose is produced from glucose which serves as the principal product of photosynthesis, and could be transported from source leaves to sink organs.[68] SUTs/SUCs (sucrose transporters or sucrose carriers) and SWEETs (Sugars Will Eventually be Exported Transporters) play significant central roles in sucrose translocation which are closely associated with crop yields.[68] Herein, three key genes (*BnSUC1,1*, *BnSUC1,4* and *BnSWEET10,2*) were selected to explore the expression of sucrose translocation genes.[68] Their expressions were up-regulated by 52.2, 53.2 and 76.3-fold in *B. chinensis* leaves, respectively (Figure S14), suggesting the activities of enzymes (SUC and SWEET) increased by exposing Se ENMs. This suggested increasing carbohydrates produced more 3C compounds which could be transformed to sucrose and transported in phloem. Therefore, there is the possibility that more sucrose was translocated to roots for supplying their growth. As shown in Figure S15, the root became larger, and the FW and DW were increased by 43.3% and 23.5% under 0.5 mg·kg^{-1} Se ENMs exposure. Meanwhile, the root length, surface area and tip number were distinctly augmented by 65.0%, 73.1% and 17.7%, respectively (Figure 4c), indicating that the uptake of water as well as nutrients by roots might be stimulated by Se ENMs. Taken together, soil application of Se ENMs can improve the rhizosphere microbiome to increase uptake of Mg, P and Fe, followed by increase in chlorophyll a and b, and ETR, ultimately

promoting the photosynthesis (Figures 4a). The translocation of carbohydrates from shoot to root was also accelerated (Figure 4b), leading to the promoted root growth (Figure 4c). The promoted roots could take up more nutrients such as Mg, P, Fe, and Se, which could be translocated to leaves, and further improving photosynthesis. In addition, the transport of carbohydrates from shoot to root could improve rhizosphere microbiomes. Therefore, Se ENMs promoted the yield and Se content of *B. chinensis* through increasing the abundance of rhizosphere beneficial microorganisms, promoting the nutrient uptake efficiency, and enhancing photosynthesis. Moreover, Se ENMs increased microelements and beneficial components along with decreasing a heavy metal content in *B. chinensis*. This research provides a promising way for producing Se-enriched vegetables.

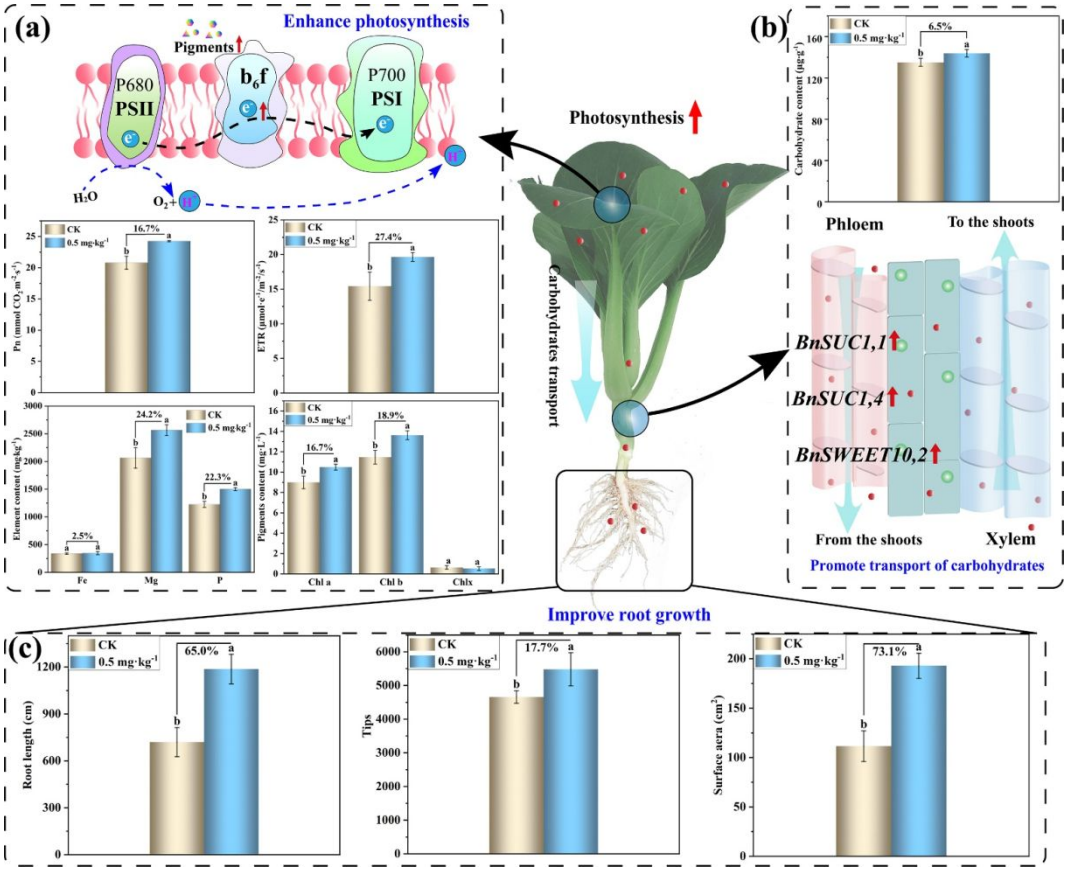

Figure 4. Effects of Se ENMs on *B. chinensis* growth, photosynthesis and transport of

carbohydrates. a, the mechanisms of enhancing photosynthesis (Increasing beneficial elements and chlorophyll content); b, improving the transport of carbohydrates in the leaves, and c, improving root growth (root length, tips number and surface area). (n=3, $p<0.05$)

4. CONCLUSIONS

Herein, the novel Se ENMs-enabled agricultural nanotechnology was developed to produce Se-enriched vegetables. Se ENMs (0.5 mg·kg^{-1}) in soil can increase net photosynthesis (16.7%), carbohydrates content (6.5%), yields (19.8%), and Se content up to 32.8 μg/100g. Moreover, the mechanism of uptake, enrichment of Se in *B. chinensis* with soil application of Se ENMs was investigated detail. We found Se ENMs could boost photosynthesis efficiency, trigger the transport of carbohydrates from shoot to root, and increase the beneficial rhizosphere microbiomes. The rhizosphere microbiomes (*Pseudomonas and Bacillus*) together with low molecular weight compounds (betaine, proline, glycine, norleucine, urocanic acid and indole-3-acrylic acid) enhanced the uptake efficiency of Se by 264.9%. Produced vegetables can meet the the recommended daily intake of Se (55–400 μg/day) for human. Therefore, this study could provide an alternative ENMs-enabled nanotechnology for producing Se-fortified crops, which is beneficial for human health and shows promising application in crop production.

CONFLICTS OF INTEREST

The authors declare no competing financial interest.

ACKNOWLEDGEMENT

This research was supported by the National Natural Science Foundation of China (41820104009, 42077296, 41807378 and 41907304), Agricultural Science and Technology Innovation Project of Jiangsu Province (CX(20)3080), UMass Conti

Faculty Fellowship, and USDA Hatch program (MAS 00549).

REFERENCES

(1) M. P. Rayman, Selenium and human health, *The Lancet.*, 2012, **379**, 1256–1268.

(2) G. V. Kryukov, S. Castellano, S. V. Novoselov, A. V. Lobanov, O. Zehtab, R. Guigó and V. N. Gladyshev, Characterization of mammalian selenoproteomes, *Science.*, 2003, **300**, 1439–1443.

(3) D. L. Hatfield, P. A. Tsuji, B. A. Carlson and V. N. Gladyshev, Selenium and selenocysteine: roles in cancer, health, and development, *Trends Biochem. Sci.*, 2014, **39**, 112–120.

(4) Y. Zhu, E. A. H. Pilon-Smits, F. Zhao, P. N. Williams and A. A. Meharg, Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation, *Trends Plant Sci.*, 2009, **14**, 436–442.

(5) E. Marguí, G. H. Floor, M. Hidalgo, P. Kregsamer, G. Román-Ross, C. Streli and I. Queralt, Analytical possibilities of total reflection X-ray spectrometry (TXRF) for trace selenium determination in soils, *Anal. Chem.*, 2010, **82**, 7744-7751.

(6) M. Malagoli, M. Schiavon, S. Dall'Acqua and E. A. H. Pilon-Smits, Effects of selenium biofortification on crop nutritional quality, *Front. Plant Sci.*, 2015, **6**, 280.

(7) K. H. Winther, M. P. Rayman, S. J. Bonnema and L. Hegedüs, Selenium in thyroid disorders-essential knowledge for clinicians, *Nat Rev Endocrinol.*, 2020, **16**, 165–176.

(8) F. Nawaz, R. Ahmad, M. Y. Ashraf, E. A. Waraich and S. Z. Khan, Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress, *Ecotoxicol. Environ. Saf.*, 2015, **113**, 191–200.

(9) J. Dong, Y. Wang, S. Wang, L. Yin, G. Xu, C. Lei and M. Zhang, Selenium increases chlorogenic acid, chlorophyll and carotenoids of Lyciumchinense leaves, *J. Sci. Food Agric.*, 2013,

93, 310–315.

(10) G. Lyons, Selenium in cereals: improving the efficiency of agronomic biofortification in the UK, *Plant Soil.*, 2010, **332**, 1–4.

(11) E. Domokos-Szabolcsy, L. Marton, A. Sztrik, B. Babka, J. Prokisch and M. Fari, Accumulation of red elemental selenium nanoparticles and their biological effects in Nicotiniatabacum, *Plant Growth Regul.*, 2012, **68**, 525–531.

(12) E. Domokos-Szabolcsy, N. Alladalla, T. Alshaal, A. Sztrik, L. Márton and H. El-Ramady, In vitro comparative study of two *Arundo donax* L. ecotypes' selenium tolerance, *Int. J. Hort. Sci.*, 2014, **20**, 119–122.

(13) H. El-Ramady, N. Abdalla, H. S. Taha, T. Alshaal, A. El-Henawy, E.-D. A. F. Salah, M. S. Shams, S. M. Youssef, T. Shalaby, Y. Bayoumi, N. Elhawat, S. Shehata, A. Sztrik, J. Prokisch, M. Fári, E. Domokos-Szabolcsy, E. A. Pilon-Smits, D. Selmar, S. Haneklaus and E. Schnug, Selenium and nano-selenium in plant nutrition, *Environ. Chem. Lett.*, 2016, **14**, 123–147.

(14) M. Haghighi, R. Abolghasemi and D. S. J. A. Teixeira, Low and high temperature stress affect the growth characteristics of tomato in hydroponic culture with Se and nano-Se amendment, *Sci. Hortic.*, 2014, **178**, 231–240.

(15) S. M. Zahedi, M. Abdelrahman, M. S. Hosseini, N. F. Hoveizeh and L. F. Tran, Alleviation of the effect of salinity on growth and yield of strawberry by foliar spray of selenium-nanoparticles, *Environ. Pollut.*, 2019, **253**, 246–258.

(16) R. L. Berendsen, C. M. J. Pieterse and P. A. H. M. Bakker, The rhizosphere microbiome and plant health, *Trends Plant Sci.*, 2012, **17**, 478–486.

(17) H. P. Bais, T. L. Weir, L. G. Perry, S. Gilroy and J. M. Vivanco, The role of root exudates in

rhizosphere interactions with plants and other organisms, *Annu. Rev. Plant Biol.*, 2006, **57**, 233–266.

(18) Y. Liu, X. Li and Y. Kou, Ectomycorrhizal Fungi: Participation in Nutrient Turnover and Community Assembly Pattern in Forest Ecosystems, *Forests.*, 2020, **11**, 453.

(19) Y. L. Liu, L. Yue, Z. Y. Wang and B. S. Xing, Processes and mechanisms of photosynthesis augmented by engineered nanomaterials, *Environ. Chem.*, 2019, **16**, 430–445.

(20) D. V. Badri and J. M. Vivanco, Regulation and function of root exudates, *Plant Cell Environ.*, 2009, **32**, 666–681.

(21) H. Zhang, M. Huang, W. Zhang, J. L. Gardea-Torresdey, J. C. White, R. Ji and L. J. Zhao, Silver nanoparticles alter soil microbial community compositions and metabolite profiles in unplanted and cucumber-planted soils, *Environ. Sci. Technol.*, 2020, **54**, 3334−3342.

(22) W. Zhang, X. Jia, S. Chen, J. Wang, R. Ji and L. J. Zhao, Response of soil microbial communities to engineered nanomaterials in presence of maize (*Zea mays* L.) plants, *Environ. Pollut.*, 2020, **267**, 115608.

(23) H. An, X. Qi, M. L. Gaynor, Y. Hao, S. C. Gebken, M. E. Mabry, A. C. McAlvay, G. R. Teakle, G. C. Conant, M. S. Barker, T. Fu, B. Yi and J. C. Pires, Transcriptome and organellar sequencing highlights the complex origin and diversification of allotetraploid *Brassica napus*, *Nat Commun.*, 2019, **10**, 2878.

(24) G. Sharma, A. R. Sharma, R. Bhavesh, J. Park, B. Ganbold, J. Nam and S. Lee, Biomolecule-mediated synthesis of selenium nanoparticles using dried *Vitis vinifera* (Raisin) extract, *Molecules.*, 2014, **19**, 2761–2770.

(25) H. Zhang, X. Si, X. Ji, R. Fan, J. Liu, K. Chen, D. Wang and C. Gao, Genome editing of

upstream open reading frames enables translational control in plants, *Nat Biotechnol*., 2018, **36**, 894-898.

(26) Y. K. Rui, Q. Q. Yu, Y. H. Jin, J. Guo and Y. B. Luo, Application of ICP-MS to the detection of forty elements in wine, *Spectrosc. Spect. Anal*., 2007, **27**, 1015-1017.

(27) Y. K. Rui, J. B. Shen and F. S. Zhang, Application of ICP-MS to determination of heavy metal content of heavy metals in two kinds of N fertilizer, *Spectrosc. Spect. Anal*., 2008, **28**, 2425-2427.

(28) Z. Wang, X. Xie, J. Zhao, X. Liu, W. Feng, J. C. White and B. Xing, Xylem- and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.), *Environ. Sci. Technol.*, 2012, **46**, 4434–4441.

(29) X. Ma, J. Geiser-Lee, Y. Deng and A. Kolmakov, Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation, *Sci. Total Environ.*, 2010, **408**, 3053–3061.

(30) J. Li, R. V. Tappero, A. S. Acerbo, H. Yan, Y. Chu, G. V. Lowry and J. M. Unrine, Effect of CeO_2 nanomaterial surface functional groups on tissue and subcellular distribution of Ce in tomato (*Solanum lycopersicum*), *Environ. Sci. Nano.*, 2019, **6**, 273–285.

(31) A. Avellan, J. Yun, Y. Zhang, E. Spielman-Sun, J. M. Unrine, J. Thieme, J. Li, E. Lombi, G. Bland and G. V. Lowry, Nanoparticle size and coating chemistry control foliar uptake pathways, translocation, and leaf-to-rhizosphere transport in wheat, *ACS Nano.*, 2019, **13**, 5291–5305.

(32) Y. Su, V. Ashworth, C. Kim, A. S. Adeleye, P. Rolshausen, C. Roper, W. Jason, and D. Jassby, Delivery, uptake, fate, and transport of engineered nanoparticles in plants: a critical review and data analysis, *Environ. Sci.: Nano*, 2019, **6**, 2311-2331.

(33) J. A. Hernandez-Viezcas, H. Castillo-Michel, J. C. Andrews, M. Cotte, C. Rico, J. R. Peralta-

Videa, Y. Ge, J. H. Priester, P. A. Holden, and J. L. Gardea-Torresdey, In Situ Synchrotron X-ray Fluorescence Mapping and Speciation of CeO_2 and ZnO Nanoparticles in Soil Cultivated Soybean (*Glycine max*), *ACS Nano* 2013, **7**, 1415-1423.

(34) P. Miralles, T. L. Church, and A. T. Harris, Toxicity, Uptake, and Translocation of Engineered Nanomaterials in Vascular plants, *Environ. Sci. Technol.*, 2012, **46**, 9224-9239.

(35) T. G. Sors, D. R. Ellis, and D. E. Salt, Selenium uptake, translocation, assimilation and metabolic fate in plants, *Photosynthesis research*, 2005, **86**, 373-389.

(36) H. El-Ramady, N. Abdalla, T. Alshaal, A. El-Henawy, E. D. F. Salah, M. S. Shams, T. Shalaby, Y. Bayoumi, N. Elhawat, Said. Shehata, A. Sztrik, J. Prokisch, M. Fári, E. A. Pilon-Smits, and É. Domokos-Szabolcsy, Selenium and its role in higher plants. Pollutants in Buildings, *Water and Living Organisms*, 2015, 235-296

(37) M. Gupta, and S. Gupta, An overview of selenium uptake, metabolism, and toxicity in plants, *Front. Plant Sci.*, 2017, **7**, 2074

(38) A. M. Debruyn, and P. M. Chapman, Selenium toxicity to invertebrates: will proposed thresholds for toxicity to fish and birds also protect their prey?. *Environ Sci Technol.*, 2007, **41**, 1766-1770.

(39) L. E. Barton, A.N. Quicksall and P. A. Maurice, Siderophore-mediated dissolution of hematite (α-Fe_2O_3): effects of nanoparticle size, *Geomicrobiology Journal.*, 2012, **29**, 314–322.

(40) B. R. Ginn and J. B. Fein, The effect of species diversity on metal adsorption onto bacteria, *Geochim. Cosmochim. Acta.*, 2008, **72**, 3939–3948.

(41) S. He, Y. Feng, J. Ni, Y. Sun, L. Xue, Y. Feng, Y. Yu, X. Lin and L. Yang, Different responses of soil microbial metabolic activity to silver and iron oxide nanoparticles, *Chemosphere.*, 2016, **147**,

195-202.

(42) M. Simonin, A. A. Cantarel, A. Crouzet, J. Gervaix, J. M. Martins and A. Richaume, Negative effects of copper oxide nanoparticles on carbon and nitrogen cycle micro-bial activities in contrasting agricultural soils and in presence of plants, *Frontiers in Microbiology.*, 2018, **9**, 3102.

(43) M. Bruto, C. Prigent-Combaret, D. Muller and Y. Moënne-Loccoz, Analysis of genes contributing to plant-beneficial functions in plant growth-promoting rhizobacteria and related Proteobacteria, *Sci. Rep.*, 2014, **4**, 6261.

(44) D. J. Saul, J. M. Aislabie, C. E. Brown, L. Harris and J. M. Foght, Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica, *FEMS Microbiol. Ecol.*, 2005, **53**, 141–155.

(45) J.-J. Kim, M. Alkawally, A. L. Brady, W. I. C. Rijpstra, J. S. S. Damsté and P. F. Dunfield, Chryseolinea serpens gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from soil, *Int. J. Syst. Evol. Microbiol.*, 2013, **63**, 654–660.

(46) J. Crovadore, B. Cochard, G. Calmin, R. Chablais, T. Schulz and F. Lefort, Whole-genome sequence of Pseudomonas xanthomarina strain UASWS0955, a potential biological agent for agricultural and environmental uses, *Genome announcements.*, 2016, **4**, e01136-16.

(47) M. A. Cipriano, M. Lupatini, L. Lopes-Santos, M. J. Da Silva, L. F. Roesch, S. A. Destéfano, S. S. Freitas and E. E. Kuramae, Lettuce and rhizosphere microbiome responses to growth promoting Pseudomonas species under field conditions, *FEMS. Microbiolo Ecol.*, 2016, **92**, fiw 197.

(48) R. Daniel and S. Gustavo, Evaluation of Bacillus and Pseudomonas to colonize the rhizosphere and their effect on growth promotion in tomato (Physalis ixocarpa Brot. ex Horm.), *Rev. Chapingo.*

Ser. Horticultura., 2016, **22**, 45–58.

(49) S. P. Chowdhury, K. Dietel, M. Rändler, M. Schmid, H. Junge, R. Borriss, A. Hartmann and R. Grosch, Effects of Bacillus amyloliquefaciens FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community, *PloS One.*, 2013, **8**, e68818.

(50) X. Yu, C. Ai, L. Xin and G. Zhou, The siderophore-producing bacterium, Bacillus subtilis CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper, *Eur. J. Soil Biol.*, 2011, **47**, 138–145.

(51) N. Zhang, D. Wang, Y. Liu, S. Li, Q. Shen and R. Zhang, Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation and colonization by beneficial rhizosphere-associated bacterial strains, *Plant Soil.*, 2014, **374**, 689–700.

(52) M. Sura-de Jong, R. J. B. Reynolds, K. Richterova, L. Musilova, L. C. Staicu, I. Chocholata, J. J. Cappa, S. Taghavi, D. Van der Lelie, T. Frantik, I. Dolinova, M. Strejcek, A. T. Cochran, P. Lovecka and E. A. Pilon-Smits, Selenium hyperaccumulators harbor a diverse endophytic bacterial community characterized by high selenium resistance and plant growth promoting properties, *Front. Plant Sci.*, 2015, **6**, 113.

(53) Ö. Baysal, D. Lai, H. Xu, M. Siragusa, M. Çalışkan, F. Carimi, J. A. T. Da Silva and M. A. Tör, Proteomic Approach Provides New Insights into the Control of Soil-Borne Plant Pathogens by Bacillus Species, *PloS One.*, 2013, **8**, e53182.

(54) P. Durán, J. J. Acuña, M. A. Jorquera, R. Azcón, F. Borie, P. Cornejo and M. L. Mora, Enhanced selenium content in wheat grain by co-inoculation of selenobacteria and arbuscular mycorrhizal fungi: A preliminary study as a potential Se biofortification strategy, *J. Cereal Sci.*,

2013, **57**, 275–280.

(55) J. Xiong, Jun. Li, H. Wang, C. Zhang and M. S. Naeem, Fullerol improves seed germination, biomass accumulation, photosynthesis and antioxidant system in *Brassica napus* L. under water stress, *Plant Physiol. Biochem.*, 2018, **129**, 130–140.

(56) C. Layet, M. Auffan, C. Santaella, C. Chevassus-Rosset, M. Montes, P. Ortet, M. Barakat, B. Collin, S. Legros, M. N. Bravin, B. Angeletti, I. Kieffer, O. Proux, J. L. Hazemann and E. Doelsch, Evidence that soil properties and organic coating drive the phytoavailability of cerium oxide nanoparticles. *Environ. Sci. Technol*., 2017, **51**, 9756-9764.

(57) H. Shang, H. Guo, C. Ma, C. Li, B. Chefetz, T. Polubesova and B. Xing, Maize (*Zea mays* L.) root exudates modify the surface chemistry of CuO nanoparticles: Altered aggregation, dissolution and toxicity, *Sci. Total Environ*., 2019. **690**, 502-510

(58) Y. Li, N. Zhu, X. Liang, L. Zheng, C. Zhang, Y. F. Li, Z. Zhang, Y. Gao, and J. Zhao, A comparative study on the accumulation, translocation and transformation of selenite, selenate, and SeNPs in a hydroponic-plant system, *Ecotox Environ Safe.*, 2020, **189**, 109955

(59) G. Moreno-Martín, J. Sanz-Landaluze, M. E. León-González, and Y. Madrid, Insights into the accumulation and transformation of Ch-SeNPs by *Raphanus sativus* and *Brassica juncea*: Effect on essential elements uptake, *Sci Total Environ.*, 2020, **725**, 138453.

(60) M. Lemireb, A. Philibert, M. Fillion, C. J. S. Passos, J. R. D. Guimarães, F. B. Jr, D. Mergler, No evidence of selenosis from a selenium-rich diet in the Brazilian Amazon, *Environ. Intel.,* **2012**, *40,* 128–136

(61) L. Zhao, L. Lu, A. Wang, H. Zhang, M. Huang, H. Wu, B. Xing, Z. Wang and R. Ji, Nano-biotechnology in agriculture: use of nanomaterials to promote plant growth and stress tolerance, *J.*

Agric. Food Chem., 2020, **68**, 1935–1947.

(62) M. H. Ghafariyan, M. J. Malakouti, M. R. Dadpour, P. Stroeve and M. Mahmoudi, Effects of magnetite nanoparticles on soybean chlorophyll, *Environ. Sci. Technol.*, 2013, **47**, 10645–10652.

(63) M. Li, L. Li, X. Shi, L. Lin and S. Lin, Effects of phosphorus deficiency and adenosine 5'-triphosphate (ATP) on growth and cell cycle of the dinoflagellate Prorocentrum donghaiense, *Harmful Algae.*, 2015, **47**, 35–41.

(64) M. Delfani, M. B. Firouzabadi, N. Farrokhi and H. Makarian, Some physiological responses of black-eyed pea to iron and magnesium nanofertilizers, *Commun. Soil Sci. Plan.*, 2014, **45**, 530–540.

(65) Z. Ca, C. Stowers, L. Rossi, W. Zhang, L. Lombardini, and X. Ma, Physiological effects of cerium oxide nanoparticles on the photosynthesis and water use efficiency of soybean (*Glycine max (L.) Merr.*). *Environ. Sci: Nano.*, 2017, **4**, 1086-1094.

(66) W. Xue, Y. Han, J. Tan, Y. Wang, G. Wang and H. Wang, Effects of nanochitin on the enhancement of the grain yield and quality of winter wheat, *J. Agric. Food Chem.*, 2018, **66**, 6637–6645.

(67) 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.

(68) H. Jian, K. Lu, B. Yang, T. Wang, L. Zhang, A. Zhang, J. Wang, L. Liu, C. Qu and J. Li, Genome-wide analysis and expression profiling of the SUC and SWEET gene families of sucrose transporters in oilseed rape (*Brassica napus* L.), *Front. Plant Sci.*, 2016, **7**, 1464.