The Impact of Selenium on the Rhizosphere Microbiome of a Hyperaccumulation Plant *Cardamine Violifolia*

Zisheng Guo
Northwest University
Bin Zhu
Virginia Commonwealth University
Jia Guo
Changzhou University
Gongting Wang
Northwest University
Meng Li
Beijing Technology and Business University
Qiaoli Yang
Northwest University
Liping Wang
Northwest University
Yue Fei
Northwest University
Shiwei Wang
Northwest University
Tian Yu
Enshi Se-Run Health Tech Development Co., Ltd.
Yanmei Sun (sunyanmei2001@126.com)
Northwest University https://orcid.org/0000-0003-2509-152X

Research Article

Keywords: Cardamine violifolia, exogenous selenium uptake and transformation, rhizosphere microbial response

Posted Date: November 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1043391/v1

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on February 4th, 2022. See the published version at https://doi.org/10.1007/s11356-022-18974-w.

Abstract

Cardamine violifolia is the only selenium hyperaccumulation plant found in China. It has been developed as a source of medicinal and edible products for selenium supplementation of human. It is essential to increase selenium content of *C. violifolia* for nutrient biofortification and many planting approaches have been developed to achieve this aim. However, the contribution of rhizosphere microbes of *C. violifolia* to the selenium enrichment has not been investigated. In this study, four types of selenium, i.e., selenate, selenite, nanoparticles element selenium from *Bacillus subtilis*, and organic selenium from yeast, was added to the soil for the growth of *C. violifolia*, respectively. Selenate had the highest accumulation in *C. violifolia,* following by selenite, *B. subtilis*-Se, and yeast-Se. Except for yeast-Se, the concentration of selenium in *C. violifolia* is positively correlated with the amount of selenium added in the soil. Furthermore, different exogenous selenium exhibited distinct effects on the rhizosphere microbiome of *C. violifolia.* Both Alpha diversity and Beta diversity analyses displayed that rhizosphere microbiome were more obviously affected by selenium from *B. subtilis* and yeast than that of selenate and selenite. Different microbial species were enriched in the rhizosphere of *C. violifolia* under various exogenous selenium treatments. *B. subtilis*-Se application enhanced the abundance of *Leucobacter*, *Sporosarcina*, *Patulibacter*, and *Denitrobacter*, and Yeast-Se application enriched the abundance of *Singulishaera*, *Lactobacillus*, *Bdellovirio*, and *Bosea. Bosea* and the taxon belonging to the order of *Solirubrobacterales* was enriched in the samples with selenite and selenite addition, respectively, and their abundance was linearly related to the concentration of selenate and selenite application in the rhizosphere of *C. violifolia*. In summary, this study revealed the response of the rhizosphere microbiome of *C. violifolia* to exogenous selenium and it is of help to develop suitable selenium fertilizers to increase selenium hyperaccumulation level of this plant.

Highlights

C. violifolia accumulated more selenium with the application of selenate than selenite, *B. subtilis*-Se, and yeast-Se.

B. subtilis-Se and yeast-Se obviously affected rhizosphere microbiome of *C. violifolia.*

Distinct genera were recruited under different exogenous selenium treatments.

The abundance of the enrichment genera positively correlated with selenium concentration.

Introduction

Selenium (Se) is an essential micronutrient with strong bioactivity for all kinds of organisms, including humans, animals, plants, and microorganisms (White and Brown 2010). However, the safety margin of Se element is relatively narrow. For adult humans the recommended dietary reference of Se intake is 55–70 μg Se/day, although the Institute of Medicine (USA) also suggested a tolerable upper intake of 400 μg Se/day (EFSA Panel on Dietetic Products 2014). Excessive Se intake can cause hair and nail loss and nervous system disorders (selenosis) in humans, a symptom similar to those caused by heavy metals (Li

et al. 2012; Rayman et al. 2018; White 2016). Conversely, Se deficiency can weaken the immune system and increase the risks of health disorders, including oxidative stress-related conditions, heart disease, reduced fertility, hypothyroidism, cardiovascular disease, or various cancers (Fairweather-Tait et al. 2011; Rayman et al. 2008; Schomburg 2017; White 2016). It has been reported that Se deficiency affects hundreds of millions of people worldwide, particularly in developing countries (Joy et al. 2014; Rita and Morse 2015). For example, approximately 2/3 Chinese dietary Se intake is about 40 μg/day (Wu et al. 2015), which is lower than the recommended Se dietary intake value of 55 μg/day according to the WHO (WHO 2009). Thus, it is essential to develop natural plant-based Se biofortification products for severe Se deficiency region worldwide (Wu et al. 2015).

For plants, Se is not considered as an essential element. In general, concentrations of Se in plants grown in seleniferous soil are less than 25 mg/kg dry weight, but some Se-hyperaccumulator species can accumulate over 1,000 mg/kg Se in plant tissues (White 2016). Many Se-hyperaccumulating plants have been discovered from the order Brassicales. For example, two famous Se hyperaccumulators desert prince's plume (*Stanleya pinnata*) and two-grooved milkvetch (*Astragalus bisulcatus*) were found to contain Se up to 0.1–1.5% and 0.6% (dry weight), respectively (Freeman et al. 2006). In China, *C. violifolia* is the only Se-hyperaccumulating plant found in 2013 in Enshi city, where the Se mine drainage areas contains high levels of Se (Yuan et al. 2013). It was found that *C. violifolia* living near Se mine tailing could accumulate Se up to 20,000, 18,000 and 44,000 mg/kg dry weight in leaves, stems, and roots, respectively, which put it as a Se hyper accumulator. *C. violifolia* has been developed as various natural products for Se biofortification (Wu et al. 2020).

Although many high Se-tolerance plants have been discovered, the studies about the mechanisms responsible for high Se-tolerance in plants are less. In *Stanleya pinnata*, the high Se-tolerance were found to be related to the levels of ascorbic acid, glutathione, total sulfur, and nonprotein thiols, and may partly be due to the increased antioxidants and up-regulated sulfur assimilation (Freeman et al. 2010). Other studies indicated that toxic Selenocysteine (SeCys) can be methylated to form non-toxic methyl-SeCys, because methyl-SeCys does not enter proteins and helps high Se-tolerance of hyperaccumulators. However, in *C. violifolia* over 85% of the total Se accumulated were in the form of selenocystine ($SeCys_2$) (Yuan et al. 2013), indicating different Se-tolerance mechanisms. Comparative transcriptomics analysis also provided some clues that storage function, oxidation, transamination, and selenation play roles in the high Se-tolerance of *C. violifolia* (Zhou et al. 2018). In addition to plants' physiological metabolism, many researchers have found that plant root-associated microbes help its tolerance to toxic compounds (Morgan et al. 2005; Stolz et al. 2006). However, the root-associated microbes in *C. violifolia* have not been investigated.

Se intake is dependent on its chemical form in the root substrate, which affects its bioavailability, mobility, and nutritional value (Thiry et al. 2012). Se commonly has four valence states in natural environment, including selenide (Se^{2-}), elemental Se (Se^0), selenite (Se^{4+}), and selenate (Se^{6+}). For human health it is generally believed that ingesting organic Se compounds (Se^{2-}) is safer than inorganic forms (Se^{4+} and Se^{6+}) (Lunøe et al. 2011), and some other studies have found that nanoparticles element Se (Se^0) had the

lest toxicity among the four types of Se (Shakibaie et al. 2013; Wadhwani et al. 2016). However, the optimum Se for uptake and transformation are different among plants, although inorganic Se compounds, such as selenate and selenite, have been commonly used for growing selenized vegetables because they are more easily available than organic Se compounds for biofortification (Michela et al. 2017; Schiavon and Pilon-Smits 2017). For example, two-grooved milkvetch preferred to uptake selenite and $SeCys_2$ in nutrient-solution, but not SeMet (Williams and Mayland 1992). Indian mustard shoots accumulated the greatest amount of Se by the application of selenate, followed by those supplied with SeMet and selenite (Zayed et al. 1998). When spring canola (*Brassica napus*) were grown hydroponically, organic forms of Se were taken up at a greater rate than inorganic forms (Kikkert and Edward 2013). For nanoparticles element Se from microbes, no application in plants has been investigated as it is known.

Which Se compound is the best for uptake and transformation of *C. violifolia* remains still unclear. In addition, to understand Se hyperaccumulation mechanisms in *C. violifolia*, it is significant to investigate the rhizosphere physical, chemical, and biological processes, which may affect soil Se bioavailability, plant uptake, distribution, and transformation of Se in the plant. Thus, in the study we examined the effect of the different types of exogenous Se (selenate, selenite, nanoparticles element Se from *Bacillus subtilis*, and organic selenium from yeast) with varied concentration levels on the growth and Se accumulation in *C. violifolia*, and analyzed the corresponding response of root-associated bacteria. These results would be helpful for understanding the Se accumulation mechanisms and developing Se biofortification or phytoremediation technologies in future.

Materials And Methods

Chemicals and reagents

Biochemical reagents used in this study were analytical grade. Selenite and selenate were bought from Sigma-Aldrich (St. Louis, MO, USA). Yeast Se (Se content 2000 mg/kg, 98% was SeMet) was purchased from Angel Yeast (Yichang, China). Nanoparticle's element Se was prepared by *B. subtilis* fermentation in the LB medium with 5 mM selenite sodium (Se content 1500 mg/kg, 90% was nanoparticles element Se).

C. violifolia growth and treatment

C. violifolia was grown and treated as previously reported (Wu et al. 2020). In brief, after germination and growth for 1 month in peat soil, the seedlings were transplanted and cultivated for 3 months in the trial field in greenhouse with the nutritional substrate mixture of peat soil and vermiculite (3:1). Then, the plants with similar growth were transplanted into the flowerpots with diameter of 32 cm and height of 29 cm for 1-month adaptation. Four types of Se solutions with varied concentration (0, 50, 100, 200, 400 and 800 mg/L) were separately added the substrates. Once every 20 days 750 mL Se solution was added into the flowerpots by root application for three times. The control group was treated by adding equal amount of deionized water. Each treatment had 3 replicates. After 90-day cultivation, the plants were harvested for subsequent analysis.

Soil sampling, DNA extraction, and 16S rRNA gene sequencing

After the root-loosely bound soil of *C. violifolia* was shaken off, the tightly associated soil samples were collected. The samples were divided into two parts: one is air-dried, ground, and sieved thought a 2-mm screen for physiochemical analysis, and the other one was stored at -20 °C for total DNA extraction.

Total genomic DNA was extracted from 0.2 g of soil using PowerSoil DNA Isolation Kit according to the manual protocol (Mobio Laboratories, USA). The extracted DNA concentration was measured using NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). After amplifying the V4–V5 region of bacterial 16S rRNA gene using the primer sets F515 (5'-GTGCCAGCMGCCGCGG-3') and R907 (5'-CCGTCAATCMTTTRAGTTT-3'), the bacterial community composition was investigated by high-throughput sequencing the amplified products using an Illumina HiSeq 2500 instrument from Novogene Biotechnology Company (Beijing, China). The raw reads were filtered and analyzed using QIIME 1.9.1 (Caporaso et al. 2010). The sequences with similarity above 97% were clustered into operational taxonomic units (OTUs) using UCLUST clustering (Edgar 2010). The bacterial taxa dominance was analyzed based on the taxonomy information.

Se quantification

The total Se concentration in plants and soils was measured using hydride generation atomic fluorescence spectrometry (HG-AFS) as previously reported (Wu et al. 2020). Briefly, approximately 1 g of samples were weighed, treated using 8 mL of nitric acid in digestion bottles, and heated in a microwave digestion system. The digestion process is 130°C for 2 min, 150°C for 2 min, and 180°C for 15 min. The solution was evaporated to 2 ml, transferred into a 50-mL volumetric flask, and diluted to 50 mL with ultrapure water. The blank group was prepared using the same method. Then, the digestion solution was extracted, mixed with 2.0 mL of 6 M HCl, and fixed to 10 mL in a 15-mLcentrifuge tube for next Se determination. The content of Se was calculated with a standard curve.

Statistical analysis

The data obtained by the sequencing was analyzed with Qiime2 (Hall and Beiko 2018). Initially, all reads were trimmed to a minimum length of 250 bp and at least a Phred score of 20 using DADA2. The remaining sequences were clustered into OTUs. Alpha diversity was characterized by Shannon index, observed OUT, and evenness. Kruskal-Wallis test was used for the differential analysis of alpha diversity. Beta diversity was quantified by Bray-Curtis distance, followed by differential analysis using Mann-Whitney U test. Additionally, the dissimilarity of the microbialprofiles was visualized by NMDS using Bray–Curtis distance. Taxonomic information for each OTU was assigned via SILVA v.132 taxonomy classifier (Parks et al. 2018). Quality OTU were clustered at 97% identity and an taxa table affiliated at species level was used for analysis in next steps. All the taxa with abundance more than or equal to 0.01% (or 0.001%) in at least 5% (or 15%) samples were kept for the following analysis. Linear regression was performed to test the correlation of Se treatment and the abundance change of each taxon using 'lm' function in R. The *p* and R values of the results of linear regression were adjusted by an FDR of 5% using

the Benjamini-Hochberg method for multiple comparisons. Additionally, an taxa table affiliated at phylum level was prepared and the rhizosphere microbiome profiles of four Se samples were compared to plant control at phylum level using Mann-Whitely U test, followed by the adjustment of *p*-values using the same FDR method.

Results And Discussion

The ability of *C. violifolia* assimilating different types of Se

The experiments were designed to examine the ability of *C. violifolia* utilizing Se and soil microbiome (Table 1). Four types of Se sources with different concentrations (0, 50, 100, 200, 400, and 800 mg/L) were added into the soils, and the Se concentration in both the soils and *C. violifolia* was compared in different treatments. Consistent with our previous work (Wu et al. 2020), among these different selenium sources, selenate was the best for increasing the Se concentration in the leaves of *C. violifolia*, following by selenite, *B. subtilis*-Se, and yeast-Se in the concentration rang tested. The concentration of Se in the leaves of *C. violifolia* was positively related to the supplementation of *B. subtilis*-Se, selenite, and selenate into the soils. However, the addition of yeast-Se in the soil was not linearly related to the Se concentration in the leaves of *C. violifolia*.

To quantified by the ratio of Se concentrations in *C. violifolia* to that in the soils, the Se uptake of plant from soil was investigated in different treatments. When there was no supplementation of exogenous Se, the concentration of Se in the soil was 1.54 ± 0.4 mg/kg. The addition of these four types of Se increased the Se concentration in the soils. However, only the uptake of the selenate group had a higher ratio than the plant control group (*C. violifolia* without Se addition) (Fig. 1). In addition, the absorption ratio was not decreased when the concentration of exogenous selenate was increased. These data suggested that unknown mechanism existed in *C. violifolia* for actively assimilating selenate. The absorption ratios of other three types of Se sources were lower than the plant control. The ratio was reduced in higher concentrations of yeast-se and selenite and fluctuated for *B. subtilis*-Se, indicating that *C. violifolia* could not utilize high concentration of yeast-Se, selenite, *B. subtilis*-Se or as well as selenate. A large part of extra yeast-Se, selenite, and *B. subtilis*-Se could not be absorbed by *C. violifolia* and they might be kept in the soils or volatilize, which might be beneficial for *C. violifolia* growth.

Se is indispensable for humans, but it is not clear if the trace element Se is indispensable for plants growth. Many studies have indeed found that Se showed beneficial effects for various plants. It can promote growth, increase tolerance to heavy metals, confer resistance to pathogens, and protect from oxidative damage (Djanaguiraman et al. 2010; Pandey and Gupta 2015; Schiavon and Pilon-Smits 2016). However, the beneficial effects of Se are usually identified at low level, and high concentration of exogenous Se application inhibited plant growth or lead to Se toxicity occurrence (Hui et al. 2011). This is consistent with our results. In addition, selenate showed the highest accumulation in *C. violifolia* among the different forms of Se. This might be because selenate was more readily assimilated through the transporter in the root cell membrane (Wu et al. 2020). Therefore, the source and concentration of Se should be taken into attention when exogenous Se fertilizers are applied.

The influence of Se sources on the diversity of *C. violifolia* rhizosphere microbiome

Rhizospheremicrobiota has been reported to help plant growth via the acquisition of nutrients or the prevention against harmful substance in soil (Mendes et al. 2013). To investigate which rhizosphere microbes could promote the Se enrichment in *C. violifolia*, the first thing is to characterize the impact of Se to the rhizosphere microbiome of *C. violifolia*. The microbial profiles were compared under different exogenous Se treatments (Table 1). We would like to identify the bacteria species with increased abundance in higher concentration of exogenous Se.

The alpha rarefaction plotting of Shannon/OTU suggested that the sequencing depth was enough to cover most of the species in the rhizosphere microbiome. Alpha diversity was performed using three index, Shannon index, observed OTU, and evenness. The related differences were analyzed using Kruskal–Wallis test. Most of the Se treatments could not affect the Shannon diversity in the rhizosphere microbiome of *C. violifolia* except fromthe two samples 800 mg/mL *B. subtilis*-Se and 200 mg/mL yeast-Se (Fig. 2a). Additionally, the different alpha diversities in these two sets of microbiomes were caused by both the changes of OTU numbers and microbial evenness (Fig. 2b and c). The supplementation of *B. subtilis*-Se reduced the observed OTUs at the levels of 100, 400 and 800 mg/mL and decreased evenness and Shannon index at 800 mg/mL level (Fig. 2b and c), indicating that some rhizosphere microbes could be inhibited or killed by *B. subtilis*-Se. In the treatment of 200 mg/mL yeast-Se, more species were observed and the microcial abundances were more uniformly distributed (Fig. 2b and c). However, higher concentrations of yeast-Se reduced the alpha diversity to the level of the control group. One explanation was that low concentration of yeast-Se (≤ 200 mg/L) might contain nutrient that helped the growth of some rhizosphere microbiota, but high concentration of yeast-Se (≥ 400 mg/L) might inhibit the survival of the rhizosphere microbes. Because both the *B. subtilis* and yeast-Se were not purified Se compounds, the impact of them on the rhizosphere microbiome might be induced by other components in the Se sources. Nevertheless, the influence from *B. subtilis*- and yeast-Se to the root microbiome should be involved in the observed changes.

Beta diversity was quantified using Bray-Curtis distance and the difference between each sample and the plant control was calculated by Mann-Whitney U test (Fig. 3a). Furthermore, ordination analysis was performed via non-metric multidimensional scaling (NMDS) (Fig. 3b). The root microbiome was more dissimilar to the plant control with the supplementation of *B. subtilis*-Se, suggested the impact of *B. subtilis*-Se on the rhizosphere microbiome of *C. violifolia* was in a concentration-dependent manner (Fig. 3). This phenomenon was consistent with the reduced alpha diversity by *B. subtilis*-Se treatment, which might because some microorganisms were inhibited or killed by exogenous *B. subtilis*-Se (Fig. 2). Although selenate was the favorite Se source for *C. violifolia* (Fig. 1), the alpha diversity of the rhizosphere microbiome was not affected by selenate (Fig. 2), and the beta diversity was only affected by 50 mg/mL of selenite (Fig. 3). A reasonable explanation was that most of selenate was assimilated by *C. violifolia*, and as a result, selenate had a low concentration in the soil (Fig. 1b), which might limit its influence on the rhizosphere microbiome. As such, selenate was the closest group to the plant control in NMDS figure (Fig. 3b). The beta diversity of no plant was significantly different from plant control (Fig. 3), suggesting that C.

violifolia affected soil microbiome. A significantly lower evenness of the microbiome in plant control samples than that in no plant samples illustrated that the existence of *C. violifolia* might enrich some microorganisms in the soil (Fig. 2).

Species affected by Se sources in the rhizosphere microbiome of *C. violifolia*

We trained a Qiime2 feature classifier using SILVA database for the identification of species in the rhizospheremicrobiome of *C. violifolia*. Top five phylum in the rhizosphere microbiome of *C. violifolia* were Actinobacteria (42.3%), Proteobacteria (32.4%), Bacteroidetes (7.1%), Firmicutes (3.8%) and Patescibacteria (3.7%) (Fig. 4). The rhizosphere microbiome profiles of four Se were compared to plant control at phylum level using Mann-Whitely U test. The application of *B. subtilis*- and yeast-Se slightly changed bacterial abundance at the phylum level compared to selenate and selenite (Fig.6). *B. subtilis*-Se application enriched the abundance of *Deinococcus-Thermus*, *Dependentiae*, and *Patescibacteria*, and reduced that of *Epsilonbacteraeota*, *Elusimicrobia*, *Chlamydiae*,and *Acidobacteria*. Yeast-Se application enriched the abundance of *Patescibacteria*, *Acinobacteria*, and *Firmicutes*, and reduced that of *Epsilonbacteraeota*, *Spirochaetes*, *Elusimicrobia*, *Chlamydiae*, and *Acidobacteria*. The change profile was similar between selenate and selenite application, and they both increased *Deinococcus-Thermus*, *Halanaerobiaeota*, *Acitnobacteria*, *Patescibacteria*, and *Dependentiae*, and reduced *Epsilonbacteriaeota*, *Spirochaetes*, *Verrucomicrobia*, *Chlamydiae*, and *Acidobacteria*. The only difference was that selenite also increased Firmicutes and reduced *Elusimicrobia* (Fig. 5).

Although the most exact species were not identified, some genera differences were found between the rhizosphere microbiome profiles of four Se application and plant control. *B. subtilis*-Se application changed the most types of microbes, and it enriched the abundance of *Leucobacter*, *Sporosarcina*, *Patulibacter*, and *Denitrobacter* and reduced that of *Mycobacterium*. Yeast-Se application enriched the abundance of *Singulishaera*, *Lactobacillus*, *Bdellovirio*, and *Bosea.* Selenite sample enriched only *Bosea*, andselenate sample was found to enrich only *Soirubrobacterales* at the order level. Furthermore, theabundance increase of *Solirubrobacterales* and *Bosea* was, respectively, positively related to the concentration of selenate and selenite application as quantified by linear regression (Fig. 6b and c).

Leucobacter was reported to be tolerant for high heavy metals, such as chromate, and therefore it was used for heavy metal reduction (Zhu et al. 2008). Another previous study found that *B. subtilis* application increased *Leucobacter* abundance (Ding et al. 2021), indicating that some components or metabolites from *B. subtilis* is benefitable for *Leucobacter* growth. In our result *B. subtilis*-Se application showed the biggest abundance increase for *Leucobacter*, indicating that *Leucobacter* might be tolerant for high concentration of Se. Some species which belong to *Singulishaera* were often identified in acidic environment (Kulichevskaya et al. 2008; Kulichevskaya et al. 2012), a similar condition with yeast's growth environment. Although the Yeast-Se is inactive, it is speculated that some organic compounds from yeast might be benefitable for *Singulishaera* enrichment*. Solirubrobacterales* was ubiquitous in natural environments, including rock, cropland, and lake (Khilyas et al. 2019; Liu et al. 2020), and it was also found to be enriched in in the high-selenium-contaminated reclaimed mine soils (Rosenfeld et al. 2018), which was consistent with our results. Some reports showed that *Bosea* was present in the rhizosphere or root

nodules of plants (Qaisrani et al. 2019; Safronova et al. 2020; Sazanova et al. 2019), and itwas also frequently found to perform denitrification function or play antioxidant protective roles (Hassan et al. 2019; Tian and Wang 2021; Wang et al. 2020; Zhang et al. 2021). These results indicated that *Bosea* might use selenite as electron receptor and play key roles during the selenite reduce in the rhizosphere of *C. violifolia*. Taken together, these results showed that various Se source application shaped the different rhizosphere microbiome profiles. Reversely, these might affect Se uptake and transform of *C. violifolia*, which is worthy of future investigation.

Conclusions

C. violifolia exhibited distinct absorption efficiency to different exogenous selenium and had more accumulation for the inorganic selenium such as selenate and selenium than nanoparticles element selenium from *Bacillus subtilis* and organic selenium from yeast. High concentration of exogenous selenium had significant effects on rhizosphere microbial profiles especially for the *B. subtilis*-Se and yeast-Se application. Differentiating microorganisms were recruited into the rhizosphere of *C. violifolia* under four different exogenous selenium treatments. Furthermore, the abundance of enrichment microbes exhibited positively correlation with the concentration of selenium application in the rhizosphere soil of *C. violifolia*. This study provided new knowledge about the root-associated microbes of *C. violifolia* with various Se source application. It is worthy investigating the role of bacteria enriched by exogenous Se on the accumulation of Se in *C. violifolia* in future.

Declarations

Authorship contribution

Zisheng Guo: methodology, data curation, conceptualization, writing-original draft; Bin Zhu: software, data curation, writing-review & editing; Jia Guo: methodology, formal analysis; Gongting Wang: investigation; Meng Li: resources; Liping Wang: Data curation; Qiaoli Yang: methodology; Yue Fei: validation; Shiwei Wang: conceptualization, funding acquisition; Tian Yu: resources, formal analysis; Yanmei Sun: project administration, funding acquisition; supervision, writing-review & editing.

Acknowledgements

This study was funded by National Natural Science Foundation of China grants (32170114, 31800098 and 31770152). Beijing Municipal Key Laboratory of Plant Resources Research and Development 2021 Open Project (PRRD-2021-YB2), National Key R&D Program of China (2021YFC1808902) and Provincial Natural Science Foundation of Shaanxi Province (2016JQ3034).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Ding H, Zhao X, Ma C, Gao Q, Yin Y, Kong X (2021) Dietary supplementation with *Bacillus subtilis* DSM 32315 alters the intestinal microbiota and metabolites in weaned piglets. J Appl Microbiol 130:217-232.
2. Djanaguiraman M, Prasad PVV, Seppanen M (2010) Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol Bioch 48:999-1007.
3. EFSA Panel on Dietetic Products NaAN (2014) Scientific opinion on dietary reference values for selenium. EFSA J 12:3846.
4. Fairweather-Tait SJ, Yongping B, Broadley MR, Rachel C, Dianne F, Hesketh JE, Rachel H (2011) Selenium in human health and disease. Antioxid Red Sig 14:1337-1383.
5. Freeman JL, Hong ZL, Marcus MA, Sirine F, Mcgrath SP, Pilon-Smits EAH (2006) Spatial imaging, speciation, and quantification of selenium in the hyperaccumulator plants astragalus bisulcatus and stanleya pinnata. Plant Physiol 142:124-134.
6. Freeman JL, Masanori T, Cecil S, Quinn CF, Cappa JJ, Jean D, Fakra SC, Marcus MA, Mcgrath SP, Doug VH (2010) Molecular mechanisms of selenium tolerance and hyperaccumulation in stanleya pinnata. Plant Physiol 153:1630-1652.
7. Hall M, Beiko RG (2018) 16S rRNA gene analysis with QIIME2. Methods Mol Biol 1849:113-129.
8. Hassan S, Ahmad B, Shah J, Bashir S, Jahan S, Hizbullah (2019) Protective role of *monotheca buxifolia* and *bosea amherstiana* against H_2O_2-induced DNA damage in human lymphocytes and its effect on oxidative enzymes. Pak J Pharm Sci 32:601-606.
9. Hui M, Zhao-Hui W, Lyons G, Mcdonald G (2011) Effects of selenium valence states and concentration on germination and root growth of six crop species. J Agro-Environ Sci 30:1958-1965.
10. Joy EJ, Ander EL, Young SD, Black CR, Watts MJ, Chilimba AD, Chilima B, Siyame EW, Kalimbira AA, Hurst R (2014) Dietary mineral supplies in africa. Physiologia Plantarum 151:208-229.

11. Khilyas IV, Sorokina AV, Elistratova AA, Markelova MI, Siniagina MN, Sharipova MR, Shcherbakova TA, D'Errico ME, Cohen MF (2019) Microbial diversity and mineral composition of weathered serpentine rock of the *Khalilovsky massif*. PLoS One 14:e0225929.
12. Kikkert J, ., Edward B (2013) Plant uptake and translocation of inorganic and organic forms of selenium. Arch Environm Contam Toxiol 65:458-465.
13. Kulichevskaya IS, Ivanova AO, Baulina OI, Bodelier PL, Damsté JS, Dedysh SN (2008) *Singulisphaera acidiphil*a gen. Nov., sp. Nov., a non-filamentous, isosphaera-like planctomycete from acidic northern wetlands. Int J Syst Evol Microbiol 58:1186-1193.
14. Kulichevskaya IS, Detkova EN, Bodelier PLE, Rijpstra WIC, Sinninghe Damsté JS, Dedysh SN (2012) *Singulisphaera rosea* sp. Nov., a planctomycete from acidic *sphagnum* peat, and emended description of the genus *singulisphaera*. Int J Syst Evol Microbiol 62:118-123.
15. Li S, Xiao T, Zheng B (2012) Medical geology of arsenic, selenium and thallium in china. Sci Tot Environ 421-422:31-40.
16. Liu YR, Delgado-Baquerizo M, Yang Z, Feng J, Zhu J, Huang Q (2020) Microbial taxonomic and functional attributes consistently predict soil CO_2 emissions across contrasting croplands. The Science of the total environment 702:134885.
17. Lunøe K, Gabeljensen C, Stürup S, Andresen L, Skov S, Gammelgaard B (2011) Investigation of the selenium metabolism in cancer cell lines. Met Int Biom Sci 3:162-168.
18. Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634-663.
19. Michela, Schiavon, Elizabeth, A., H., Pilon-Smits (2017) The fascinating facets of plant selenium accumulation–biochemistry, physiology, evolution and ecology. New Phytol.
20. Morgan JAW, Bending GD, White PJ (2005) Biological costs and benefits to plant-microbe interactions in the rhizosphere. J Exp Bot 56:1729-1739.
21. Pandey C, Gupta M (2015) Selenium and auxin mitigates arsenic stress in rice (*Oryza sativa* L.) by combining the role of stress indicators, modulators and genotoxicity assay. J Hazard Mat 287:384-391.
22. Parks DH, Chuvochina M, Waite DW, Rinke C (2018) A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotech 36:996-1004.
23. Qaisrani MM, Zaheer A, Mirza MS, Naqqash T, Qaisrani TB, Hanif MK, Rasool G, Malik KA, Ullah S, Jamal MS, Mirza Z, Karim S, Rasool M (2019) A comparative study of bacterial diversity based on culturable and culture-independent techniques in the rhizosphere of maize (*Zea mays L.*). Saudi journal of biological sciences 26:1344-1351.
24. Rayman MP, Heidi Goenaga I, Mike S (2008) Food-chain selenium and human health: Spotlight on speciation. Br J Nutr 100:254-268.
25. Rayman MP, Winther KH, Pastor-Barriuso R, Cold F, Thvilum M, Stranges S, Guallar E, Cold S (2018) Effect of long-term selenium supplementation on mortality: Results from a multiple-dose, randomised

controlled trial. Free Rad Biol Med 135:S0891584918300704.

26. Rita S, Morse NL (2015) A review of dietary selenium intake and selenium status in europe and the middle east. Nutrients 7:1494-1537.
27. Rosenfeld CE, James BR, Santelli CM (2018) Persistent bacterial and fungal community shifts exhibited in selenium-contaminated reclaimed mine soils. Appl Environ Microbiol 84.
28. Safronova VI, Guro PV, Sazanova AL, Kuznetsova IG, Belimov AA, Yakubov VV, Chirak ER, Afonin A, Gogolev YV, Andronov EE, Tikhonovich IA (2020) Rhizobial microsymbionts of *kamchatka oxytropis* species possess genes of the type iii and vi secretion systems, which can affect the development of symbiosis. Molecular plant-microbe interactions : MPMI 33:1232-1241.
29. Sazanova AL, Safronova VI, Kuznetsova IG, Karlov DS, Belimov AA, Andronov EE, Chirak ER, Popova JP, Verkhozina AV, Willems A, Tikhonovich IA (2019) *Bosea caraganae* sp. Nov. A new species of slow-growing bacteria isolated from root nodules of the relict species *Caragana jubata* (Pall.) Poir. Originating from mongolia. Int J Syst Evol Microbiol 69:2687-2695.
30. Schiavon M, Pilon-Smits EAH (2016) Selenium biofortification and phytoremediation phytotechnologies: A review. J Environ Qual 46:10.
31. Schiavon M, Pilon-Smits EA (2017) Selenium biofortification and phytoremediation phytotechnologies: A review. J Environ Qual 46:10.
32. Schomburg L (2017) Dietary selenium and human health. Nutrients 9:22.
33. Shakibaie M, Shahverdi AR, Faramarzi MA, Hassanzadeh GR, Rahimi HR, Sabzevari O (2013) Acute and subacute toxicity of novel biogenic selenium nanoparticles in mice. Pharm Biol 51:58-63.
34. Stolz JF, Basu P, Santini JM, Oremland RS (2006) Arsenic and selenium in microbial metabolism. Ann Rev Microbiol 60:107-130.
35. Thiry C, Ruttens A, Temmerman LD, Schneider YJ, Pussemier L (2012) Current knowledge in species-related bioavailability of selenium in food. Food Chem 130:767-784.
36. Tian L, Wang L (2021) Multi-omics analysis reveals structure and function of biofilm microbial communities in a pre-denitrification biofilter. The Science of the total environment 757:143908.
37. Wadhwani SA, Shedbalkar UU, Singh R, Chopade BA (2016) Biogenic selenium nanoparticles: Current status and future prospects. Appl Microbiol Biotech 100:2555-2566.
38. Wang J, Liu X, Jiang X, Zhang L, Hou C, Su G, Wang L, Mu Y, Shen J (2020) Facilitated bio-mineralization of N,N-dimethylformamide in anoxic denitrification system: Long-term performance and biological mechanism. Water Res 186:116306.
39. White PJ, Brown PH (2010) Plant nutrition for sustainable development and global health. Ann Bot 105:1073.
40. White PJ (2016) Selenium accumulation by plants. Ann Bot 117:217-235.
41. WHO. 2009. *Global health risks: Mortality and burden of disease attributable to selected major risks*. Available: http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_annex.pdf [accessed March 5 2014].

42. Williams MC, Mayland HF (1992) Selenium absorption by two-grooved milkvetch and western wheatgrass from selenomethionine, selenocystine, and selenite. J Ran Manag 45:374-378.
43. Wu M, Cong X, Li M, Rao S, Liu Y, Guo J, Zhu S, Chen S, Xu F, Cheng S, Liu L, Yu T (2020) Effects of different exogenous selenium on se accumulation, nutrition quality, elements uptake, and antioxidant response in the hyperaccumulation plant cardamine violifolia. Ecotoxicol Environ Saf 204:111045.
44. Wu Z, Bãnuelos GS, Lin ZQ, Liu Y, Yuan L, Yin X, Li M (2015) Biofortification and phytoremediation of selenium in china. Front Plant Sci 6:136.
45. Yuan L, Zhu Y, Lin ZQ, Banuelos G, Li W, Yin X (2013) A novel selenocystine-accumulating plant in selenium-mine drainage area in Enshi, China. Plos One 8:e65615.
46. Zayed A, Lytle CM, Terry N (1998) Accumulation and volatilization of different chemical species of selenium by plants. Planta 206: 284–292.
47. Zhang Y, Sun K, Li Z, Chai X, Fu X, Kholodkevich S, Kuznetsova T, Chen C, Ren N (2021) Effescts of acute diclofenac exposure on intestinal histology, antioxidant defense, and microbiota in freshwater crayfish (*Procambarus clarkii*). Chemosphere 263:128130.
48. Zhou Y, Tang Q, Wu M, Mou D, Liu H, Wang S, Zhang C, Ding L, Luo J (2018) Comparative transcriptomics provides novel insights into the mechanisms of selenium tolerance in the hyperaccumulator plant cardamine hupingshanensis. Sci Rep 8:2789.
49. Zhu W, Yang Z, Ma Z, Chai L (2008) Reduction of high concentrations of chromate by *Leucobacter* sp. Crb1 isolated from Changsha, China. World J Microb Biot 24:991-996.

Tables

Table 1 The experiment design about the effects of exogenous selenium on enrichment and rhizosphere microbiome of *C. violifolia*

Treatments	Exogenous selenium concentration (mg/L)				
Control (soil)	0	0	0	0	0
Plant control (soil +plant)	0	0	0	0	0
B. subtilis-Se (soil + *B. subtilis*-Se +plant)	50	100	200	400	800
Yeast-Se (soil + Yeast-Se + plant)	50	100	200	400	800
Sodium selenate (soil + sodium selenite + plant)	50	100	200	400	800
Sodium selenite (soil + sodium selenite + plant)	50	100	200	400	800

Figures

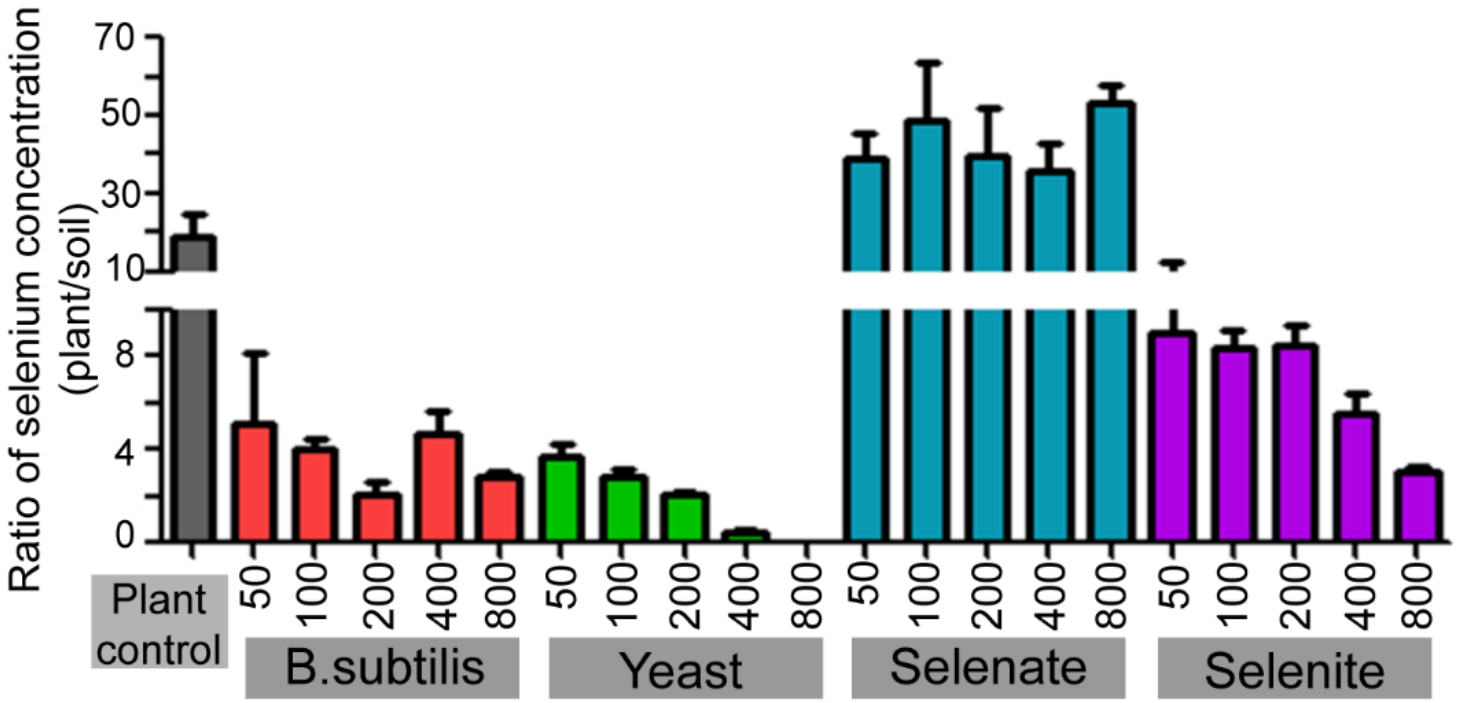

Figure 1

The ratio of selenium concentrations in C. violifolia to that in the soil after the supplementation of four types of selenium sources, including nanoparticles element selenium from Bacillus subtilis, organic selenium from yeast, selenate, and selenite. There is no selenium addition in the plant control.

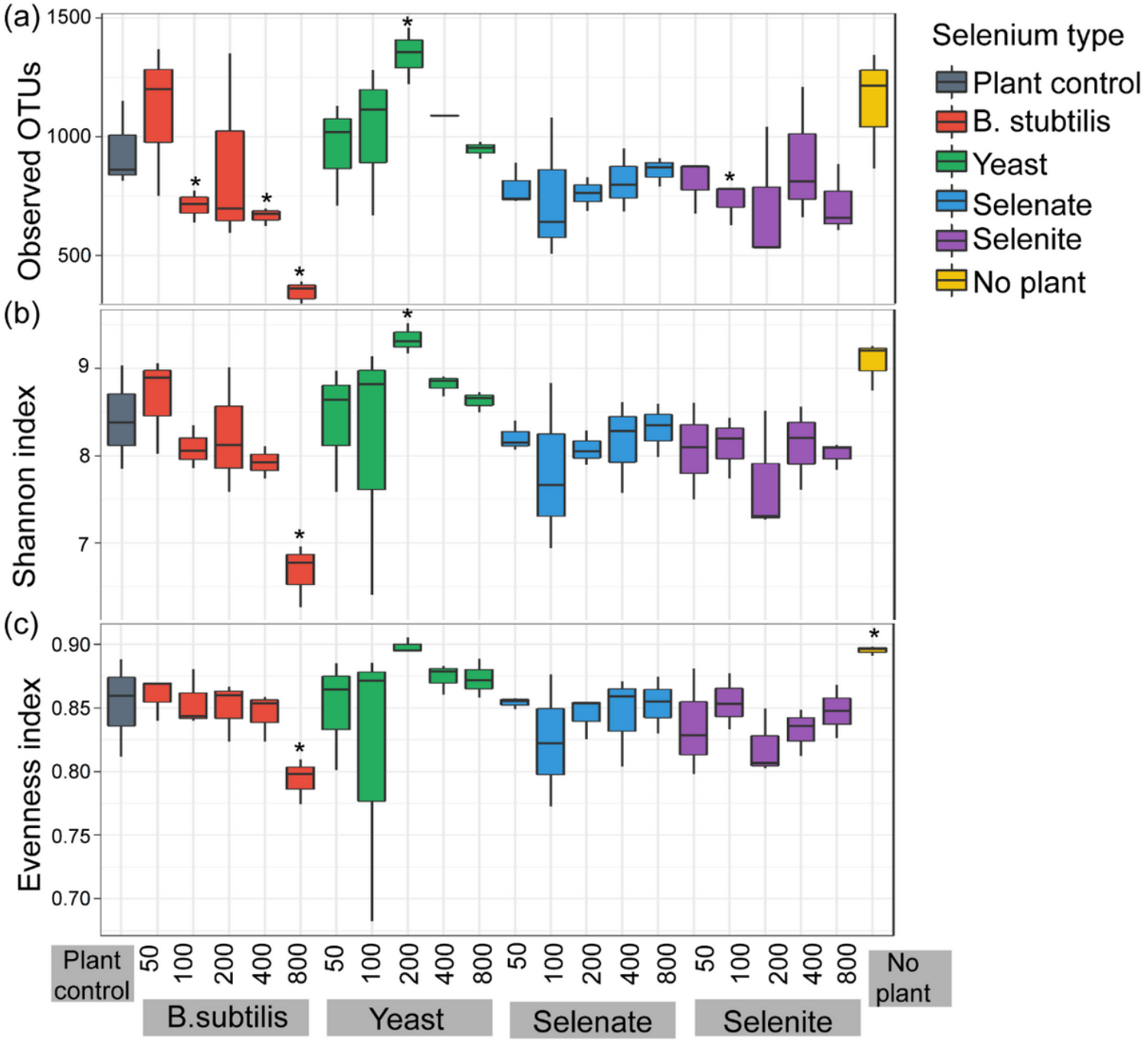

Figure 2

Observed OTUs (a), Shannon index (b), and evenness index (c) of the rhizosphere microbiome affected by four types of selenium addition, including nanoparticles element selenium from Bacillus subtilis, organic selenium from yeast, selenate, and selenite. There is no selenium addition in the plant control group. 'No plant' indicates samples without neither plant nor selenium addition. *p-value ≦ (change the symbol) 0.05, Kruskal-Wallis test.

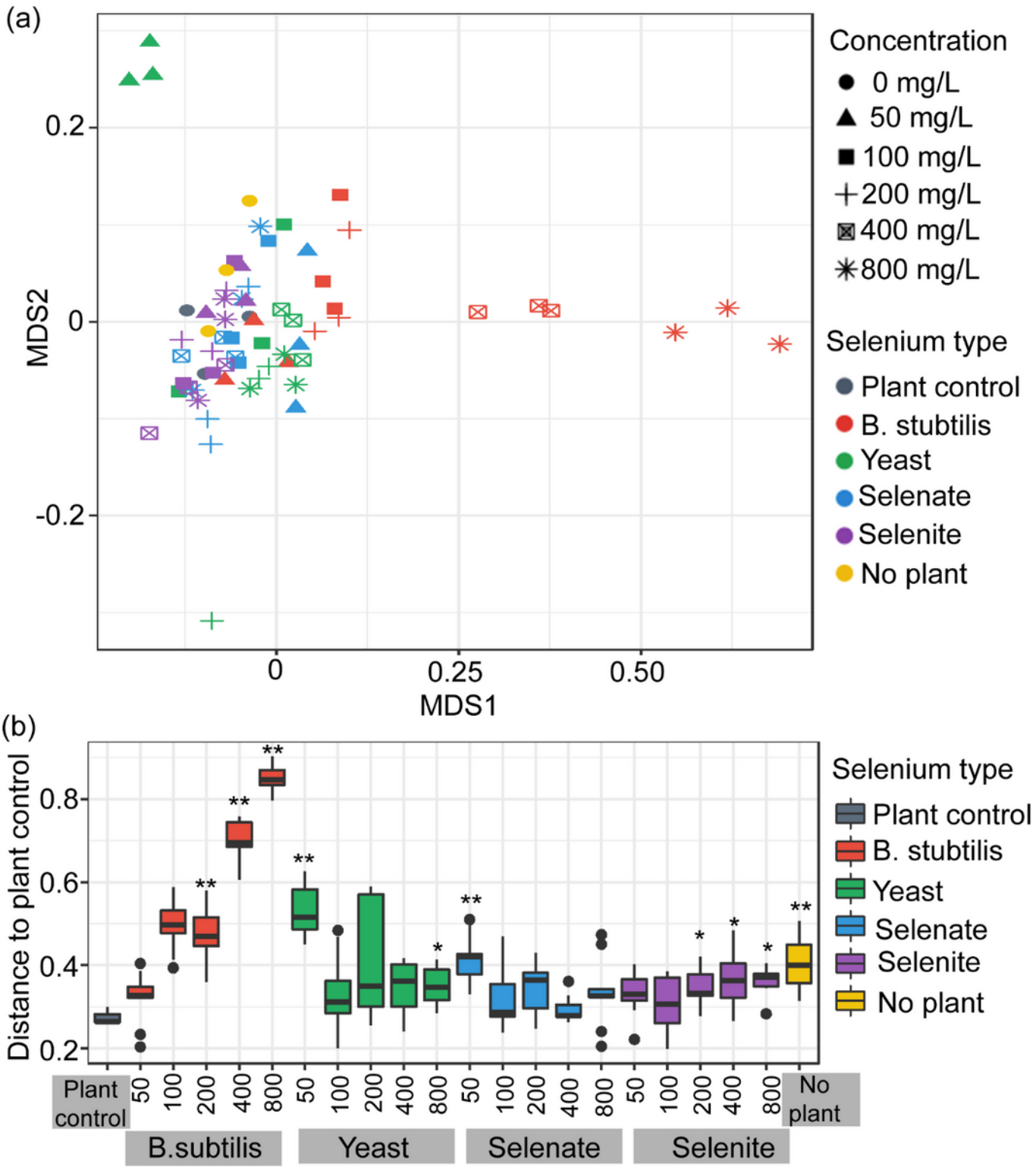

Figure 3

(a) Beta diversity among the rhizosphere microbiomes measured by Bray-Curtis distances and visualized by NMDS. The rhizosphere microbiomes are color coded by the supplementation of selenium sources and the concentration of selenium sources are marked by different shapes. (b) Bray-Curtis distance from each rhizosphere microbiome to the rhizosphere microbiome of plant control. 'No plant' indicates samples

without neither plant nor selenium addition. *p-value ≤ (change the symbol) 0.05, **p-value ≤ (change the symbol) 0.01, Mann-Whitney U test.

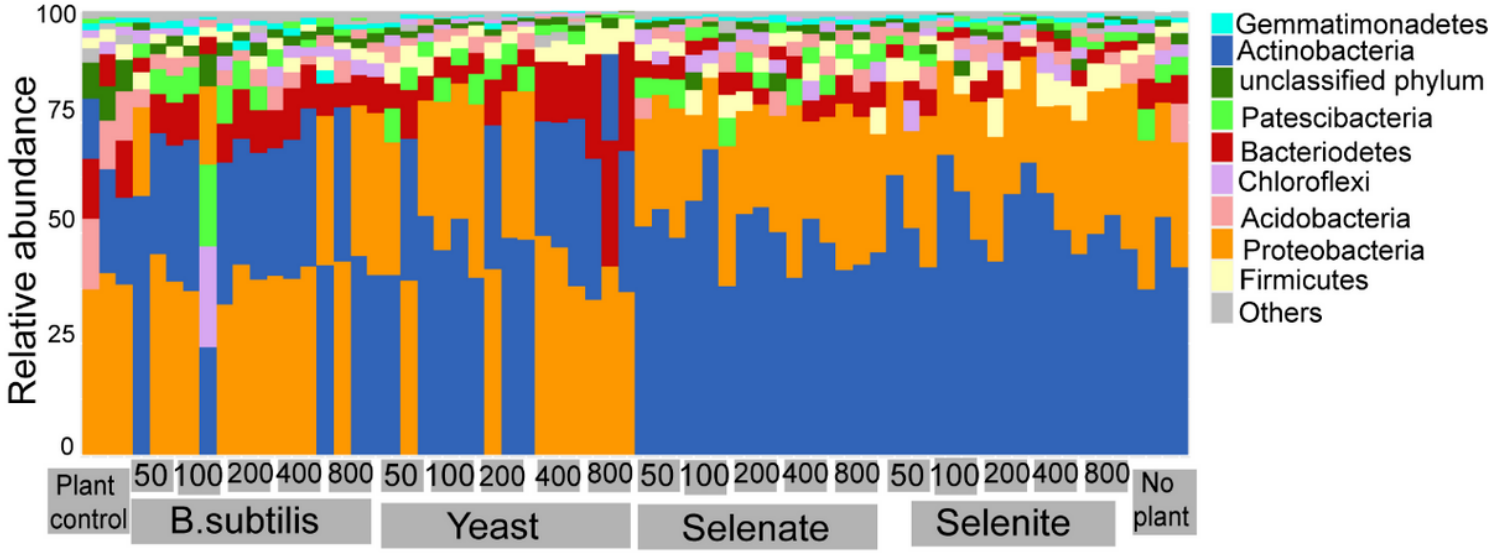

Figure 4

The relative abundance of the rhizosphere microbiomes at phylum level in the four group samples with four types of selenium addition, respectively, including nanoparticles element selenium from Bacillus subtilis, organic selenium from yeast, selenate, and selenite. There is no selenium addition in the plant control. There is no selenium addition and no plant in the No plant group.

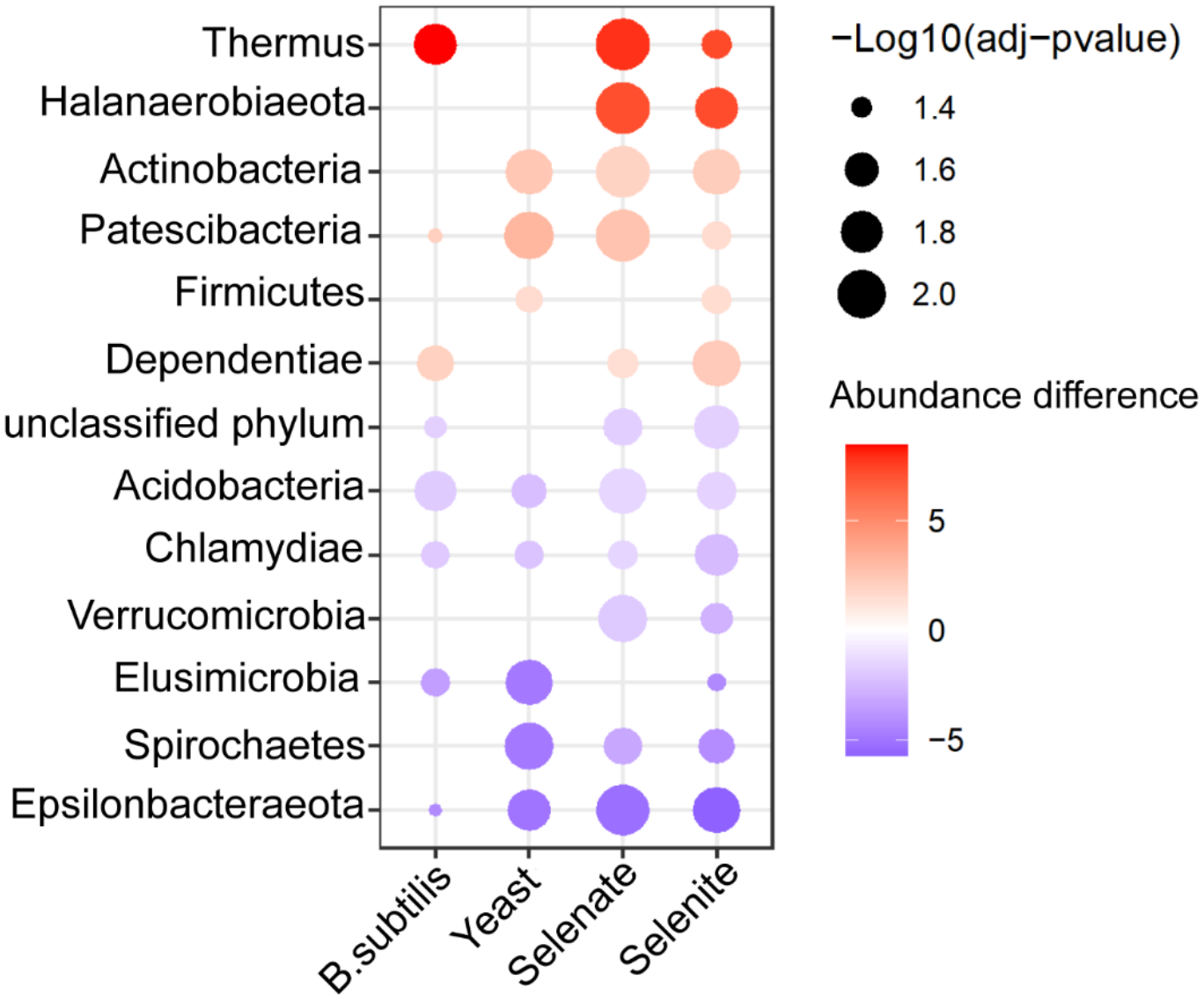

Figure 5

Enrichment of taxa by selenium sources at phylum level. The abundance of taxa in the rhizosphere microbiomes with the supplementation of 800mg/L of four selenium sources was compared to that in plant control. Abundance difference was calculated using the ADLEx2 package in R and quantified by the per-feature median difference between two conditions. Adjusted p values were generated by Benjamini-Hochberg corrected p-values of the Wilcoxon test.

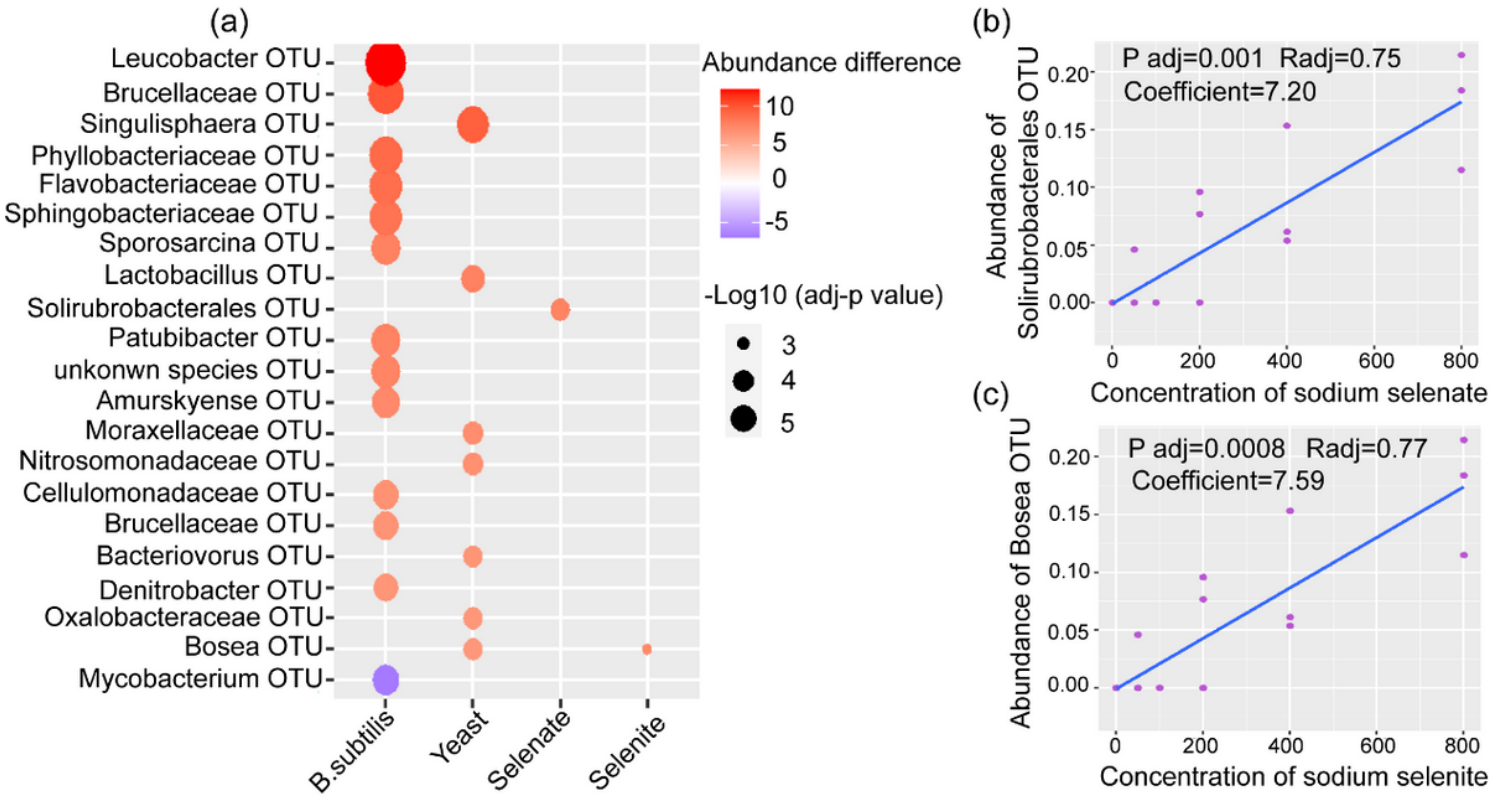

Figure 6

(a) Enrichment of taxa by selenium sources at species level. The abundance of taxa in the rhizosphere microbiomes with the supplementation of 800 mg/L of four selenium sources was compared to that in plant control. Abundance difference was calculated using the ADLEx2 package in R and quantified by the per-feature median difference between two conditions. Adjusted p values were generated by Benjamini-Hochberg corrected p-values of the Wilcoxon test. (b and c) Linear regression of taxa abundance in the rhizosphere microbiomes supplemented by different concentrations of selenium sources. The p values, R-values and coefficients were measured by the stats package in R and the p values were corrected using the Benjamini–Hochberg procedure.