Article

Application of Selenium Can Alleviate the Stress of Cadmium on Rapeseed at Different Growth Stages in Soil

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Abstract: Cadmium (Cd) stress greatly limits the growth of rapeseed, and selenium is a micronutrient that is essential for rapeseed growth, but whether and how selenium application alleviates Cd-induced inhibition remains poorly understood. The present study investigated the alleviating effects of exogenous selenium on rapeseed growth under cadmium (Cd) stress based on the aspects of agronomic traits and soil bacterial community structure and diversity. The results show that low-selenium treatment increased the rapeseed yield by 20.92% by increasing the number of pods per plant under Cd stress, but such effects were not prominent when the selenium application rate was high. Meanwhile, selenium application significantly reduced the cadmium content by 4.74–26.89% in different organs of rapeseed. Further analysis suggested that the benefits of selenium in alleviating Cd stress might be induced by changes in soil bacterial community structure and diversity. In addition, in the functional metabolism spectrum of rapeseed microorganisms treated with selenium, there were 5 primary metabolic pathways with significant differences, and there were 32 and 169 pathways for secondary and tertiary metabolic pathways, respectively. Therefore, selenium treatment in rapeseed soil can alter the composition and metabolic function spectrum of soil microbial communities, ultimately affecting plant growth and Cd tolerance.

Keywords: cadmium stress; rapeseed; selenium; yield traits; soil bacterial community

1. Introduction

In recent years, cadmium pollution in soil has been a common problem in agricultural environmental pollution in China, and the soil ecological balance has been impacted [1]. The rhizosphere soil environment of plants has been severely damaged, causing extremely adverse effects on organisms. Rapeseed, a significant oil crop, is extensively cultivated worldwide and serves as a crucial feedstock for bioenergy production. Its yield heavily relies on the application of nitrogen, phosphorus, and potassium fertilizers. Proper utilization of these fertilizers can substantially enhance rapeseed productivity [2,3]. Cadmium, a highly toxic heavy metal element, poses a serious threat to human health as it can enter the body through soil and plants [4]. However, understanding the mechanism of interaction between soil function and heavy metals remains a paramount concern in the field of soil ecology.

Cadmium is considered one of the most hazardous elements in soil remediation processes and has become a growing public concern as the risks associated with soil remediation continue to increase [5]. Additionally, despite its low concentration, cadmium pollution poses a significant threat to both human and animal health due to its persistent
release into the soil. The toxicity of cadmium can impede the growth and development of plants, with the root system serving as the primary site for cadmium pollution propagation in most plant species [6,7]. Studies have shown that cell walls are the main site for cadmium accumulation in metal-tolerant plant roots. The pectin in the cell wall can fix cadmium, thereby reducing the toxic damage of cadmium to plants [8]. At present, research on heavy metals in fields mostly focuses on the migration of heavy metals from the soil to crops, while cadmium pollution has less of an impact on the soil microbial community [9].

Soil microorganisms can regulate the composition of soil microbial community structures, maintain soil fertility, and promote the steady state of soil microorganisms and the growth and development of crops such as rapeseed [10]. At the same time, soil microorganisms have an important impact on biogeochemical processes and nutrient transformation [11]. Moreover, the abundance, diversity, and composition of soil microorganisms are crucial indicators for assessing soil quality as they play a vital role in organic matter decomposition, mineralization, and nutrient cycling. These factors are influenced by both biotic and abiotic factors such as pH, soil organic matter content, and other potentially toxic elements [12,13]. In soil contaminated with cadmium, heavy metal accumulation beyond a certain threshold can lead to adverse effects on microorganisms, altering the structure and diversity of soil microbial communities. This subsequently disrupts the balance of the soil ecosystem by reducing microbial populations, diminishing soil enzyme activity, and impairing overall soil environmental functionality [14–16].

Selenium is a micronutrient that is essential for the healthy growth of organisms. Due to the fact that plants are the main source of dietary selenium, plant selenium metabolism is crucial for the nutrition of organisms. However, excessive intake of selenium can also lead to organism toxicity, so we need to consume an appropriate amount of dietary selenium [17,18]. The use of different enzymatic and non-enzymatic antioxidants can be used to combat selenium-induced excess production of reactive oxygen species, thereby affecting the anti-lipid peroxidation process of plants [19]. Some studies have combined selenium and methyl jasmonic acid to control the occurrence of tomato gray mold, analyzed the bacterial microbial community structure using amplification sequencing technology, and isolated strains against gray mold from tomato leaves, revealing the key role of selenium and methyl jasmonic acid in jointly recruiting beneficial plant bacteria and improving tomato disease resistance [20]. Selenium also regulates the opening of plant stomata, thereby increasing plant carbon dioxide flux and the net photosynthesis rate [21]. In addition, an increase in selenium content can enhance the activity of glutathione peroxidase (GSHP)X and reduce the absorption of heavy metals such as cadmium by plants [22].

The main groups of soil microorganisms in plants are bacteria and fungi, and alterations in soil quality can impact the composition and activity of these microorganisms. The supply of selenium changes the structure and diversity of soil microbial communities. Low concentrations of selenium increase the diversity and quantity of microorganisms, while high concentrations of selenium have inhibitory effects [23]. Research has shown that selenium can reduce the toxicity of cadmium to plants and reduce cadmium accumulation. Through analysis of cadmium absorption, nutrient balance, and other aspects, the impact of selenium treatment on plants under cadmium toxicity has been revealed [24]. However, the impact of selenium on rapeseed growth under cadmium stress through the modulation of soil microorganisms remains to be fully elucidated.

The “High Oleic Acid No.1” rapeseed variety currently has a higher content of oleic acid among Chinese rapeseed varieties. And previous studies have mainly focused on the absorption of nutrients to analyze the reasons why selenium alleviates the growth of rapeseed under cadmium stress. However, this study analyzed the changes in soil microorganisms after selenium application at different growth stages to analyze the growth of rapeseed. Therefore, this study investigated the effects of different selenium treatments on the physical and chemical indicators of rapeseed and soil microbial communities at different growth stages under cadmium stress. The findings establish a theoretical
foundation for comprehending the diversity of soil microbial communities, the impact of pollutants on such communities, and their implications for rapeseed cultivation and food safety.

2. Materials and Methods

2.1. Materials

The oilseed rape cultivar used in this study was Gaoyousuan1 (Brassica napus L.), which was obtained from the Hunan Branch of the National Oilseed Crops Improvement Center. Field experiments were carried out at the Yunyuan Teaching and Experimental Base of Hunan Agricultural University (28°10' N, 113°04' E) during the period from October 2021 to June 2022. The experimental soil used in this study was field soil, where rice and oilseed rape were rotated between irrigated and upland fields throughout the year. The soil exhibited a pH of 6.11, a total cadmium content of 0.49 mg/kg, a total nitrogen content of 1.98 g/kg, an alkali-hydrolyzed nitrogen content of 127.90 mg/kg, an available phosphorus content of 43.10 mg/kg, an available potassium content of 137.51 mg/kg, and an organic matter content of 23.83 g/kg. Carbamide (46% N), superphosphate (12% P2O5), and muriate (60% K2O) were employed as fertilizers in this study.

2.2. Experimentation

The experiment was arranged with a randomized block design with three replications. Based on previous pre-experiments, the treatments included a no-selenium control (CK), low-selenium treatment (L, 500 g/hm²), and high-selenium treatment (H, 1000 g/hm²). The source of selenium was sodium selenite, and selenium treatment was applied to the soil before transplantation. Oilseeds were sown on 28 September, and one vigorous seedling was transplanted into each pot on 25 October.

2.3. Plant Sampling

At seven days before full maturity, three plants in each plot were collected to measure the number of pods per plant, the number of pods per pod, and the weight of 1000 seeds. After harvesting, all plants in each plot were harvested, threshed, and naturally dried to calculate the actual yield. During the mature period of rapeseed, 3 rapeseed plants were selected with the same growth size from each community. After threshing the rapeseed, it was divided into three parts: root, stem, and leaf. The plant samples were soaked in a 20 mmol/L Na₂EDTA·2H₂O solution for 15 min and then rinsed three times with deionized water. Then, the plant samples were dried at 65°C to a constant weight, weighed, and ground into powders. For the determination of the cadmium content in the plants, the digestion of samples was performed using the wet ashing method. After weighing the samples, a nitric acid–perchloric acid mixed acid (9:1) was added, and they were put in a fume hood and boiled until the solutions were colorless and transparent and the brown smoke almost disappeared, and a small amount of slightly cold distilled water was added, and the filtrate was filtered and combined in a volumetric bottle. After digestion, the cadmium contents in the samples were determined with an atomic absorption spectrometer (Z-2000, HITACHI, Tokyo, Japan) [25]. The specific operation method was as follows: absorb cadmium (5 μg/mL) standard solution of 0.0, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mL in a 50 mL volumetric flask; add 1 mL of mixed acid (9:1 HNO₃:HClO₄) and 20 mL of 1 mol/L HCl; bring to volume with deionized water; and prepare Q (Cd) 0.0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 μg/mL standard series solutions, respectively. Under the same conditions, measure the absorption values using the flame atomic absorption method to obtain the concentration values of the sample solutions, and perform three internal duplicates.

2.4. Soil Sampling

For the processing and collection of soil samples, soil samples were selected in the bolting stage, flowering stage, and mature stage, as B_CK, B_L, B_H, F_CK, F_L, F_H,
M_CK, M_L, and M_H. All instruments need to be disinfected and sterilized before sampling. During the bolting, flowering, and ripening stages of rapeseed, a soil knife was used to excavate the upper layer of the soil layer by layer from the base of the rapeseed. The fibrous roots along the lateral roots were found, 20 cm deep soil was collected, the rapeseed roots were gently shaken, large pieces of soil and loose soil from the rapeseed plant roots were removed, and a brush was used to collect the rhizosphere soil adhered to the roots into sterile centrifuge tubes. Afterward, the plant roots, animal debris, and other impurities were removed through a sieve and packaged in sterile centrifuge tubes. The samples were subsequently placed in sample bags and subjected to freezing at −40 °C, followed by storage in a refrigerator. This entire process was repeated five times.

2.5. DNA Extract and PCR Products Mixing and Purification

The soil DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions. The purity and quality of the genomic DNA were assessed using 1% agarose gels and a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA).

The V4 hypervariable region of the bacterial 16S rRNA gene was amplified with the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGAC- TACHVGGGTWTCTAAAT-3′) [26]. For each soil sample, an 8-digit barcode sequence was added to the 5′ ends of the forward and reverse primers (provided by Allwegene Company, Beijing, China). The PCR was carried out with a Mastercycler Gradient (Eppendorf, Germany) using 25 µL reaction volumes, containing 12.5 µL of 2× Taq PCR MasterMix, 3 µL of BSA (2 ng/µL), 1 µL of the forward primer (5 µM), 1 µL of the reverse primer (5 µM), 2 µL of template DNA, and 5.5 µL of ddH2O. The cycling parameters were 95 °C for 5 min, followed by 28 cycles at 95 °C for 45 s, 55 °C for 50 s, and 72 °C for 45 s, with a final extension at 72 °C for 10 min. The PCR products were purified using an Agencourt AMPure XP Kit.

2.6. High-Throughput Sequencing

Deep sequencing was performed on the MiSeq platform at Allwegene Company (Beijing, China). Subsequently, image analysis, base calling, and error estimation were conducted using Illumina Analysis Pipeline Version 2.6.

2.7. Statistical Analysis

The physiological experimental data were plotted using Excel 2010 software and analyzed using LSD and Duncan tests with SPSS 20.0. The graphs were generated using GraphPad Prism V8.0.0 software. The statistical analysis was conducted at a significance level of p < 0.05, and the results are presented as means ± standard error.

The raw data underwent rigorous screening, and sequences that did not meet the quality criteria were excluded from further analysis. Subsequently, qualified reads were subjected to clustering into operational taxonomic units (OTUs) using the UParse algorithm of the Vsearch (v2.7.1) software with a similarity threshold set at 97% [27]. The Ribosomal Database Project (RDP) Classifier tool was employed to classify all sequences into distinct taxonomic groups based on the SILVA138 database, in accordance with established scientific protocols [28].

Rarefaction curves and richness and diversity indices based on the OTU information were generated using QIIME (v1.8.0). Heatmaps were constructed in R to compare community membership and structure across samples, utilizing the top 20 OTUs [29]. The barplot diagrams were generated using R (v3.6.0) software based on taxonomic annotation and relative abundance data. To evaluate the similarity between samples, clustering analyses and PCA were conducted in R (v3.6.0) with the OTU information from each sample [30].
Analysis of molecular variance (AMOVA) is a non-parametric analysis method based on a beta distance matrix to test the significance of differences between different groups. It is a non-parametric simulation of traditional analysis of variance, similar to ANOVA. AMOVA can perform inter-group difference analysis using the AMOVA function (v.1.48.0) of the other software based on the Bray–Curtis distance. The Kruskal–Wallis rank-sum test is a non-parametric test on three or more sets of data and can calculate the existence of differences between the means of multiple independent data sets. It only processes the volatility of the data displayed on the graph, unlike ANOVA, where non-parametric testing does not make assumptions about the data distribution.

3. Results

3.1. Effects of Different Amounts of Selenium Application on Agronomic Characteristics and Yield of Rapeseed during Maturity

Compared with the control group, the low-selenium treatment increased the yield by 20.92%, mainly by increasing the silicle number per plant. The low-selenium treatment increased the seeds per silicle and seed weight by 4.82% and 4.11%, respectively. There were no significant differences in the seeds per silicle and seed weight between each treatment, but there was a significant difference in the silicle number per plant between the low-selenium and CK treatments, and the silicle number per plant with CK was 10.81% higher than that with the L treatment. However, the high-selenium treatment had no significant influence on the yield and yield components compared with CK (Table 1).

Table 1. Effects of different selenium treatments on the yield and composition of rapeseed.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Silicle Number Per Plant</th>
<th>Seeds Per Silicle</th>
<th>Seed Weight (mg)</th>
<th>Yield (kg/hm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>610.4 ± 29.5 b</td>
<td>23.26 ± 0.81 a</td>
<td>2.92 ± 0.18 a</td>
<td>2280.18 ± 82.51 b</td>
</tr>
<tr>
<td>L</td>
<td>676.4 ± 22.1 a</td>
<td>24.38 ± 0.59 a</td>
<td>3.04 ± 0.23 a</td>
<td>2757.23 ± 148.21 a</td>
</tr>
<tr>
<td>H</td>
<td>611.8 ± 4.9 b</td>
<td>23.53 ± 0.12 a</td>
<td>2.94 ± 0.25 a</td>
<td>2327.78 ± 40.43 ab</td>
</tr>
</tbody>
</table>

Note: The significance of differences between different treatments is represented by different lowercase letters at 0.05 level.

3.2. Effects of Different Amounts of Selenium on Cadmium Contents in Different Parts of Rapeseed

The cadmium contents in various parts of rapeseed at the maturity stage under different selenium treatments are shown in Figure 1. The cadmium contents in all parts of the rapeseed followed the order of seeds < roots < shoot. The H treatment significantly reduced the cadmium contents in all parts of the rapeseed, and the cadmium contents in all parts followed the order of H < L < CK. The cadmium contents in roots, stems, and leaves with the H treatment were 0.21, 0.15, and 0.13 mg/kg, indicating that the cadmium reduction effect of selenium increased with the increase in selenium application in a certain range. Meanwhile, the high-selenium treatment has the best effect on reducing cadmium content.
3.3. Diversity and Community Differences in Rhizosphere Microbial Communities

As shown in Table 2, the sample data after sequencing were statistically summarized. The Clean_tags obtained from the sequencing were all above 60,000. The study of microbial diversity in community ecology can reflect the abundance and diversity of microbial communities using single-sample diversity analysis (alpha diversity), including a series of statistical analysis indices to estimate the species abundance and diversity of environmental communities, such as Chao1, observedSpecifications, PD, Whole Tree, Shannon, etc. Alpha diversity analysis revealed that selenium and cadmium had no significant impact on microbial diversity in rhizosphere soil samples. Furthermore, all samples exhibited a coverage index exceeding 90%, indicating a sufficient sequencing depth (Figure 2).

As shown in Figure 3A, based on the PCA analysis, it can be concluded that there were significant differences in the microbial communities after cadmium treatment at different stages. In addition, our PCA analysis of cadmium-contaminated rapeseed treated with low and high selenium amounts showed that there were certain differences in microbial communities between the low-selenium and high-selenium treatments, while there was no significant difference in microbial communities in rapeseed treated with selenium during flowering (Figure 3B). ANOSIM analysis further showed that there was a significant impact on the bacterial community structure of cadmium-contaminated soil in rapeseed at different stages ($R^2 = 0.62; p = 0.001$). At the same time, after selenium treatment, there was also a significant impact on the bacterial community in rapeseed at different stages ($R^2 = 0.92, p = 0.001$), verifying the effectiveness of the experiment.

Table 2. Concatenate charge result statistics.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw_Tags</th>
<th>Clean_Tags</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_CK</td>
<td>105,498</td>
<td>101,630</td>
<td>96.33</td>
</tr>
<tr>
<td>B_H</td>
<td>361,809</td>
<td>343,433</td>
<td>94.92</td>
</tr>
<tr>
<td>B_L</td>
<td>91,700</td>
<td>89,008</td>
<td>97.06</td>
</tr>
<tr>
<td>F_CK</td>
<td>124,037</td>
<td>120,711</td>
<td>97.32</td>
</tr>
<tr>
<td>F_H</td>
<td>185,158</td>
<td>181,078</td>
<td>97.80</td>
</tr>
<tr>
<td>F_L</td>
<td>61,673</td>
<td>60,132</td>
<td>97.50</td>
</tr>
<tr>
<td>M_CK</td>
<td>512,856</td>
<td>496,359</td>
<td>96.78</td>
</tr>
<tr>
<td>M_H</td>
<td>216,585</td>
<td>210,290</td>
<td>97.09</td>
</tr>
<tr>
<td>M_L</td>
<td>350,507</td>
<td>336,036</td>
<td>95.87</td>
</tr>
</tbody>
</table>
Figure 2. The boxplots of alpha diversity indices (chao1, observed_species, PD_whole_tree, and shannon) reflect the differences in alpha diversity indices between different groups.

Figure 3. PCA analysis between different groups. (A) PCA analysis of non-selenium treatment in rapeseed at different stages. (B) PCA analysis of selenium treatment in rapeseed at different stages.
3.4. Effect of Root Selenium Treatment on Soil Bacterial Community Structure

Simultaneously, Figure 4A illustrates the utilization of a Wayne plot to depict the abundance and uniqueness of operational taxonomic units (OTUs) in soil microbiota across different treatment groups. The cumulative count of OTUs among all groups amounted to 4498, enabling an analysis of community structure similarity, overlap, and specificity between distinct treatment cohorts.

As illustrated in Figure 4B, we subsequently examined the relative abundance of the top 20 bacterial phyla, with 9 exhibiting an average relative abundance exceeding 1%. Among them were Proteobacteria, Acidobacteria, Bacteroidetes, Planctomycetes, Gemmatimonadetes, Actinobacteria, Chloroflexi, Verrucomicrobia, and unidentified taxa. The Proteobacteria, Acidobacteria, Gemmatimonadetes, Bacteroidetes, Chloroflexi, and Acidobacteria sequences collectively accounted for more than 75% of the total sequence pool and represented the predominant microbial populations.

Additionally, we conducted an analysis of the relative abundance of the top 20 bacterial genera, with 2 exhibiting an average relative abundance exceeding 1%. The largest genus remained unidentified, while Haliangium was also detected. These dominant populations accounted for over 55% of the total sequence reads and were therefore considered major contributors (Figure 4C).

At the phylum level, for rapeseed during the bolting stage compared with the control group, the relative abundance of Chloroflexi and unidentified bacteria in the low-selenium treatment decreased, while the relative abundance of the other seven dominant bacteria increased. The relative abundance of Proteobacteria, Bacteroidetes, and Gemmatimonadetes in the high-selenium-treated bacterial phyla increased, while the relative abundance of other dominant bacterial phyla decreased. Compared with rapeseed treated with the low selenium amount, the relative abundance of Proteobacteria, Acidobacteria, unidentified bacteria, and Chloroflexi in the high-selenium-treated bacterial phyla increased, while the relative abundance of other dominant bacterial phyla decreased. For rapeseed in the flowering stage compared with the control group, the relative abundance of Verrucomicrobia, Gemmatimonadetes, Bacteroidetes, and Proteobacteria in the low-selenium treatment group decreased, while the relative abundance of the other five dominant bacterial phyla increased. However, the relative abundance of Actinobacteria, Bacteroidetes, and Proteobacteria in the high-selenium-treated bacteria decreased, while the relative abundance of other dominant bacteria increased. Compared with rapeseed treated with the low selenium amount, the relative abundance of Proteobacteria, Bacteroidetes, Actinobacteria, and Chloroflexi in the high-selenium-treated bacterial phyla decreased, while the relative abundance of other dominant bacterial phyla increased. For mature rapeseed compared with the control group, the relative abundance of Proteobacteria, Acidobacteria, and unidentified bacteria with the low-selenium treatment decreased, while the relative abundance of the other six dominant bacteria increased. However, the relative abundance of Proteobacteria in the high-selenium-treated bacteria increased, while the relative abundance of other dominant bacteria decreased. Compared with rapeseed treated with the low selenium amount, the relative abundance of Proteobacteria in the high-selenium treatment increased, while the relative abundance of other dominant bacterial phyla decreased (Figure 5A).

In summary, the low-selenium treatment inhibited the abundance of Chloroflexi and unidentified bacteria in rapeseed soil during the bolting period, while the high-selenium treatment promoted an increase in the abundance of Proteobacteria, Bacteroidetes, and Gemmatimonadetes. The low-selenium treatment inhibited increases in the abundance of Verrucomicrobia, Gemmatimonadetes, Bacteroidetes, and Proteobacteria in rapeseed soil during flowering, while the high-selenium treatment inhibited increases in the abundance of Actinobacteria, Bacteroidetes, and Proteobacteria. The low-selenium treatment inhibited increases in Proteobacteria, Acidobacteria, and unidentified bacteria abundance in mature rapeseed soil, while the high-selenium treatment promoted an increase in Proteobacteria abundance.
At the genus level, for rapeseed during the bolting stage compared with the control group, the relative abundance of unidentified bacterial genera and Haliangium in the low-selenium treatment decreased. However, the relative abundance of the bacterial genus Haliangium increased in the high-selenium treatment, while the relative abundance of unidentified bacteria also increased. Moreover, compared with rapeseed treated with the low selenium amount, the relative abundance of identified bacterial genera and Haliangium in the high-selenium treatment increased. For flowering rapeseed compared with the control group, the relative abundance of the unidentified bacterial genera and Haliangium increased in the low-selenium treatment, while the relative abundance of the unidentified bacterial genera and Haliangium increased in the high-selenium treatment. Moreover, compared with rapeseed treated with the low selenium amount, the relative abundance of identified bacterial genera and Haliangium in the high-selenium treatment increased. For mature rapeseed compared with the control group, the relative abundance of bacteria belonging to the unidentified and Haliangium genera decreased in the low-selenium treatment. At the same time, the relative abundance of the unidentified bacterial genera and Haliangium decreased in the high-selenium treatment. Compared with rapeseed treated with the low selenium amount, the relative abundance of identified bacterial genera and Haliangium in the high-selenium treatment increased (Figure 5B).

In summary, the low-selenium treatment promoted an increase in the abundance of unidentified bacteria and Haliangium in rapeseed soil during the bolting period, while the high-selenium treatment inhibited an increase in unidentified bacteria abundance and promoted an increase in Haliangium abundance. The low-selenium treatment promoted an increase in the abundance of unidentified bacteria and Haliangium in rapeseed soil during flowering, while the high-selenium treatment promoted an increase in the abundance of Haliangium and unidentified bacteria. For mature rapeseed compared with the control group, the low-selenium treatment inhibited an increase in unidentified bacteria and Haliangium abundance, while the high-selenium treatment promoted an increase in Haliangium and unidentified bacteria abundance.

**Figure 4.** Species composition analysis of selenium treatment in rapeseed at different stages. (A) Venn diagram showing common and unique OTUs of different groups. (B) Species composition at different phylum levels. (C) Species composition at different genus levels.
3.5. Effects of Non-Selenium Treatment on Different Soil Microbial Communities

Using AMOVA analysis, differences in the soil bacteria of non-selenium-treated rape-seed at three different stages were compared, and the p-value was less than 0.001. Therefore, we believe that there were significant differences between the non-selenium treatments at different growth stages. Kruskal–Wallis tests for different periods of non-selenium treatment showed that there were 13 species with differences at the phylum level and 114 species with significant differences at the genus level. Meanwhile, via LEfSe analysis, the threshold was set to three, resulting in a total of 62 key biomarkers. Among them, the B_CK group had 19 key biomarkers, the F_CK group had 29 key biomarkers, and the M_CK group had 14 key biomarkers (Figure 6A).

Figure 5. Heatmap of cluster analysis for different groups. (A) Cluster analysis heatmap of species at different phylum levels. (B) Cluster analysis heatmap of species at different genus levels.

Figure 6. Analysis of species differences between different groups. (A) LEfSe analysis for key biomarkers under non-selenium treatment. (B) LEfSe analysis for key biomarkers under selenium treatment.
3.6. Effects of Selenium Treatment on Different Soil Microbial Communities

The soil bacteria of rapeseed treated with selenium at three different growth stages were compared using AMOVA analysis, yielding a p-value of less than 0.001. Hence, we assert that significant differences existed between the selenium treatments at various growth stages. Kruskal–Wallis tests at different stages of selenium treatment showed that there were 40 species with differences at the phylum level and 301 species with significant differences at the genus level. Meanwhile, via LEfSe analysis, the threshold was set to four, resulting in a total of 41 key biomarkers. Among them, B_H had 7 key biomarkers, B_L had 5 key biomarkers, F_H had 3 key biomarkers, F_L had 4 key biomarkers, M_H had 5 key biomarkers, and M_L had 17 key biomarkers (Figure 6B).

3.7. Effects of Selenium Treatment on Soil Metabolic Pathways

Through picrust2 for 16S functional prediction, 6 primary metabolic pathways, 36 secondary metabolic pathways, and 186 tertiary metabolic pathways were ultimately obtained. The top 10 metabolic pathways in abundance were the biosynthesis of ansamycins; the biosynthesis of vancomycin group antibiotics; valine, leucine, and isoleucine biosynthesis; fatty acid biosynthesis; C5-Branched dibasic acid metabolism; bacterial chemotaxis; pantothenate and CoA biosynthesis; D-Glutamine and D-glutamate metabolism; lipoic acid metabolism; and streptomycin biosynthesis (Figure 7). For rapeseed during the bolting stage, both the low-selenium and high-selenium treatments reduced the abundance of these 10 metabolic pathways. For flowering rapeseed, the low-selenium and high-selenium treatments increased the abundance of nine other metabolic pathways, except for bacterial chemotaxis. For mature rapeseed, except for D-Glutamate metabolism and streptomycin biosynthesis, the abundance of the other eight metabolic pathways first showed a decreasing and then increasing trend.

Meanwhile, using the Kruskal test, it was found that there were five different primary metabolic pathways in different groups, namely, metabolism, genetic information processing, environmental information processing, cellular processes, and organizational systems. There were 32 significantly different secondary metabolic pathways in different groups, while there were 169 significantly different tertiary metabolic pathways, and the top 10 metabolic pathways in abundance were all significantly different metabolic pathways.

![Figure 7. Picrust2 prediction of differential microbial functions.](image-url)
4. Discussion

Cd is one of the most toxic heavy metal elements in nature. It usually exists in soil in both organic and non-organic forms and combines with oxides or carbonates to affect the structural composition of soil microbial communities [31]. Cd cannot be absorbed by plant roots in the soil. As soil pH decreases, the adsorption capacity of soil components for Cd\textsuperscript{2+} decreases, and their mobility increases [32], resulting in an increase in the solubility of Cd\textsuperscript{2+} in the soil. After plants further absorb Cd\textsuperscript{2+}, it poses a greater threat to the plants [33]. The toxicity of cadmium can also inhibit plant photosynthesis and respiration. In cadmium-contaminated soil, the activities and contents of chlorophyll a and chlorophyll b decrease [34].

The concentration of selenium in plants is directly related to the concentration of selenium in soil. The application of inorganic selenium fertilizers can increase the selenium concentration in edible crops such as rice and corn. At the same time, it can increase the selenium content in feed and improve the selenium content in animals, which is of great significance for the health development of animals [35]. This study measured the yield indicators of cadmium-contaminated rapeseed treated with selenium, including the number of pods per plant, the number of pods per pod, and the weight of 1000 seeds. It was found that there were significant differences between the low-seelenium and high-seelenium treatments in the number of pods per plant, while there were no significant differences in the number of pods per pod and the weight of 1000 seeds. This indicates that low-seelenium treatment reduces the toxicity of cadmium-contaminated rapeseed, while high-seelenium treatment has an inhibitory effect. Studies have shown that under different concentrations of selenium treatment, the grain yield and concentration of black and white wheat significantly increase and decrease from high-seelenium to low-seelenium regions. Meanwhile, the grain yield and crude protein content of wheat in high-seelenium regions are both higher [36]. This is inconsistent with our experiment, possibly due to the inconsistent selection of selenium concentrations.

We found that increasing the concentration of selenium can change the community composition and functional metabolism of microorganisms in the rhizosphere soil of plants and affect the incidence rate of rot mold in some plants. Some studies have analyzed the bacterial and fungal communities in the rhizosphere soil of rapeseed using 16S and ITS sequencing technology, and the results show that selenium has a strong impact on the incidence rate of rot mold [17–20]. In addition, selenium treatment also increased the diversity of bacterial microbial communities, which is consistent with this study. Soil Se enhanced the microbiome diversities and the related bases of Bryobate, Nitrospirae, Rhizobiales, Xanthobacteriaceae, Nitrosomonadaceae, and Basidiomycota. Regarding the metabolic function spectrum, selenium also increased nitrogen metabolism, carbon hydrate metabolism, and cellular-process-related functions in the soil [37]. In this study, for mature rapeseed, the relative abundance of Proteobacteria and Acidobacteria in the low-seelenium treatment decreased, while the relative abundance of the other six dominant bacterial phyla increased. However, the relative abundance of Proteobacteria in the high-seelenium-treated bacteria increased, while the relative abundance of other dominant bacteria decreased. For mature rapeseed, the low-seelenium treatment inhibited an increase in Haliangium abundance, while the high-seelenium treatment promoted an increase in Haliangium abundance. For mature rapeseed, except for D-glutamine and D-glutamate metabolism and streptomycin biosynthesis, the abundance of the other eight metabolic pathways first showed a decreasing and then increasing trend.

Studies have shown that treatment with Se alters the structural composition of soil microbial communities but does not have a significant impact on the diversity of soil bacterial microbial communities. Proteobacteria and Bacteroidetes decreased under Cd toxicity, whereas they increased after applying Se in [38], which is consistent with the results of this study. Proteobacteria and Bacteroidetes are involved in the degradation of organic matter and the biotransformation of mineral elements and play a role in the degradation of macromolecular organic matter in soil. At the same time, the application of selenium
increases the abundance of Fibrobacteres in the soil, thereby promoting plant growth [39]. Kang Liu et al. [40] found that selenium treatment in soil increased the relative abundance of beneficial microorganisms but decreased the relative abundance of pathogenic fungi while altering the functional spectrum of microorganisms. According to the results of PICRUSt, the functional abundance related to carbohydrate metabolism, nitrogen metabolism, signal transduction, metabolism, and peptidase in HSe soil increased. These processes may play a synthetic role in promoting plant resistance.

5. Conclusions

The results of this study indicate that moderate selenium application can increase the yield of rapeseed by increasing the number of pods per plant, and selenium application exceeding a certain threshold affects the yield increase effect. Selenium application can significantly reduce the cadmium content in the roots, stems, and leaves of rapeseed, and within a certain range, the cadmium reduction effect of selenium increases with an increase in the selenium application amount. The low-selenium treatment inhibited increases in Proteobacteria, Acidobacteria, and unidentified bacteria abundance in mature rapeseed soil, while the high-selenium treatment promoted an increase in Proteobacteria abundance. After applying selenium at different stages of rapeseed growth, both the high- and low-selenium treatments had a certain inhibitory effect on certain species of the bacterial top 10. At the same time, it was found that there were differences in the metabolic pathways of the top 10 between different groups in the functional metabolism spectrum of rapeseed microorganisms treated with selenium. The abundance of all eight metabolic pathways in mature rapeseed, except for D-glutamine, D-glutamate metabolism, and streptomycin biosynthesis, first showed a decreasing and then increasing trend. Therefore, selenium treatment in rapeseed soil can alter the composition and metabolic function spectrum of soil microbial communities, ultimately affecting plant growth and Cd tolerance.

There are still some shortcomings in this study. At a later stage, experimental verification will be conducted on differential microorganisms and in-depth exploration will be conducted on the impacts of specific microorganisms on the growth of rapeseed. At the same time, it is necessary to study the effect of selenium on different forms of cadmium in soil and the mechanism of cadmium absorption by rapeseed in order to provide a theoretical basis for cadmium pollution control.

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