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# Exogenous Selenium Endows Salt-Tolerant and Salt-Sensitive Soybeans with Salt Tolerance through Plant-Microbial Coactions

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**Abstract:** Soil salinization is a common abiotic stress that seriously affects soybean growth and yield, underscoring the need to enhance plant salt tolerance for sustainable agriculture development. Selenium is a beneficial element that has been shown to promote plant growth, development and stress resistance. This study employed pot experiments to investigate the effects of different salt levels (0, 50, 100 and 150 mM NaCl) on salt-tolerant (Zhonghuang 13) and salt-sensitive soybean (Dongnong 63) varieties. Additionally, the critical salt concentration (100 mM NaCl) was selected to explore the effects of exogenous selenium (0, 0.5, 1 and 3 mg·kg$^{-1}$) on improving salt tolerance in salt-tolerant and salt-sensitive soybeans under salt stress. Results showed that as salt concentration increased, plant height, shoot and root fresh weight, SPAD value and enzyme activity of both salt-tolerant and salt-sensitive soybeans significantly decreased. The increasing concentration of exogenous selenium significantly decreased the proline content of salt-sensitive and salt-tolerant soybeans by 40.65–58.87% and 38.51–50.46%, respectively, and the MDA content by 19.33–30.36% and 16.94–37.48%, respectively. Selenium supplementation also reduced the content of $Na^+$ in salt-sensitive and salt-tolerant soybeans and improved $K^+$ absorption in soybeans, which increased the $K^+/Na^+$ ratio. Moreover, high-throughput sequencing of the 16S ribosomal RNA gene demonstrated that selenium application optimized the rhizosphere microecology structure of salt-tolerant and salt-sensitive soybean varieties and enhanced functional genes related to lipid metabolism, energy metabolism and cell motility of rhizosphere microorganisms. In summary, selenium application improved the salt tolerance of the two soybean varieties by enhancing the physiological resistance to salt stress and optimizing the structure and function of the rhizosphere microbial community.

**Keywords:** soil salinization; soybean varieties; physiological resistance; rhizosphere microorganisms; microbial community



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## 1. Introduction

According to recent statistics data, approximately 20% of arable land has been affected by salinization [1]. This phenomenon is exacerbated by various factors, including unreasonable irrigation practices in intensive agriculture, excessive use of fertilizers and groundwater depletion [2]. Given the increasing demand for food production and the escalating deterioration of ecological conditions, more sustainable agriculture is becoming necessary [3]. Extracting salt from heavily saline soils is a challenging process that requires significant amounts of time, labor and capital investment [4]. Consequently, the enhancement of crop salt tolerance is emerging as a crucial direction for future agricultural development, which is fundamental to ensuring the sustainable development of agriculture [5]. Primarily, salt stress impairs plant photosynthesis, whereas water shortage and ionic imbalances resulting from osmotic effects may also impact crop growth [6]. Currently,

various methods are being employed to improve crop salt tolerance. These methods include the addition of soil conditioners, such as calcium chloride and boric acid, and the use of biofertilizers containing beneficial bacteria [7,8]. Among them, adding trace elements to the soil is considered an effective approach for enhancing plant adaptability to salt stress [9].

Selenium (Se) is recognized as a crucial plant-beneficial element that plays a crucial role in the physical and biochemical processes of plants [10]. Appropriate levels of Se not only promote plant growth and development but also effectively increase the accumulation of starch and nitrogen utilization in crops, improve the physiological traits of plants and enhance crop yield and quality [11,12]. The improvement of plant salinity tolerance through selenium (Se) application has garnered considerable attention as a potential solution to counteract the adverse effects of salinity stress on crop productivity. Rahman et al. reported that foliar Se application enhances antioxidant defense systems by increasing the activities of key enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). This augmentation leads to efficient scavenging of reactive oxygen species (ROS), thereby mitigating oxidative stress induced by salt [13]. Additionally, it has been reported that soil application of $Na_2Seo_3$ enhanced the up-regulation of genes responsible for $Na^+$ compartmentalization in the maize root system, thereby reducing $Na^+$ content in maize, which are vital for maintaining ion homeostasis and minimizing sodium (Na) accumulation in plant tissues [14]. However, most studies have focused only on the direct effects of Se on plants (changes in plant physiological indicators or gene up-regulation and down-regulation), there are few studies on the modification of plant rhizosphere nutrient cycling and changes in microbial community structure and function through the application of Se to the soil to improve plant salt tolerance.

Soybean is a primary oil crop globally used in various food processing fields [15]. However, soil salinization caused by environmental degradation has adversely impacted soybean quality and yield, posing a serious threat [6]. In saline soil, the redox balance within soybean plants is disrupted, resulting in excessive production of reactive oxygen species and triggering oxidative stress responses that damage cell membranes and organelles [16]. Moreover, the imposition of salt stress disrupts the crucial processes of water and nutrient absorption in the intricate root architecture of soybeans, particularly concerning essential metal cations, thereby precipitating imbalances in nutrient distribution [17,18]. This disruption affects physiological metabolic processes in soybeans, inhibiting photosynthesis, respiration and nitrogen metabolism, thereby limiting plant growth and development [19]. Therefore, improving the salt tolerance of soybeans is crucial to ensuring global food security [20]. Based on the degree of salt tolerance, soybeans can be categorized as salt-sensitive and salt-tolerant varieties. Under salt stress, salt-tolerant soybeans can sequester $Na^+$ from the cytoplasm into vacuoles, thereby enhancing their adaptability to salt stress [21]. Additionally, high-affinity $K^+$ transporters in salt-tolerant soybeans can maintain the $Na^+/K^+$ balance in the plant, supporting normal growth [22]. Furthermore, plants with distinct salt tolerance may have different rhizosphere microbial compositions [4]. Tolerant plants recruit specific microorganisms in the rhizosphere and the dynamic interplay between these microorganisms and plants can facilitate plant growth under abiotic stress. Based on the positive role of Se in improving plant resistance, the hypothesis of this study was that the application of Se could enhance salt tolerance in different salt-tolerant soybean varieties. The specific objectives of this study were (1) to investigate the effect of different salt levels on salt-tolerant and salt-sensitive soybeans; (2) to evaluate the growth-promoting effect of Se application on salt-tolerant and salt-sensitive soybeans under salt stress; and (3) to explore the modulation of Se supplementation on rhizosphere microecology characteristics of salt-tolerant and salt-sensitive soybeans.

## 2. Materials and Methods

### *2.1. Soil Preparation*

The soil was obtained from the yellow-brown soil of the Ecological Research Center, College of Resources and Environment, Huazhong Agricultural University, located at

30°28′35″ N, 114°21′54″ E in Wuhan, China. The soil was collected from a depth of 0–20 cm after removing any floating soil and sieved through a mesh with a diameter of less than 2 mm. The basic physical and chemical properties of the soil are presented in Table 1.

**Table 1.** Chemical properties of the soil used for pot experiments.

| Physicochemical Properties | Value |
|---|---|
| pH | 6.03 |
| electrical conductivity [ms·cm$^{-1}$] | 0.47 |
| Organic matter [g·kg$^{-1}$] | 14.52 |
| Alkaline hydrolyzed nitrogen [mg·kg$^{-1}$] | 34.18 |
| Available P [mg·kg$^{-1}$] | 50.50 |
| Available K [mg·kg$^{-1}$] | 201.41 |
| Total Se [mg·kg$^{-1}$] | 0.090 |
| Available Se [mg·kg$^{-1}$] | 0.073 |

### *2.2. Experiment Designation*

Two soybean varieties, Dongnong 63 (DN) and Zhonghuang 13 (ZH), were obtained from the Soybean Research Institute of Northeast Agricultural University. ZH is a salt-resistant cultivar that is commonly grown in northeastern China, while DN is a salt-sensitive cultivar. Prior to sowing, the seeds of both cultivars were subjected to surface sterilization, involving treatment with 75% (*v*/*v*) ethanol for 50 s followed by 3% sodium hypochlorite for 30 min and finally rinsed three times with sterile deionized water. After undergoing the aforementioned sterilization process, the soybean seeds were planted in plastic pots filled with vermiculite to facilitate germination. The sowing density of each pot was maintained at 50 seeds. Ten days after germination, two seedlings of comparable growth potential were transferred into each pot.

The pot experiment was carried out in the greenhouse of the College of Resources and Environment, Huazhong Agricultural University. Uniform seedlings were transplanted into plastic pots (15 cm diameter, 12 cm height), each pot containing 1.5 kg of air-dried and sieved soil. Before sowing, basal macroelement fertilizers consisting of 0.1 g·kg$^{-1}$ N as $CH_4N_2O$, 0.34 g·kg$^{-1}$ P as $NH_4H_2PO_4$ and 0.12 g·kg$^{-1}$ K as $K_2SO_4$ were incorporated into the soil. Additionally, microelement fertilizers (0.025 mg Fe-EDTA, 1.81 mg $MnCl_2{\cdot}4H_2O$, 0.08 mg $CuSO_4{\cdot}5H_2O$, 0.22 mg $ZnSO_4{\cdot}7H_2O$ and 2.86 mg $H_3BO_3$ per kg of soil) were applied to the soil together.

Two experiments were conducted in this study (Figure 1A,B). Experiment 1 aimed to investigate the physiological responses and soil fertility changes of two soybean varieties under different levels of salt stress. The experiment included two soybean varieties (DN and ZH) and four salt levels (0, 50, 100 and 150 mM NaCl). A total of eight treatments (DNT0, DNT1, DNT2, DNT3, ZHT0, ZHT1, ZHT2, ZHT3) were established. Specifically, the seedlings transplanted into pots initiated the salt stress treatment one week after the commencement of their growth. A plastic tray was placed under each pot to prevent the loss of the salt solution. Every three days, 200 mL of salt solution with varying concentrations was poured into the pots. The soybeans were weighed and rehydrated on a daily basis. During rehydration, the salt grains on the tray were rinsed and subsequently poured into the basin to prevent the loss of salt. The soybeans in the control group were irrigated with an equivalent volume of deionized water and each treatment was replicated four times. The soybean plants and rhizosphere soil samples were collected 30 days after salt treatment for the determination of subsequent indicators.

Experiment 2 aimed to explore the impact of Se on the salt tolerance of two soybean varieties under salt stress. The two soybean varieties, DN and ZH, were selected as test subjects. Based on the results of Experiment 1, 100 mM NaCl was selected as the salt concentration. Before transplanting, different concentrations of Se solutions (0, 0.5, 1.0 and 3.0 mg kg$^{-1}$, Se as $Na_2SeO_3$) were applied to the soil along with basal fertilizer. The salt stress treatment details were the same as in the choice procedure used in Experiment

1 (above). Each treatment was replicated four times. The soybeans were harvested after 30 days of transplanting and samples of the rhizosphere soil were collected. After determining the physical and chemical indicators, three representative treatments (treatment without Se addition in normal soil, treatment without Se addition in salt stress soil and treatment with Se addition in salt stress soil) of the two soybean varieties were selected for microbiome analysis. The composition of the soil bacterial community was characterized using 16S rRNA gene sequencing. All experiments were carried out in a greenhouse with the environmental conditions set as: average temperature 26 °C/22 °C (day/night, 14/10 h), relative humidity 60% and light intensity 8000 lx.

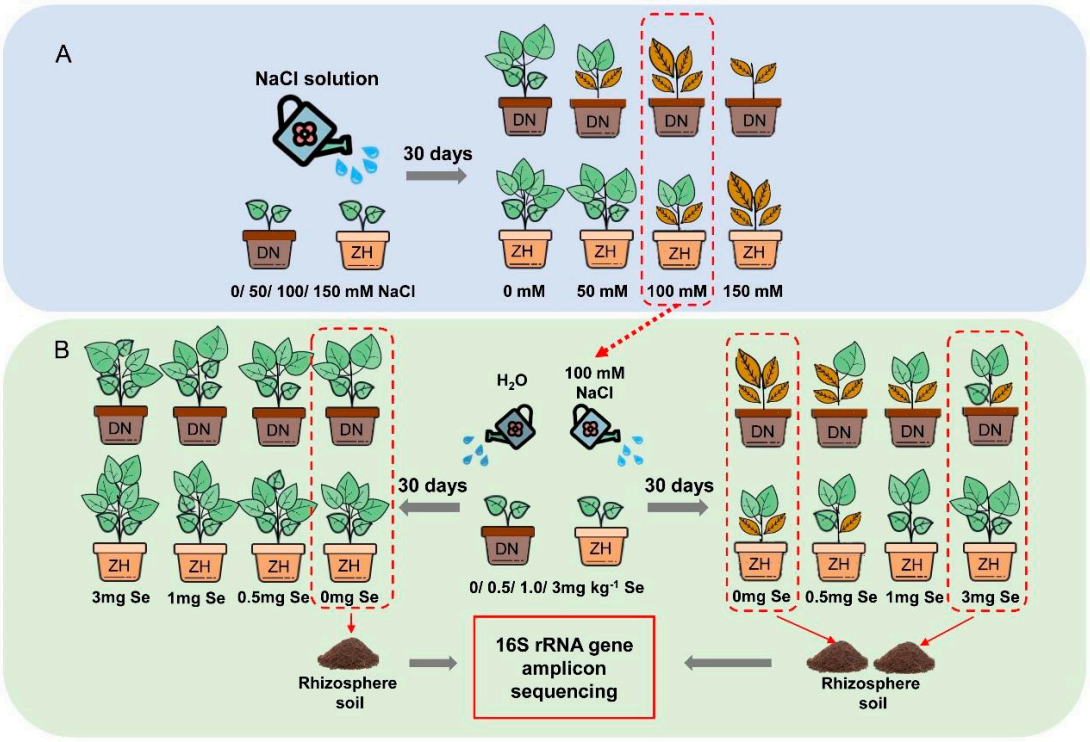


**Figure 1.** The design of the experiments for this study. (**A**) Effects of Salt Stress on Salt Sensitive and Salt Tolerant Soybean. (**B**) Growth-promoting effect of Se on salt-tolerant and salt-sensitive soybean under no-salt and salt stress. DN was salt-sensitive soybean, ZH was salt-tolerant soybean. Four replicates were performed for each experimental treatment.

### *2.3. Sample Collection*

The plant samples of the two experiments were rinsed four times with deionized water after collection and divided into the shoot and root parts for the determination of physiological indicators. The soil was collected before planting for analysis of basic soil physical and chemical properties. To collect the rhizosphere soil from Experiment 1 and 2, the soybean roots were manually shaken to remove excess soil and the remaining soil attached to the roots (1 mm) was collected and stored at −80 °C until use.

### *2.4. Physical and Chemical Indexes Analysis*

The pH value and electrical conductivity of the soil were measured with a pH meter and electrical conductivity meter and the soil–water ratio was 1:5. The soil organic matter was determined as described by Yeomans et al. [23]. Soil alkaline hydrolyzed nitrogen was determined using the alkaline solution diffusion method [24]. The available phosphorus and potassium in the soil were determined as described by Cai et al. [11]. Before sampling, the chlorophyll content of the leaves was measured with SPAD-502Plus (Konica Minolta, Tokyo, Japan). The proline content in the soybean leaves was determined according to the method described previously [25]. Proline was extracted from fresh plant leaves with

3% sulfosalicylic acid. After stratification, the content of proline in the toluene layer was measured with a spectrophotometer at 520 nm. The MDA content in fresh leaves of soybean was determined according to the method described by Heath et al. [26]. The sodium and potassium content in plants were determined using atomic absorption spectrometry (AAS). Plant tissues were subjected to nitric perchloric acid digestion and determination of potassium and sodium at 766.5 nm and 589 nm, respectively.

### *2.5. Determination of Soil Enzyme Activity*

The determination of acid phosphatase, catalase, sucrase and urease activities in the rhizosphere soil was based on the previous method [11,27]. For the determination of soil acid phosphatase activity, soil samples were extracted with toluene for 15 min, followed by the addition of 10 mL of disodium phenylphosphate solution and acetate buffer and incubated at 37 °C for 3 h. After filtration, 0.5 mL of 2% 4-aminoantipyrine and 0.5 mL of 8% potassium ferricyanide were added and the absorbance was measured at 510 nm. The results of the acid phosphatase activity were expressed as the content of $P_2O_5$ per 1 g soil sample.

For the determination of soil catalase activity, 5 g of soil sample was weighed and 40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide solution were added. After shaking for 30 min, 5 mL 1.5 mol/L $H_2SO_4$ was added to terminate the reaction and the mixture was then filtered. It was titrated with 0.002 mol/L potassium permanganate solution until reddish, the reading was recorded and the activity of catalase was further calculated. The results of catalase activity were expressed by the titrated $K_2MnO_4$ content per gram of soil sample.

For the determination of soil sucrase activity, 1 g of soil sample was placed in a 50 mL conical flask, 15 mL of 8% sucrose solution was added followed by 5 mL of phosphate buffer and toluene, it was shaken well and stabilized at 37 °C for 24 h. After filtering, 3 mL 3,5-dinitrosalicylic acid was added and the mixture was heated in a boiling water bath for 5 min. The obtained samples were colorimetrically measured at a wavelength of 508 nm. The results of the sucrase activity were expressed by the glucose concentration produced.

For the determination of soil urease activity, a 2 g soil sample was weighed and 1 mL phenol was added to stabilize for 15 min. Then, 10 mL of 10% urea and 20 mL of citrate buffer were added, mixed thoroughly and then stabilized at 37 °C for 24 h. After filtering, 3 mL of sodium hypochlorite solution was added and colorimetrically measured at 578 nm. The results of the urease activity were expressed as the content of $NH_3$-N per gram of soil sample. All chemical reagents used in the experiment were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

### *2.6. DNA Extraction and 16 S rRNA Sequencing*

The total genomic DNA in soybean rhizosphere soil samples was extracted using the MogaBio soil/Feces genomic DNA Purification Kit (Bioer, Hangzhou, China). The DNA quality and quantity were determined using the NanoDropTM One Spectrophotometer. Universal primers (515 F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and 806 R: 5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3-V4 region of the 16S rRNA gene. The samples were submitted to Magigene Biotechnology Co., Ltd. (Guangzhou, China) for library preparation and using the Illumina Nova 6000 platform for sequencing.

### *2.7. Microbiome Analysis*

The α and β diversity among treatments were calculated using QIIME2 software (https://view.qiime2.org, accessed on 1 March 2023) and visualized using the OmicStudio online analysis network (https://www.omicstudio.cn, accessed on 1 March 2023). OTU abundance tables were normalized using PICRUSt and the influence of the copy number of the 16S marker gene in the species genome was removed. Subsequently, the KEGG Ortholog (KO) information corresponding to the OTU was obtained from the Greengene

ID and the corresponding abundance was calculated according to the OTU table. The microbiome data were visualized using Origin (version 9.65) and R (version 4.2.3).

### *2.8. Statistical Analysis*

A two-way analysis of variance (ANOVA) was used to examine the differences in plant properties and rhizosphere soil enzyme activity with genotype and salinity or Se concentration as fixed effects. The effect of salinity or Se concentration on a particular genotype was determined using a one-way ANOVA. Differences were considered significant at the level of $p < 0.05$. The standard error of the mean was shown throughout the paper ($n = 4$). All data obtained from the experiment were analyzed using SPSS 26.0.

## 3. Results

### *3.1. Effects of Salt Stress on Growth of Soybean*

Salt stress and genotype had significant effects on plant height, fresh weight of shoot, fresh weight of root and SPAD values of the two varieties of soybean (Figure 2). These growth indicators exhibited the most significant decreases at 150 mM NaCl compared to other salt stress. Under different salt stress, the average decreases of DN and ZH were 21.21–59.03% and 12.98–35.48% for plant height, 12.75–69.07% and 7.84–49.94% for fresh weight of root, as well as 13.77–43.65% and 7.84–49.94% for fresh weight of shoot, respectively. SPAD values of both soybean species showed an opposite trend to the increase in salt stress. The minimum SPAD values were observed for DN (12.6 ± 0.82) and ZH (21.35 ± 2.02) under high salt stress. Overall, the growth inhibitory effect of salt stress on DN was higher than that of ZH. Moreover, ZH could maintain a certain level of growth even when the salt stress reached 150 mM NaCl, while DN exhibited severe growth inhibition.

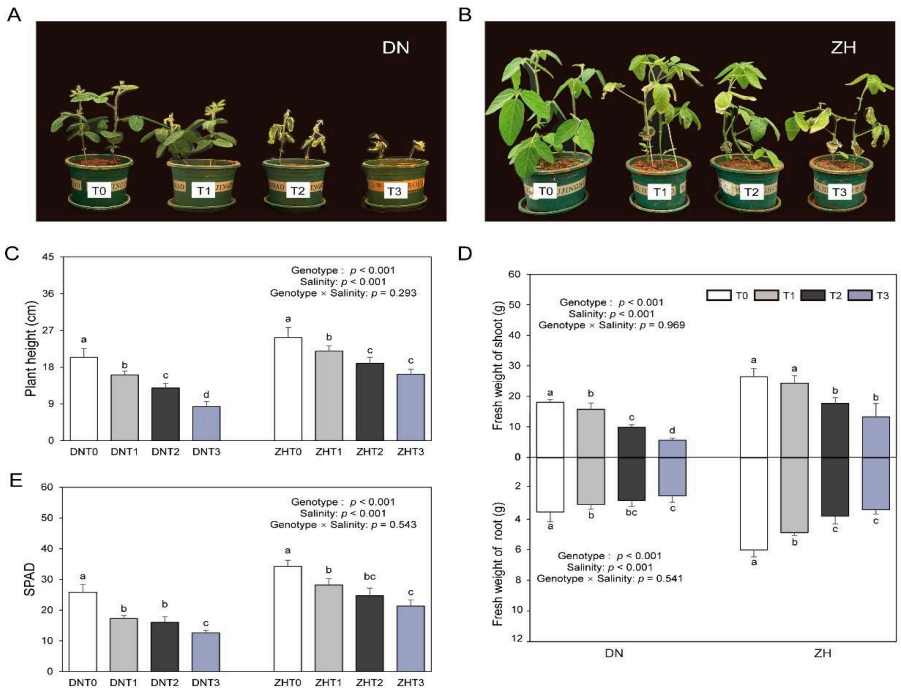


**Figure 2.** Effects of salinity levels on the growth of two varieties of soybean. (**A**,**B**) Soybean under different NaCl concentrations after 30 days of growth. (**C**) Plant height. (**D**) Fresh weight. (**E**) SPAD value. T0–T3 were different salt concentration levels. T0, 0 mM NaCl; T1 50 mM NaCl; T2 100 mM NaCl; T3, 150 mM NaCl (the same below). Data represents means ± the standard error (SE) ($n = 4$). The different lowercase letters indicate significant differences among different treatments at the $p < 0.05$ level.

### *3.2. Effects of Salt Stress on Rhizosphere Soil Enzyme Activities of Soybean*

Figure 3 illustrates that ZH rhizosphere soil enzyme activities were higher than DN under each treatment condition. With an increase in salt concentration, the rhizosphere soil urease, sucrase, catalase and acid phosphatase activities of both soybean varieties showed a certain degree of decrease. Notably, salt stress had the most pronounced effect on the urease activities of both soybean varieties. At 150 mM NaCl, DN and ZH urease activities decreased by 63.72% and 40.48%, respectively. Furthermore, except for peroxidase activity, the decrease in soil urease, sucrase and acid phosphatase activities of DN rhizosphere soil were higher than that of ZH when the salt concentration reached 150 mM NaCl.

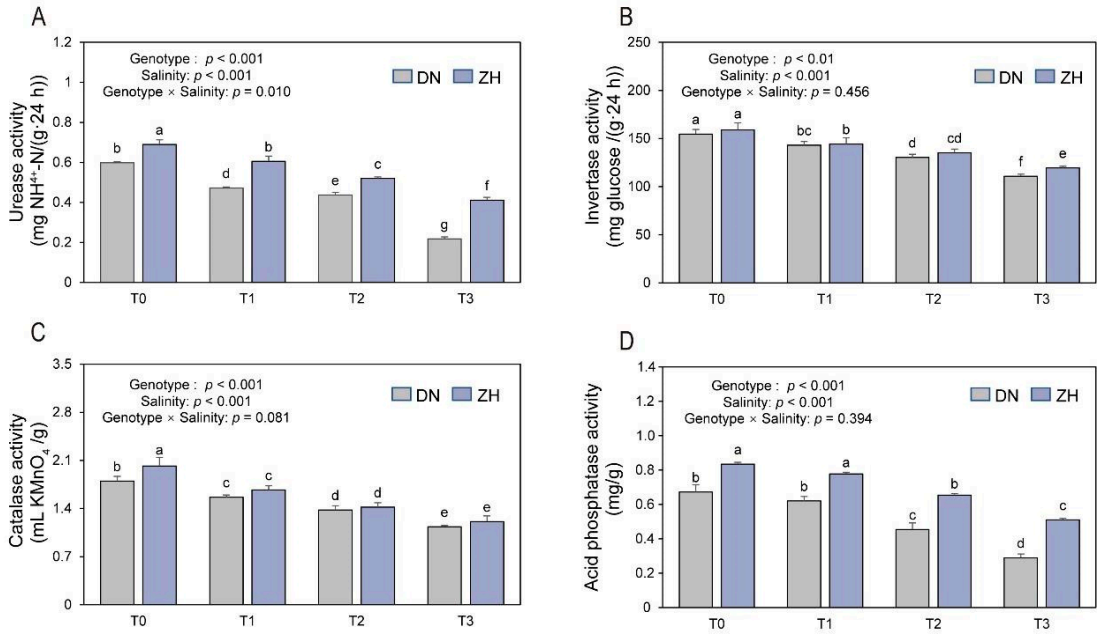


**Figure 3.** Effects of salinity levels on enzyme activities of two varieties of soybean rhizosphere soil. (**A**) Urease activity. (**B**) Sucrase activity. (**C**) Catalase activity. (**D**) Acid phosphatase activity. Data represents means ± the standard error (SE) ($n$ = 4). The various letters indicate significant differences among different treatments at the $p < 0.05$ level.

### *3.3. Effects of Se on Growth of Soybean*

To assess the impact of exogenous Se on two soybean varieties under salt stress, we analyzed various physiological parameters of soybeans (Figure 4). The application of Se exhibited a consistent growth-promoting trend in both soybean varieties. In response to the increasing Se concentration, both soybean varieties demonstrated varying degrees of improvement in plant height, SPAD value and fresh weight of shoot and root. The most significant growth-promoting effect was observed at a Se concentration of 3 mg·kg$^{-1}$. The DN variety exhibited a 21.16% increase in plant height, a 23.03% increase in the fresh weight of the shoot, an 11.44% increase in the fresh weight of the root and a 37.79% increase in SPAD value under salt stress. Similarly, the ZH variety displayed respective increases of 17.20%, 18.33%, 21.26% and 36.55% under these parameters. However, it is worth noting that the Se application did not change the root/shoot ratio of the two soybean varieties.

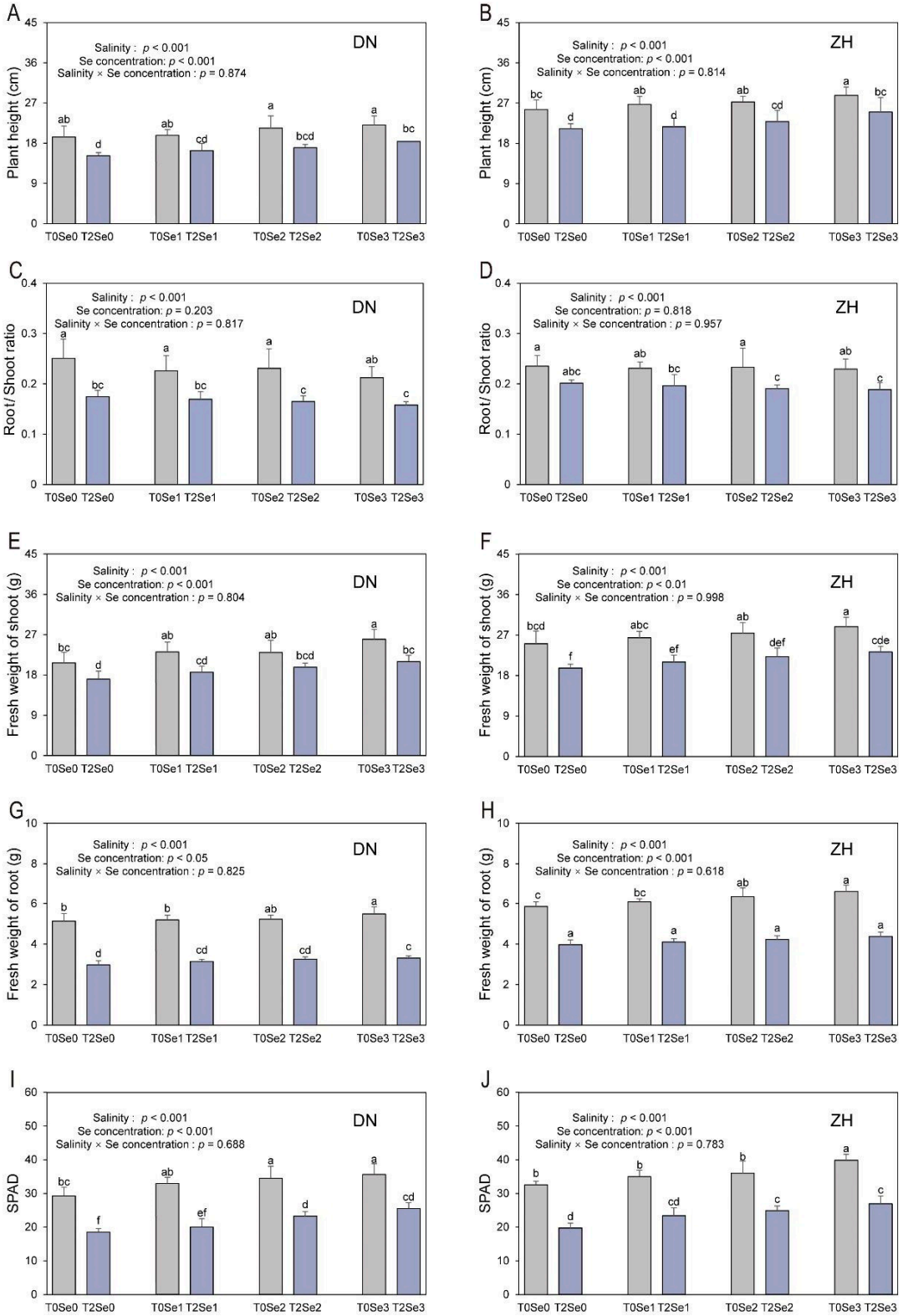


**Figure 4.** Effects of Se level and salinity level on the growth of two varieties of soybean. (**A**,**B**) Plant height. (**C**,**D**) Root/Shoot ratio. (**E**,**F**) Fresh weight of shoot. (**G**,**H**) Fresh weight of root. (**I**,**J**) SPAD value. Se0, 0 mg·kg$^{-1}$ Se; Se1, 0.5 mg·kg$^{-1}$ Se; Se2, 1mg·kg$^{-1}$ Se; Se3, 3 mg·kg$^{-1}$ Se (the same below). Data represents means ± the standard error (SE) ($n$ = 4). The various letters indicate significant differences among different treatments at the $p < 0.05$ level.

### *3.4. Effects of Se on Soybean Chemical Indexes under Salt Stress*

To further investigate the effect of Se on the two varieties of soybean under salt stress, we investigated a series of physiological stress resistance indicators of DN and ZH. For the osmoregulation of the two soybean varieties, the application of Se significantly decreased the proline content (Figure 5A). As the Se concentration increased, the proline content of DN and ZH decreased by 40.65–58.87% and 38.51–50.46%, respectively. Furthermore, the MDA content in DN and ZH decreased by 19.33–30.36% and 16.94–37.48%, respectively (Figure 5B). Moreover, Se application significantly increased the $K^+$ content of DN and ZH and decreased the excessive intake of $Na^+$ under salt stress, which increased the $K^+/Na^+$ ratio of DN (20.02–34.31%) and ZH (6.09–34.61) (Figure 5C–E).

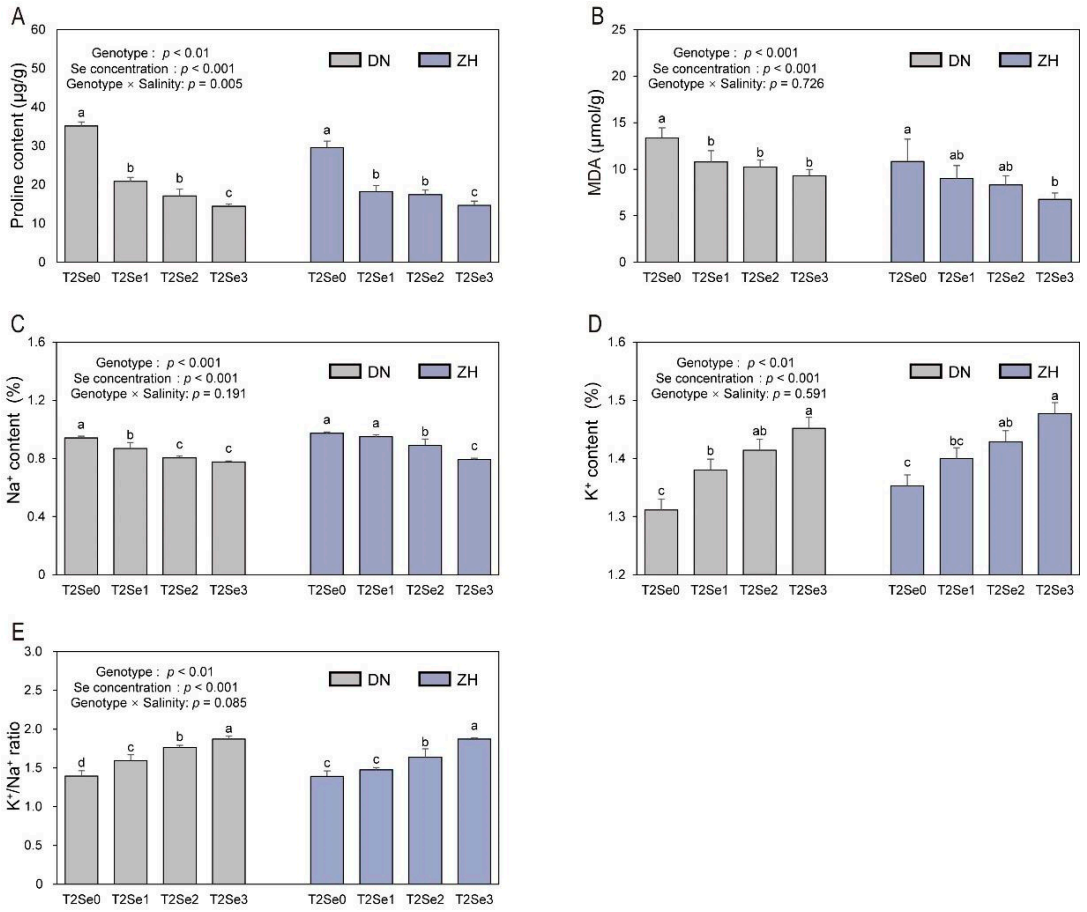


**Figure 5.** Effect of Se levels and salinity levels on the physiological parameters of the two varieties of soybean. (**A**) proline content, (**B**) MDA content, (**C**) $Na^+$ content, (**D**) $K^+$ content, (**E**) $K^+/Na^+$ ratio. Data represents means ± the standard error (SE) ($n$ = 4). The various letters indicate significant differences among different treatments at the $p < 0.05$ level.

### *3.5. Effects of Se on Rhizosphere Microecology of Soybean under Salt Stress*

To characterize the changes of Se on the rhizosphere microbial community of two soybean varieties, the samples of rhizosphere soil from three treatments of two soybean varieties (T0Se0, T2Se0, T2Se3) were collected for 16S rRNA sequencing. For the DN soybean cultivar, OTUs enriched accounted for 16.42% (529 out of 3221 OTUs) in T0Se0, 14.90% (466 out of 3128 OTUs) in T2Se0 and 11.06% (331 out of 2992 OTUs) in T2Se3 (Figure 6A), respectively. For soybean cultivar ZH, OTUs enriched accounted for 15.23% (518 out of 3402 OTUs) in T0Se0, 11.61% (372 out of 3205 OTUs) in T2Se0 and 12.56% (418 out of 3329 OTUs) in T2Se3 (Figure 6B), respectively. Under salt stress, the addition of exogenous Se resulted in a higher Simpson index of DN compared to treatment without Se addition, while ZH showed the opposite trend (Figure 6C). PCA was applied to compare

the changes in the rhizosphere microbial community between the different treatments, which clearly showed that the rhizosphere soil microbial communities under salt stress and Se application were significantly separated from the control treatment along PC1, accounted for 30.6% of the total variance (Figure 6D).

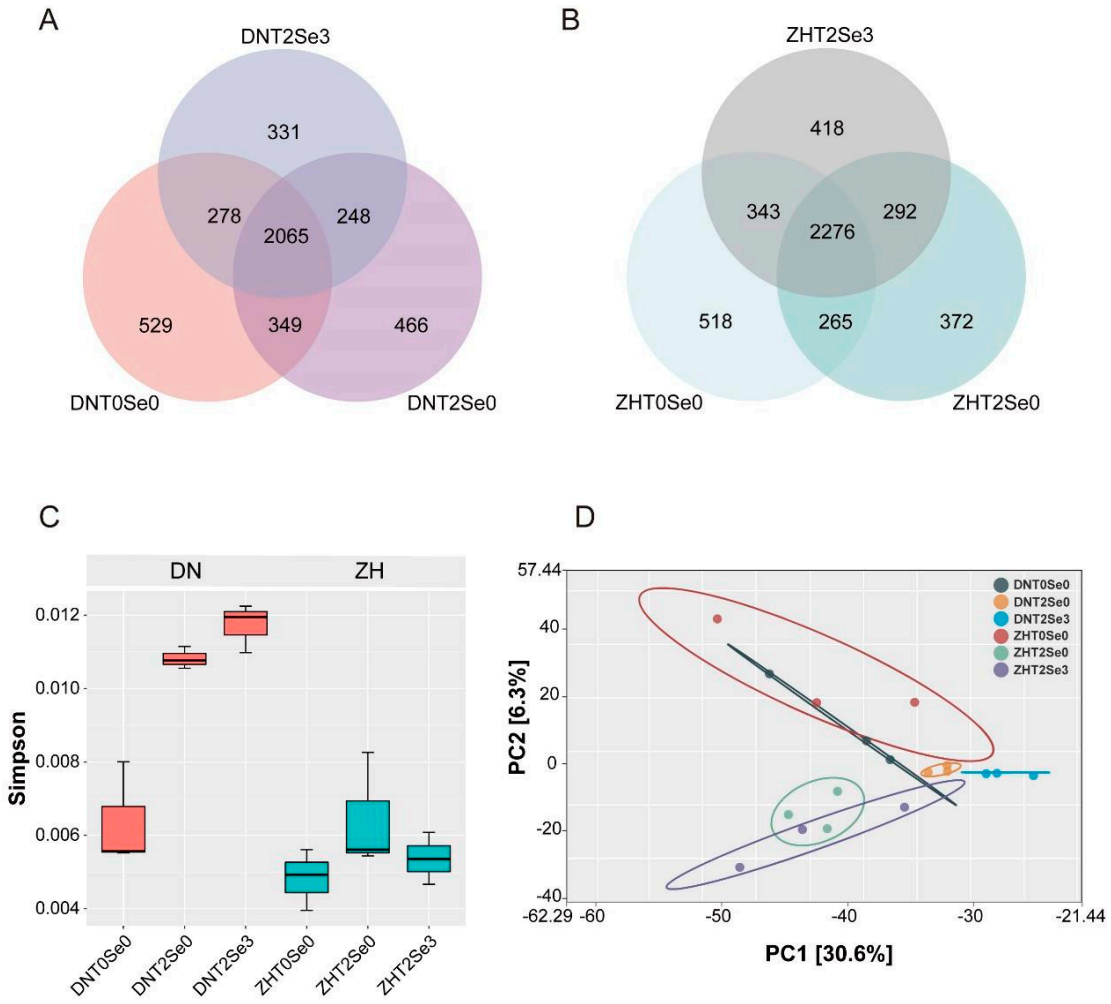


**Figure 6.** Effects of different treatments on enriched OTUs and diversity of rhizosphere bacterial community. (**A**,**B**) Venn diagrams showing the unique and shared OTUs of different treatment groups. (**C**) Evaluation of the microflora diversity of different treatment by Simpson index. (**D**) Principal component analysis of rhizosphere microorganisms in different treatments.

To explore the changes in the community structure of the two soybean varieties under salt stress and Se application, the relative abundances of dominant microorganisms of the top fifteen phyla and fifteen genera were selected (Figure 7A,B). Notably, the genus *Burkholderia* and *Noviherbaspirillum* were significantly enriched in the rhizosphere of soybean cultivar DN after Se application, while the genus *Dyella* and *Sphingomonas* were significantly enriched in the rhizosphere of soybean cultivar ZH. In summary, these results show that Se application remarkably impacted the diversity and structure of the rhizosphere microbial communities in soybean varieties DN and ZH.

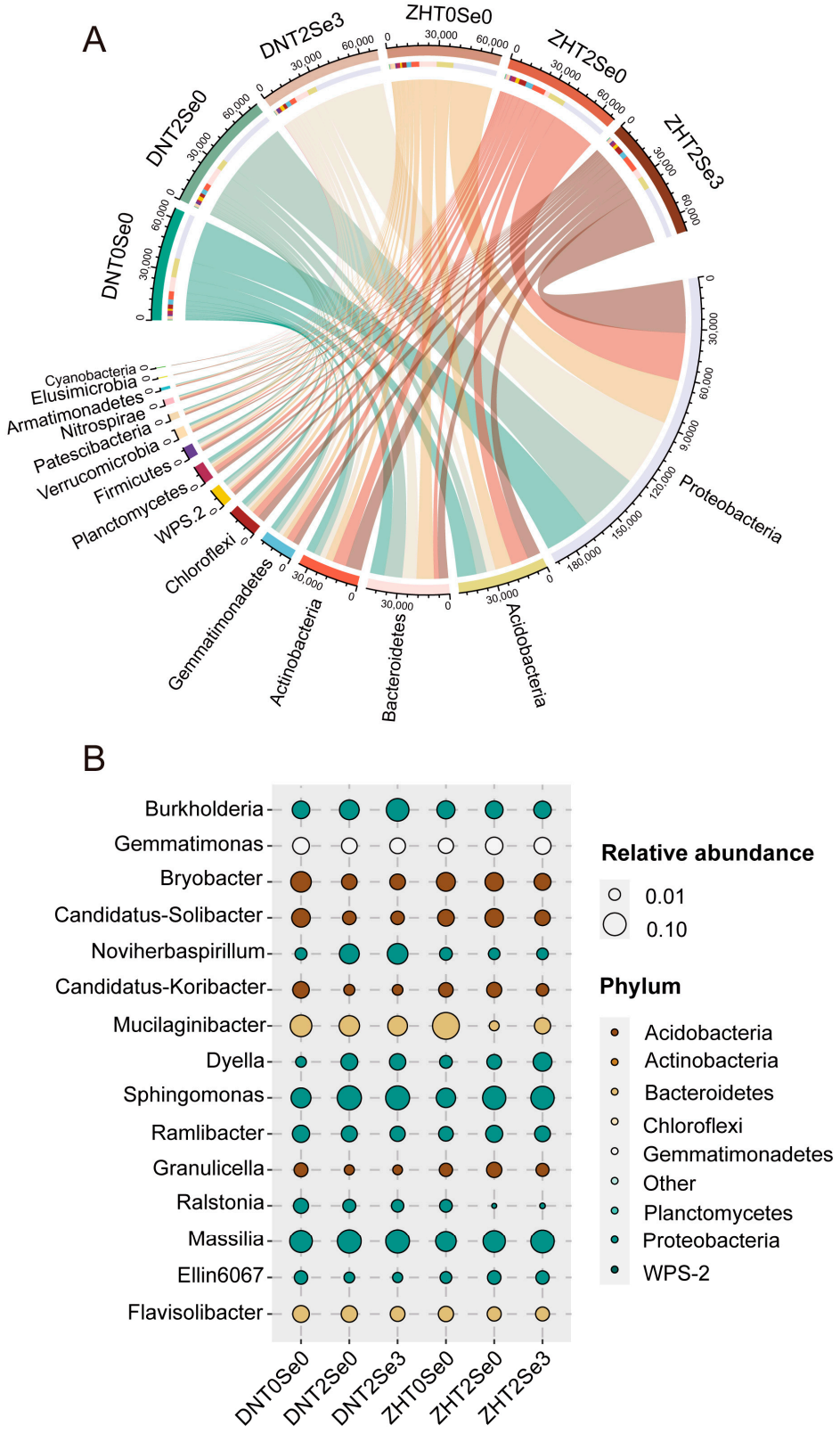


**Figure 7.** Rhizosphere microbial community structure under different treatments. (**A**) Relative abundance of the top 15 most abundant bacterial phyla. (**B**) Distribution and abundance of rhizosphere bacterial community compositions at the genus level.

### *3.6. Effects of Se on Rhizosphere Microorganisms Functions of Soybean under Salt Stress*

KEGG cluster analysis revealed significant differences in functional abundance between the two soybean varieties rhizosphere microbes under different treatments. In KEGG level 2, the metabolic pathways related to amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, nucleotide metabolism, carbohydrate metabolism and lipid metabolism were upregulated in the rhizosphere microorganisms of two soybean varieties under salt stress. Meanwhile, these metabolic pathways were further upregulated after Se application (Figure 8A). Specifically, pathways related to the synthesis and degradation of ketone bodies, C5-Branched dibasic acid metabolism, aminoacyl-tRNA biosynthesis, citrate cycle-TCA cycle, amino acid metabolism and selenocompound metabolism in the rhizosphere microorganisms of two soybean varieties were significantly enriched after Se application under salt stress (Figure 8B).

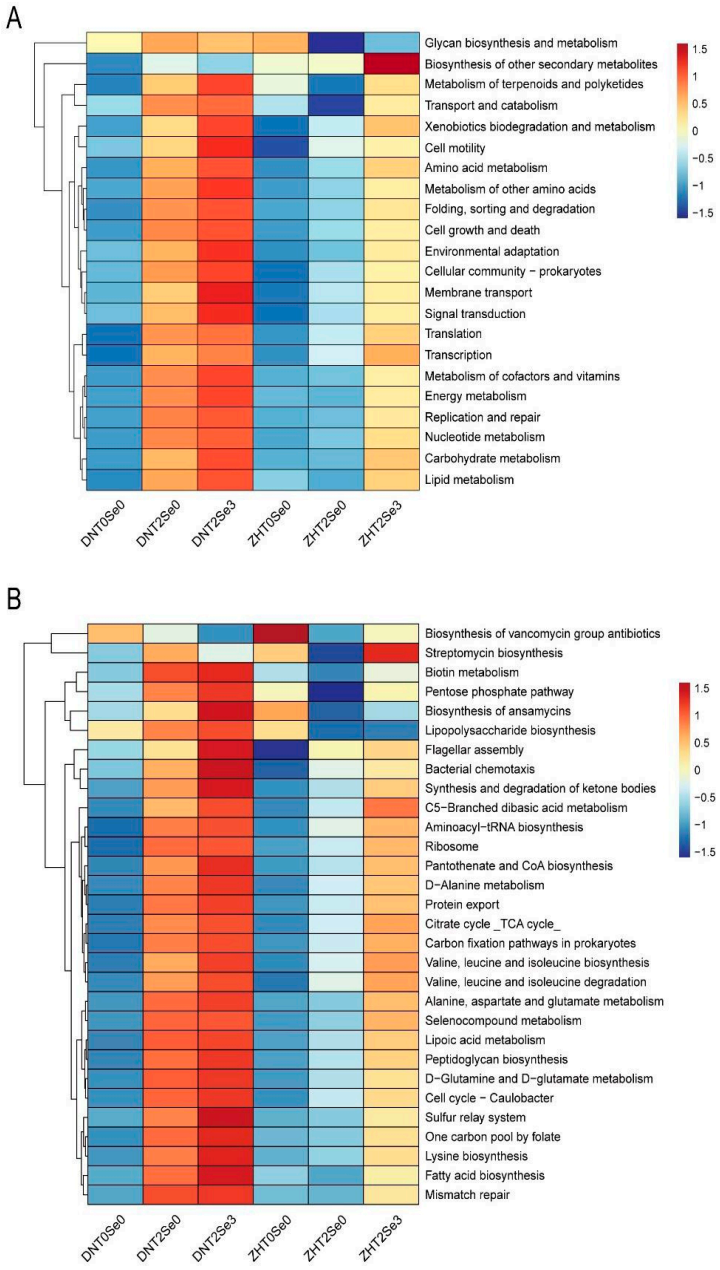


**Figure 8.** The heatmap of clustering for KEGG pathways under different treatments. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway functions were categorized per level. (**A**) KEGG pathway level 2. (**B**) KEGG pathway level 3.

The KEGG functional prediction analysis of the metabolic function changes in different treatments using STAMP analysis showed that bacterial chemotaxis and ABC transporters, D-arginine and D-ornithine metabolism, chlorocyclohexane and chlorobenzene degradation and chloroalkane and chloroalkene degradation and biosynthesis of other secondary metabolites in soybean varieties in DN rhizosphere microorganisms were significantly upregulated after Se application. However, the other glycan degradation, drug metabolism-other enzymes, glycolysis/gluconeogenesis and sulfur metabolism of soybean variety ZH rhizosphere microorganisms were significantly upregulated after Se application (Figure 9A,B).

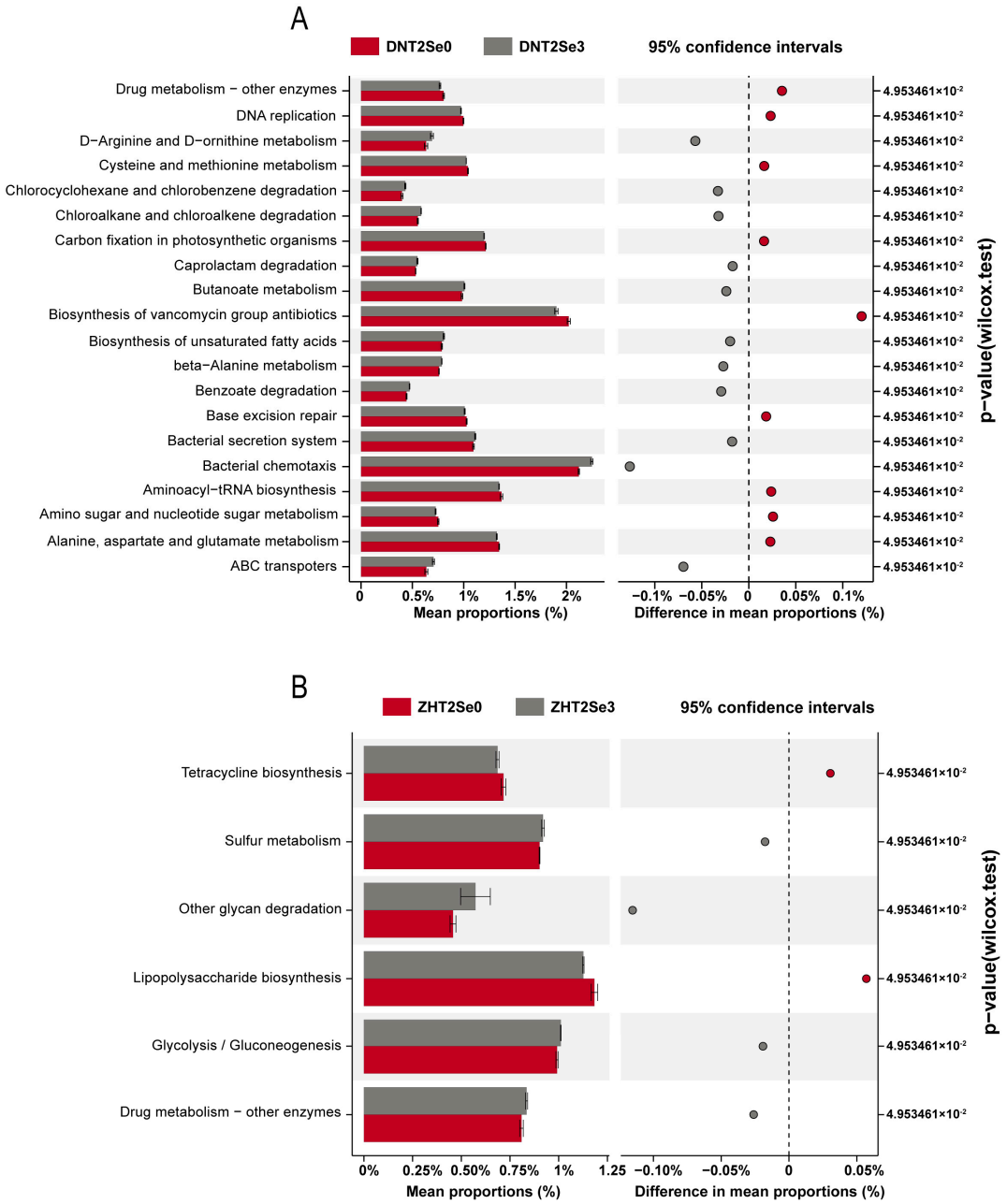


**Figure 9.** Difference analysis of functional genes of rhizosphere microorganisms under different treatments. (**A**) Soybean Variety DN. (**B**) Soybean Variety ZH. The bar plot showed the difference in the mean proportion of functional genes in rhizosphere microorganisms between the two groups. The error bars shown the 95% confidence interval for this effect size. Only $p$ value < 0.05 after Storey's FDR correction were shown (Welch's t-test rank sum test).

## 4. Discussion

The vegetative growth stage of soybeans is particularly susceptible to salinity [28]. Findings in this study indicated that soybean plants experience a significant reduction in plant height, SPAD value and fresh weight of shoot and root as salt stress intensifies (Figure 2). Moreover, the results demonstrated that the ZH variety had higher salt tolerance than the DN variety under salt stress. Specifically, at 150 mM NaCl, the tolerance threshold of DN is exceeded, resulting in yellowing, wilting, poor growth and eventual death of the plants. In contrast, ZH maintained normal growth even at 150 mM NaCl.

Furthermore, salt stress exerts a significant impact on the biological activities of soil microbes, as salt-stress-induced massive microbial consumption compels microorganisms to shift from growth strategies to survival strategies [29]. Soil urease, sucrase, acid phosphatase and catalase are microbial-produced enzymes whose activities are closely linked to soil nutrient cycling and they can assess the overall health of soil [30]. The rhizosphere is the closely associated soil surrounding the roots of plants, provides nutrients and energy for plant roots and its physiological indicators play a crucial role in plant health [31,32]. In this study, rhizosphere soil enzyme activities of the two soybean varieties decreased with the increase in salt stress (Figure 3). In comparison to the sharp decline observed in soil urease and acid phosphatase activities, the activities of sucrase and catalase in the rhizosphere soils of the two soybean varieties responded more moderately to salt stress. This difference can be explained by the fact that salt stress changed the community structure of soybean symbiotic rhizobia by reducing the activity of soybean symbiotic rhizobia, which reduced the nitrogen content fixed by the rhizobia in soil [33]. Soil nitrogen content directly influenced the activities of urease and acid phosphatase in soybean rhizosphere soil, which resulted in a significant decrease in urease and acid phosphatase activities under salt stress [34–36].

Se application has been shown to alleviate the negative impacts of salt stress on plants, resulting in improved plant growth parameters and physiological responses [37]. In this study, the root/shoot ratio of the two soybean varieties did not change significantly at different Se levels. However, the fresh weight of both shoot and root increased for both soybean varieties. Furthermore, exogenous Se promoted the growth of both varieties of soybean and increased the plant height and SPAD value significantly, indicating that the accumulation of organic matter was improved, a phenomenon that strongly demonstrates the mitigation of salt stress in both varieties of soybean (Figure 4).

In addition, salt-stress-induced osmotic stress, ionic stress, nutrient imbalance, disruption of ionic balance and excessive production of ROS threaten the antioxidant defense system of plants [14]. Among them, the excessive accumulation of ROS results in membrane lipid peroxidation, which ultimately leads to plant cell death. As an index to evaluate the degree of plant cell membrane damage, MDA can be used to measure the damage caused by oxidized lipids due to stress [38,39]. A low concentration of Se can eliminate ROS in plants, prevent plants from oxidative stress by scavenging free radicals in cells and activate related enzyme activities [40]. In this study, Se application significantly reduced the MDA content in plants, which prevented cell membrane damage under salt stress and protected the safety of the two soybean varieties' cells (Figure 5B). Proline is an essential amino acid in plants that can maintain plant metabolism under abiotic stress [41]. The proline synthesized through the glutamate pathway helps maintain the redox balance of plants under salt stress [42]. The introduction of exogenous Se alleviated the osmotic stress caused by high salt in the roots of plants and reduced the proline content in plants (Figure 5A), which is consistent with the study result of Hussain and Yao et al. [43,44].

Salinity-induced $Na^+$ accumulation and $K^+$ uptake suppression lead to ionic imbalance. K serves not only as a cofactor for numerous enzymes in plants but also as a key ion regulating plant cellular homeostasis, influencing the opening and closing of leaf stomata and governing photosynthesis [45,46]. Se application has been demonstrated to enhance potassium uptake in plants under salt stress [47]. In addition, the application of Se contributes to the regulation of ion transporters and channels in plant roots, promoting the

uptake and translocation of potassium ions. This improved potassium uptake supports plant growth and helps mitigate the negative effects of salt stress [6]. Additionally, the application of Se has been reported to decrease sodium uptake in plants exposed to salt stress. Se regulates ion transporters, such as sodium/proton antiporters, and restricts sodium entry into plant cells. By reducing sodium uptake, Se contributes to the maintenance of ion homeostasis and prevents sodium toxicity in plant tissues [48]. Therefore, reducing the $Na^+$ content in plants and maintaining the $K^+$ content in cells is crucial for the normal functioning of key plant processes. The findings of this experiment suggested that exogenous Se significantly reduced the excessive uptake of $Na^+$ by plant roots, preventing excess $Na^+$ from competing with $K^+$ for binding sites and thereby maintaining a high intracellular $K^+/Na^+$ ratio to achieve intracellular ion homeostasis (Figure 5C–E).

To explore the impact of Se application on the rhizosphere microecology of two soybean varieties, we employed 16S rRNA sequencing to analyze the rhizosphere microorganisms. Our findings revealed significant variations in the soybean rhizosphere microbial community among different treatments. Notably, compared to the reduction in rhizosphere microbial abundance observed in the soybean variety DN, the soybean variety ZH exhibited an increase in rhizosphere microbial abundance (Figure 6A–D). The result was probably due to the fact that salt-tolerant soybeans maintain a stable and diverse microbial community under salt stress, while salt-sensitive soybeans experience disruptions, leading to reduced diversity and specific populations [49]. The exogenous Se effect on the microbial community depends on the soybean varieties and microbial mechanisms. Se application has a positive effect on salt-tolerant soybeans, restoring stability, increasing specific microbes and enhancing diversity. However, salt-sensitive soybeans may experience an altered community structure, inhibiting certain populations and impacting abundance and diversity [50]. The interaction of Se with soybean physiology further perturbs microbial community stability [51].

Soil microbes influence soil nutrient cycling, decomposition processes and plant health by modifying their metabolic functions [52]. In the present study, pathways related to cell metabolism and genetic information processing appeared significantly increased under salt stress in both salt-sensitive and salt-tolerant varieties of soybean (Figure 8A). In particular, the up-regulation range of salt-sensitive soybean DN was significantly higher than that of the salt-tolerant soybean ZH under salt stress. The reason for this may be that the diversity of the salt-tolerant soybean rhizosphere microorganisms promotes community stability, which improves functional resilience [53]. Salt stress results in a significant decrease in the abundance of rhizosphere microorganisms in salt-sensitive the soybean DN and drastic changes in the rhizosphere microecology led to drastic changes in microbial functions, which showed a stronger response than the salt-tolerant soybean ZH. This finding is consistent with most previous studies [54,55]. Root exudates were considered a trigger for microbial chemotactic colonization [56]. Exogenous Se can improve the rhizosphere microecology structure of plants and promote the synthesis of organic acids in plant root exudates [57]. Malic acid and citric acid among organic acids were demonstrated to act as sources and chemoattractants for rhizosphere microbes, thereby assisting plants to recruit microorganisms in the rhizosphere [58]. Therefore, in this study, Se application affected the prediction results of the rhizosphere microbial functions by altering the structure of the rhizosphere microbial community of the two soybean varieties. These functional genes responsible for nutrient acquisition and hormone balance can promote plant growth or protect plants against abiotic stress, specifically including functional genes responsible for nutrient exchange and signal transduction between plants and microorganisms such as ABC transporters, or genes related to bacterial chemotaxis, bacterial motility and biofilm formation (Figure 9A,B). Alterations in these functional genes explain the increased salt tolerance in both varieties of soybean after Se application from a microbial perspective.

## 5. Conclusions

Salt stress elicited inhibitory effects on both soybean variety growth and rhizosphere soil enzyme activity. Exogenous Se could improve the physiological conditions by altering physicochemical properties and maintaining the $K^+/Na^+$ ratio in two varieties of soybean by reducing the excess intake of $Na^+$, which promotes the growth and tolerance to salt stress of two soybean varieties. Furthermore, exogenous Se regulated the structure of the rhizosphere microbial community and enhanced the abundance of functional genes associated with nutrient acquisition and hormone balance, thereby synergistically mitigating the salt-stress-induced damage.

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