

Combined Foliar and Soil Selenium Fertilizer Improves Selenium Transport in Oats and the Diversity of the Bacterial Community in Rhizosphere Soil

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Abstract

Reasonable application of selenium (Se) fertilizer is beneficial for improving Se contents in grains and can affect soil ecology. No study has compared Se fertilizer application methods on biofortification, yield, and soil bacterial community. This study investigated the effects of topsoil (T), foliar (S), and soil+foliar (TS) application of Se fertilizer on oats. TS treatment significantly increased oat yield compared with the control and S. The Se content in grains was increased in the order of TS > S > T. T and TS increased the nutrients, soil organic matter, activities of urease, alkaline phosphatase, and sucrose, as well as the diversity and abundance of soil bacterial communities. According to PCA analysis, TS and T increased the relative abundance of bacteria involved in the decomposition of organic matter, such as Proteobacteria, Chloroflexi, and Bacteroidetes, while reduced *Granulicella*, *Bacillus*, *Raoultella*, *Lactococcus*, *Klebsiella*, and *Pseudomonas*. Furthermore, TS significantly increased the relative abundance of *Aciditeromonas*, *Gemmatimonas*, *Geobacter*, and *Thiobacter*. While, T significantly increased the abundance of *Lysobacter*, *Holophaga*, *Candidatus-Koribacter*, *Povalibacter*, and *Pyrinomonas*. S did not significantly change the bacterial communities. The redundancy analysis revealed that soil nutrients and enzyme activities were positively correlated with the abundance of *Actinobacteria*, *Acidobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, *Planctomycetes*, and *Chloroflexi*, but negatively correlated with the abundance of *Proteobacteria* and *Firmicutes*. Thus, a combined application of foliar and soil Se proved most conducive for achieving higher yield, grain Se content, and improving bacterial community structure and functional gene expression in rhizosphere soil.

1 Introduction

Oats (*Avena sativa* L.) ranks sixth in the world among cereal crops according to the planting area following wheat, corn, rice, barley, and sorghum. Oats are rich in nutrients, such as fat, protein, vitamins, and fiber. At the same time, oats have important medical value and health effects and play important roles in preventing and treating hyperlipidemia and controlling obesity. Owing to its high nutritious values oat-based food and feed uses are gaining increasing consideration at the Loess Plateau of China.

Selenium (Se) is a trace element necessary for the human body (Brown & Arthur 2001), and its deficiency can cause various diseases (Arroyo et al. 2015; Kieliszek & Blazejak 2013). In China, Keshan and Keshin–Beck diseases become prevalent due to Se deficiency, for instance, the Keshan disease becomes common in the Tibetan Plateau and Heilongjiang province (Ye et al. 2020). The distribution of Se in the soil is highly uneven and site-specific (Banuelos et al. 2015). Approximately 51% area of China is Se deficient (Dinh et al. 2018). An estimated 15% of the global population (500–1100 million people) have Se-deficiency problems (Wang et al. 2017). Most of the Se in the human body is derived from plants which absorb Se from their external environment (Jozwiak & Politycka 2019).

In some soil the bioavailability of Se is low and Se also forms stable complexes with clay or reacts with hydrous oxides to form complexes of low solubility and availability to plants. The application of Se fertilizer has proved to be a safe, low-cost, efficient, and convenient method to produce Se-rich plants (Chen et al. 2002). The soil and foliar application of Se fertilizers not only significantly increase the content of Se in crop grains but also increase yield (Escalante-Valdez et al. 2019; Lara et al. 2019; Ligowe et al. 2020; Reis et al. 2020). Foliar application of Se can be quickly absorbed by the leaf surface and efficiently transported to the grains to improve the utilization of Se (Zhang et al. 2019). While applying Se to the soil can increase soil enzyme activity and promote N, P, and C nutrients in the soil ecosystem cycle (Shi et al. 2018). The Se enrichment effect of these two Se application methods has been confirmed in many crops, such as corn (Longchamp et al. 2015), wheat (Manojlovic et al. 2019), rice (Fang et al. 2008), potato (Golubkina & Skriabin 2010), and rape (Ding et al. 2017).

Soil microorganisms are an important part of the soil ecosystem and play a vital role in the process of material conversion, and energy flow in ecosystems (Palomo et al. 2016; Talbot et al. 2013). In the plant rhizosphere, microorganisms significantly affect the bioavailability of mineral elements in the soil-plant system and produce a healthy micro-ecological environment for the growth of the plant (Mendes et al. 2011; Nacke et al. 2017). The function of the microbial community is determined by the structure and diversity of the microbial community (Bakker et al. 2015). Any factors that affect the rhizosphere soil microbial community will affect the function of the community and the growth of plants. Studies have shown that micronutrients have

an important effect on soil microbial community structure. Song et al (2020) showed that the presence of Cd, Cu, and Zn, and micronutrients account for 30% of the change in microbial community structure in the soil. The richness and diversity of soil bacterial communities were significantly reduced at a high concentration of Cu or Zn (Golebiewski et al. 2014). The appropriate concentration of Fe, Mn, Cu, and Zn can improve the soil bacterial community by promoting the diversity of probiotics (*Paenibacillus polymyxa*, *Bacillus amyloliquefaciens*, *Bacillus mycoides*, and *Lysinibacillus*) and improve the disease prevention ability of crops.

Variations in soil micronutrients can lead to change the soil physical and chemical properties, such as pH, carbon, nitrogen, and the activity of soil enzymes which also influence the plant physiology and the composition and relative abundance of rhizosphere microorganisms (Lareen et al. 2016; Liu et al. 2019). Selenium could influence soil bacterial communities, and conversely, microbial activity can alter Se bioavailability and distribution (Rosenfeld et al. 2018). However, at present, there is very little research on the effects of selenium on soil bacteria and therefore, further studies are needed to confirm the effectiveness of different selenium biofertilization strategies under field conditions by elucidating the main mechanisms of Se uptake by plants and the response of soil bacterial communities to Se fertilizer.

Based on previous studies, we hypothesized that selenium fertilizer would have significant effects on oat growth and rhizosphere soil properties, enzyme activities, and microbial diversity. Therefore, the purpose of this study was to: (1) investigate the effects of selenium fertilizer on soil properties, and soil enzyme activities; (2) to compare the responses of bacterial diversity and community composition to selenium application; and (3) to evaluate the response of selenium content and yield of oats to selenium application.

2 Materials And Methods

2.1 Study area

The experiment was conducted from June to October 2017 in Haijiayao Village, Guanjiabao Township, Zuoyun County, Datong City, Shanxi Province. Zuoyun County is located in the northernmost part of Shanxi Province (39°44′–44°15′ N, 112°34′–112°59′ E). It belongs to the Loess hilly region and has a typical, temperate, semi-arid continental climate with an average annual temperature of 5°C, annual rainfall of 430 mm, and a frost-free period of 105 d. The soil is light chestnut type. The 0–20 cm layer has 9.93 g kg⁻¹ organic matter, 0.746 g kg⁻¹ total nitrogen, 0.51 g kg⁻¹ total phosphorus, 38.62 mg kg⁻¹ alkaline nitrogen, 12.50 mg kg⁻¹ available phosphorus, 92.41 mg kg⁻¹ available potassium, a pH of 8.68, and 356.23 µg kg⁻¹ Se.

2.2 Oat variety and Se fertilizers

The seeds of oat (*Avena sativa* L.) variety 'Jinyan 21' were provided by Youyu Agricultural Experimental Station of the Shanxi Academy of Agricultural Sciences. An organic Se fertilizer (Se content 36.4 mg kg⁻¹, NPK nutrient content ≥ 5%, organic matter content ≥ 45%) was used as a topdressing. Additionally, a Se-enriched composite synergist aqueous solution containing 8 mg mL⁻¹ Se (Yifeng Selenium-Rich Agricultural Products R & D Co., Ltd. Taiyuan, Shanxi Province) was used as a foliar spray (diluted 100 times before the use). Both Se fertilizers contained Se in the form of selenite (Se⁴⁺).

2.3 Experimental design and treatments

The treatments were arranged in a randomized complete blocks design. The four Se application treatments were: topdressing of 1.20 mg m⁻² Se fertilizer at the booting stage (T); foliar spraying of 1.20 mg m⁻² Se fertilizer at the flowering stage (S); both topdressing of 1.20 mg m⁻² Se fertilizer at the booting stage and foliar spraying of 1.20 mg m⁻² Se fertilizer at the flowering stage (TS); and no Se fertilizer as a control (CK). Se fertilizer is provided by Yifeng Selenium-Rich Agricultural Products R & D Co., Ltd, and the application amount is the reasonable application amount obtained from the company's long-term practice in China. Each treatment was repeated three times, with a total of 12 plots. Each plot was 100 m². The seeds of oat were sown on June 30, 2017. Before sowing, the soil was prepared by applying 6 kg of NPK fertilizer (N:P₂O₅:K₂O =

18:18:18, total nutrient content 54%, organic matter content $\geq 45\%$, Shanxi Tianji Coal Chemical Group Co., Ltd.) per plot. Seeds were sown using mechanized drilling. Row spacing was 20 cm, with 1.2 kg of seeds sown per plot. Other field management measures, such as irrigation and weeding, were carried out according to the common practice in the region.

2.4 Sample collection and processing

Soil and plant samples were collected 1 d before oat harvesting. Three sampling points were randomly selected from each plot from which 10 plants were dug out carefully along with soil from a 20 cm radius around each plant. The rhizosphere soil was removed from the roots and roots were gently rinsed with clean water. Each plant was divided into root, stem, leaves, and ears. Then, the plants were dried in the oven at 65°C until the weight was constant and ground into a powder with an electric grinder (FZ102, Shanghai, China). The ground powder was passed through a 0.15 mm sieve and then sealed in Ziplock bags for analysis of total Se content. Similarly, 100 g of oat grains were ground into flour using an electric grinder (HCP 100, Zhejiang, China), pass through a 0.15 mm sieve, and sealed in a Ziploc bag for analysis of total Se content. Bulk soil samples were collected adjacent to excavated plants from the depth of 0–20 cm with a sterilized spiral drill having 8 cm diameter. The plant roots were removed by hand and rhizosphere and bulk soil samples were passed through a sieve (2 mm) and then divided into two parts. One part was placed in a refrigerator at 4°C for soil nutrient determination, and the other part was stored in liquid nitrogen at -80°C for soil microbial analyses.

2.5 Plant and soil analyses

2.5.1 Determination of yield components, and plant and soil nutrients

Plant height, total spike number, yield, and 1000-grain weight were determined according to conventional methods. A mixture of HNO₃-H₂O₂ (v/v ratio of 4:1) was used to digest the preserved plant and flour samples (100 mg) in the digester reaction system (LWY84B, China). Then, the content of Se was determined using microwave digestion-inductively coupled plasma mass spectrometry (ICP-MS).

Conventional methods were used to determine nutrients and organic content in soil (Bell et al. 2005). The organic matter content was determined using the potassium dichromate external heating method; the nitrogen content was determined using the alkali hydrolysis diffusion method; the available phosphorus content was determined in a 0.5 mol L⁻¹ NaHCO₃ extraction using the leaching-molybdenum antimony anti-colorimetric method; the available potassium content was determined in a 1.0 mol L⁻¹ NH₄OAc extraction using the leaching-flame photometric method. Soil urease activity was determined using the indophenol colorimetric method, soil sucrose activity was determined using the 3,5-dinitrosalicylic acid colorimetric method, and soil alkaline phosphatase activity was determined using the p-nitrophenyl phosphate colorimetric method (Guan 1986).

2.5.2 Conversion rate of exogenous Se

The conversion rate of exogenous Se was calculated using the following equation:

$$R = \frac{C_i - C_0}{CE_i} \times 100\%$$

1

where, R : conversion rate, C_i : Se content in Se treated plants, C_0 : Se content of control plants, CE_i : application amount of exogenous Se.

The rate of increase was calculated using the following equation:

$$N = \frac{A_i - B_i}{B_i} \times 100\%$$

where, N : increase rate, A_i : Se content in Se treated plant organs, B_i : Se content in control oat plant organs.

2.5.3 Extraction and sequencing of soil genomic DNA

FastDNA SPIN kits (MP Biomedical, Santa Ana, CA, USA) were used to extract total soil DNA, and then DNA concentration and purity were determined using a NealDel-ND1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis.

The 16S rRNA V4 region of the extracted genomic DNA was amplified using the general primer sequences: 520F (AYTGGGYDTAAAGNG), and 802R (TACNVGGGTATCTAATCC). The polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 98°C for 2 min; followed by 25 cycles of denaturation at 98°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min. PCR products were then stored at 4°C. PCR products were purified using an Agencourt AMPure Beads kit (Beckman Coulter, Indianapolis, IN, USA), and quantified using a PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA). The samples were entrusted to Shanghai Passino Biotechnology Co., Ltd. for sequencing and analysis using the Illumina MiSeq high-throughput sequencing technology platform.

2.5.4 Optimized processing of sequencing data

Quality filtering and merging of paired-end sequences were performed using QIIME (version 1.9.0, //qiime.org/). Data filtering was conducted following the method of Feng et al. (2009). First, the sequence of the 5'-terminal primers was removed using a mismatch base number > 1. Then, sequences containing Ns (fuzzy bases) were removed. Finally, sequences containing > 8 consecutive, identical bases, and sequences with a length \leq 150 bp were removed. The UCHIME method in MOTHUR (version 1.31.2, <http://www.mothur.org/>) was used to remove chimera sequences and to obtain a high-quality sequence that was used for subsequent analysis.

2.5.5 OTU cluster analysis and annotation

The UCLUST method in QIIME was used to cluster high-quality sequences with a sequence similarity of 97%. The longest sequence in each category was selected as the representative sequence. The BLAST method in QIIME was used to obtain taxonomic information for each OTU using the Greengenes database (release 13.8, <http://greengenes.secondgenome.com/>). Then, OTUs with an abundance of less than 0.001% of the total number of sequences were removed to obtain a simplified OTU table for subsequent analysis.

2.5.6 PICRUSt function prediction

Bacterial functions and metabolic pathways were predicted using PICRUSt (Muller et al. 2002); the closed OTU table obtained from QIIME was compared to the KEGG database to determine different bacterial community functions.

2.6 Calculations and statistical analysis

The *Single* command in MOTHUR was used to calculate the Chao1, ACE, Simpson, and Shannon diversity indices for soil bacteria using the OTU table obtained from QIIME (Schloss et al. 2011; Schloss & Handelsman 2005). Beta diversity analysis was performed to investigate the structural variation in microbial communities across samples using UniFrac distance metrics (Lozupone & Knight 2005; Lozupone et al. 2007). Principal component analysis (PCA) was conducted based on the species-level compositional profiles (Ramette 2007). A UPGMA cluster analysis was performed in QIIME with a weighted UniFrac distance matrix, and then R packages (v3.2.0) were used for visualization. Microsoft Excel 2003 was used for basic calculations; Origin (2019) was used to construct charts of the taxonomic composition and differences among communities at various levels. One-way analysis of variance (ANOVA) was performed using SAS 9.4 with a significance level of $P < 0.05$. CANOCO 4.5 was used to perform a redundancy analysis (RDA) of soil chemical properties, and bacterial community structure and diversity.

3 Results

3.1 Effects of Se fertilizer application on yield components of oats

The topdressing of Se fertilizer at booting (T) and foliar spray at the flowering stage (S) has no significant effect on the plant height and the total number of spikes per unit area (Table 1). Compared with the control, the 1000-grain weight and grain yield were significantly influenced by Se. The topsoil application of Se (T), foliar spray of Se (S), and their combination (TS) showed a 12%, 9%, and 18% increase in 1000-grain weight. S showed a non-significant effect on 1000-grain weight. The grain yield was significantly increased by TS, whereas T and S showed a non-significant increase. Thus, the maximum 1000-grain weight and grain yield were observed in TS.

Table 1
Effects of Selenium Fertilizer Application on Economic Characters of Oats

Treatment	Plant height (cm)	Number of spikes ($\times 10^6 \text{ ha}^{-1}$)	Number of grains (spike^{-1})	1000-grain weight (g)	Grain production (kg ha^{-1})
Control	102.27 ^a	27.15 ^a	48.00 ^a	19.85 ^c	2.76 ^b
T	106.77 ^a	26.95 ^a	49.00 ^a	22.17 ^b	2.86 ^{ab}
TS	104.43 ^a	27.69 ^a	50.33 ^a	23.43 ^a	3.00 ^a
S	109.73 ^a	27.04 ^a	48.67 ^a	21.73 ^{bc}	2.76 ^b

Note: Different lowercase letters in the same column indicate significant differences among different treatments. ($P < 0.05$). CK: Control, T: soil application, TS: combined soil and foliar application, S: foliar application

3.2 Effect of Se fertilizer application on Se content in oat plants

Without Se fertilizer, the maximum Se was accumulated in roots and the minimum in the stem (Table 2). The order of Se accumulation in various organs of oats without Se fertilizer was stem > grain > leaf > ear > root. The Se accumulation in various organs was increased to different degrees after Se application. Among them, soil topdressing (T) significantly increased the Se accumulation in roots, ears, and grains compared with the control, while foliar spray (S) significantly increased the Se contents of stems, leaves, ears, and grains. The combined topdressing + foliar spray (TS) significantly increased the Se content in all parts of the oat plant.

Table 2
Effects of selenium fertilizers on selenium allocation in different organs of oat

Treatment	Root ($\mu\text{g kg}^{-1}$)	% Increase	Stem ($\mu\text{g kg}^{-1}$)	% Increase	Leaf ($\mu\text{g kg}^{-1}$)	% Increase	Ear ($\mu\text{g kg}^{-1}$)	% Increase	Grain ($\mu\text{g kg}^{-1}$)	% Increase
Control	132.08 ^b	-	36.18 ^c	-	45.01 ^b	-	98.00 ^d	-	41.72 ^d	-
T	152.27 ^a	15.3	47.11 ^b	30.2	52.84 ^b	17.4	101.28 ^c	3.35	48.27 ^c	15.7
S	135.24 ^b	2.4	56.01 ^a	54.8	79.39 ^a	76.4	107.86 ^b	10.1	53.44 ^b	28.1
TS	158.15 ^a	19.7	51.59 ^{ab}	42.6	72.10 ^a	60.2	125.83 ^a	28.4	57.71 ^a	38.3

Note: Different lowercase letters in the same column indicate significant differences among different treatments. ($P < 0.05$). CK: Control, T: soil application, TS: combined soil and foliar application, S: foliar application

The conversion rate of exogenous Se was above 100% for all Se treatments and all plant organs (Table 3). The conversion rate of exogenous Se under the S treatment followed the order leaf > stem > grain > ear > root; under the T treatment, it followed the order root > stem > leaf > grain > ear; and under the TS treatment, it followed the order ear > leaf > root > grain > stem. Under the S

treatment, the conversion rate of exogenous Se in leaves reached 644.63%, and the conversion rates in stems, leaves, and grains were higher than those in the other treatments ($P < 0.05$). Under the T treatment, the conversion rate of Se in grains was significantly lower than that under the TS and S treatments. Under the TS treatment, the conversion rate of exogenous Se in leaves was lower than that under the S treatment ($P < 0.05$), and the conversion rates in spikes and grains were slightly improved.

Table 3
Conversion Rate of Exogenous Selenium

Treatment	Root (%)	Stem (%)	Leaf (%)	Ear (%)	Grain (%)
T	416.00 ^a	225.21 ^b	161.33 ^c	67.58 ^c	134.96 ^b
TS	255.92 ^b	151.28 ^c	265.94 ^b	273.20 ^a	156.97 ^b
S	59.25 ^c	371.81 ^a	644.63 ^a	184.88 ^b	219.75 ^a

Note: Different lowercase letters in the same column indicate significant differences among different treatments. ($P < 0.05$). T: soil application, TS: combined soil and foliar application, S: foliar application.

3.3 Effects of Se fertilizer application on soil nutrients, soil enzyme activities, and bacterial community diversity

The contents of available nitrogen, available potassium, and organic matter in the soil treated with T and TS were significantly increased. However, there were no significant differences in the above indices after Se foliar spray (S). All Se fertilizer treatments had no significant effect on the content of available phosphorus in the soil. The T and TS can significantly increase the activity of urease, alkaline phosphatase, and sucrase in the soil as compared to the control. There was no significant difference in soil enzyme activities between the control and S. The indices of Chao1, ACE, and Shannon were increased by TS but did not achieve a significant difference, while T and S had no significant effect.

Table 4

Effects of selenium fertilizers on nutrients, enzyme activities and diversity of bacterial communities in rhizospheric soil of oat

Indices		control	T	TS	S
Nutrients	Available N (mg kg ⁻¹)	36.66 ^b	38.12 ^a	38.86 ^a	36.99 ^b
	Available- P (mg kg ⁻¹)	5.32 ^a	5.45 ^a	5.47 ^a	5.34 ^a
	Available -K (mg kg ⁻¹)	74.8 ^b	83.0 ^a	80.8 ^a	76.3 ^b
	SOM (g kg ⁻¹)	11.03 ^b	12.50 ^a	13.01 ^a	11.46 ^b
Enzymes	Urease (mg g ⁻¹ 24h ⁻¹)	0.57 ^c	0.78 ^b	0.90 ^a	0.56 ^c
	Phosphatase (mg g ⁻¹ 24h ⁻¹)	3.68 ^b	3.82 ^a	4.00 ^a	3.62 ^b
	Sucrase (mg g ⁻¹ 24h ⁻¹)	4.58 ^b	5.14 ^a	4.92 ^a	4.62 ^b
Diversity Index	Chao 1	2373 ^{ab}	2229 ^b	2885 ^a	1845 ^b
	ACE	2684 ^{ab}	2356 ^b	3213 ^a	1994 ^b
	Simpson	0.99 ^a	1.00 ^a	1.00 ^a	0.99 ^a
	Shannon	9.45 ^{ab}	9.98 ^a	10.10 ^a	9.10 ^b

Note: Different lowercase letters in the same column indicate significant differences among different treatments. ($P < 0.05$). CK: Control, T: soil application, TS: combined soil and foliar application, S: foliar application

3.4 Effect of Se fertilizer application on soil bacterial community composition

3.4.1 Bacterial community structure at the phylum and species level

Under Se treatments, 26 bacterial phyla were identified. The relative abundance of eight phyla was ≥ 0.01 (dominant bacterial phyla), whereas that of the remaining 18 phyla was < 0.01 . In the cluster analysis of the top 20 phyla (Fig. 1A), CK and S were clustered together, and T and TS were clustered together. The relative abundance of bacteria could be influenced by exogenous Se fertilizer application. The T and TS treatments significantly increased the relative abundance of Bacteroidetes, Proteobacteria, Chloroflexi, and Gemmatimonadetes. In addition, T significantly increased the relative abundance of Acidobacteria; S increased the relative abundance of Firmicutes.

Under different treatments, 637 bacterial genera were identified. The relative abundance of 19 genera was ≥ 0.01 (dominant bacterial genus), whereas that of the remaining 618 genera was < 0.01 . The cluster analysis of the top 30 genera (Fig. 1B) revealed that CK and S were clustered together, and T and TS were clustered together. Se application to the soil had a significant effect on bacterial abundance. T treatment significantly increased the relative abundance of *Lysobacter*, *Holophaga*, *Candidatus Koribacter*, *Povalibacter*, and *Pyrinomonas*. The TS treatment significantly increased the relative abundance of *Aciditeromonas*, *Gemmatimonas*, *Geobacter*, and *Thiobacter*. Furthermore, T and TS reduced the relative abundance of *Granulicella*, *Bacillus*, *Raoultella*, *Lactococcus*, *Klebsiella*, and *Pseudomonas*; S increased the relative abundance of *Bacillus*.

3.4.2 Correlations between soil bacterial communities and soil nutrients

The soil microbial communities under the four treatments were analyzed using a PCA (Fig. 2A). The variation in the first principal component (PC1) was mainly caused by soil application of Se, while the variation in the second principal component (PC2) was mainly caused by the foliar application of Se. The contribution rates of PC1 and PC2 were 88.21% and 9.58%, respectively (97.79% in total). All three Se fertilizer treatments differed significantly from the CK treatment. In Fig. 2A, S and CK

are both located on the negative side of PC1 with CK on the positive and S on the negative side of PC2. T and TS are both located on the positive side of PC1, with T on the positive and TS on the negative side of PC2. This shows that the method of applying Se fertilizer has a significant effect on the structure of soil microbial communities ($P < 0.05$). Soil application of Se had a greater impact on soil microbial community structure than the foliar application of Se.

The relationship between bacterial community structure at the phylum level and soil nutrients, enzyme activities, and bacterial diversity indices were analyzed using an RDA (Fig. 2B). Soil nutrients and enzyme activities were significantly positively correlated with Actinobacteria, Acidobacteria, Gemmatimonadetes, Bacteroidetes, Planctomycetes, and Chloroflexi, and were significantly negatively correlated with Proteobacteria and Firmicutes. This shows that soil nutrients, enzyme activities, and bacterial communities are closely related, and bacterial communities play an important role in the cycling of soil materials. Soil nutrients have a significant effect on soil microbial communities.

3.4.3 PICRUSt analysis: Composition of functional gene families

The predicted functional gene families were compared with the KEGG database (Fig. 3). The functions annotated by all bacterial gene sequences can be divided into three categories related to metabolism, environmental information processing, and genetic information processing. From the metabolism category, the microorganisms involved in carbohydrate and amino acid metabolism were more abundant ($> 6\%$). Among environmental information processing, the bacteria involved in membrane transport had a significant advantage ($> 6\%$). Among the flora involved in the processing of genetic information, the bacteria involved in replication and repair were the most significant ($> 6\%$).

A comparison among the four treatments revealed that the topdressing of Se (T and TS) significantly improved the relative abundance of bacteria involved in amino acid metabolism, replication, repair, and energy metabolism, but had a certain weakening effect on membrane transport. There was no significant difference between foliar spray of Se fertilizer (S) and control treatment (CK).

4 Discussion

4.1 Effects of Se fertilizer application on yield components of oats

In the present study, a combined application of topdressing and foliar spray (TS) significantly increased oat yield compared to the control. Previous studies have shown that the appropriate amount of soil Se fertilizer promotes oat plant development, increases seed setting rate and crop yield (Escalante-Valdez et al. 2019; Lara et al. 2019; Ligowe et al. 2020; Liu et al. 2020). The increments in yield could be explained by the improvement in seed setting rates and grain weights (Luo et al., 2020). In the present study, the highest grain yield and 1000-grain weight were obtained under the combined soil + foliar (TS) application of Se (Table 1). While the 1000-grain weight was significantly higher for the soil-applied Se (T) than the foliar spray of Se fertilizer (S). The grain weight is the main determining yield determining traits that had a linear correlation with grain yield. The higher net photosynthetic rate during the grain filling stage produced heavier grains because the amount of net photosynthesis has a decisive effect on the seed setting rate and grain weight (Luo et al., 2020).

This might be because Se can affect the synthesis of chlorophyll in plants and regulate the electron transfer in photosynthesis and respiration, and thus increase the photosynthetic capacity and yield of crops (Zhang et al. 2014b). In addition, Se fertilizer can promote the growth of oats by increasing the absorption of mineral elements (Lopes et al. 2017; Rayman 2008) and activity of superoxide dismutase and peroxidase, while reducing senescence and improve anti-aging by reducing lipid peroxidation and free proline in leaves (Jozwiak & Politycka 2019). At the same time, the booting stage is an important period for the reproductive growth of oats. The application of organic Se fertilizer in the soil not only supplements Se but also improves organic matter in the soil. Therefore, single soil application of Se fertilizer is better than the single foliar spray of Se, and soil application + foliar spray application is better than soil application or foliar application.

4.2 Effect of Se fertilizer application on the distribution of Se in oats and the conversion rate of exogenous Se

The Se content in oat plants was always the highest in the roots and lowest in the stems in this study irrespective of the application method (soil or foliar) of organic Se. The effect of topdressing of organic Se fertilizer on the Se content of oat roots was more prominent than that of foliar spray. Foliar spray of Se fertilizer mainly affected the Se content of plant stems, leaves, spikes, and grains. The plant root system absorbs Se in the form of selenite (SeO_3^{2-}) and then Se is converted to insoluble selenomethionine (Sors et al. 2005). While Se absorbed through the foliar spray is easily carried through the vascular system without a long process of soil fixation, root absorption, and transportation to stems and leaves, thereby increasing the effectiveness of Se. The total Se content was highest by the soil + foliar application of Se (TS), followed by foliar spray of Se (S), and the lowest by soil application (T). These results indicate that the foliar spray of Se fertilizer is more beneficial for the accumulation of Se in grains than the soil application. At the same time, there is an additive effect of the combined soil and foliar application of Se on the Se accumulation in oat grains. The present study also showed that the application of exogenous Se increased the uptake of Se from the soil by oat roots. The low recovery efficiency of exogenous Se in grains by the soil treatment (T) and high accumulation of Se in leaves by foliar spray (S) can be improved by soil + foliar treatment of Se (TS).

4.3 Effects of Se fertilizer application on soil nutrients and soil bacterial communities

The topdressing of Se and topdressing + foliar application significantly increased the nutrient content, soil organic matter, and activities of urease, phosphatase, and sucrase in soil. This may be because the organic Se fertilizer provides some carbon, nitrogen sources, and Se for the microorganisms. Meanwhile, organic matter can protect the soil sucrose from decomposition and denaturation. In addition, soil enzymes are produced by the metabolism of soil microorganisms, while organic Se fertilizer can improve the metabolism of soil microorganisms, so the activities of soil urease, sucrase, and alkaline phosphatase are improved.

The diversity and richness of soil bacterial community composition is an important indicator to measure the stability and health of the soil ecosystem (Geisseler et al. 2017; Panico et al. 2018). The Se in the soil changes the cell metabolism and microbial function of soil microorganisms, causing changes in the survival and competitiveness of microorganisms and resulting in changes in population diversity (Wang et al. 2018). Gonzalez-Gil et al. (2016) reported changes in the microbial community structure after 21 d exposure of selenite. The Se in soil could increase the diversity of Se reducing or oxidizing bacteria. The Se reducing bacteria play a pivotal role in the Se cycle in the environment. Bacteria can reduce soluble selenate and selenite to elemental Se and soluble selenide. Selenide and elemental Se oxidized back to selenite or selenate by selenium-oxidizing bacteria (Nancharaiah and Lens, 2015).

In the present study, the topdressing of Se organic fertilizers (TS) increased the diversity indices (Chao1, ACE, and Shannon) of the bacterial community in the soil. This may be related to the changes in soil nutrients after the application of Se organic fertilizer. The content of N, K, and organic matter in the soil increased significantly after the application of Se fertilizer, which accelerated the activities of the bacterial community in the soil (Dong et al. 2014; Zhang et al. 2016), and also increased the activity of urease, alkaline phosphatase, and sucrose in soil.

From the PCA analysis, it can be seen that the soil + foliar application of Se fertilizer significantly affected the soil microbial community structure and increased *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, and other microbial groups involved in the decomposition of organic matter in the soil, providing more carbon and nitrogen sources for microbial activities in the soil (Fierer et al. 2012; Wessén et al. 2010). Besides, soil Se fertilizer (T and TS) significantly increased the relative abundance of *Bacteroidetes*, *Proteobacteria*, *Chloroflexi*, and *Gemmatimonadetes*. These bacteria are closely associated with the content of soil nutrients, and their relative abundance can reflect the level of soil nutrients. *Proteobacteria* and *Bacteroidetes* inhabit nutrient-rich conditions, and their relative abundance increases at high organic matter levels. *Proteobacteria* are metabolically versatile gram-negative microorganisms that reduce selenate and selenite (Gonzalez-Gil et al. 2016). *Bacteroidetes* members have a phosphorus-dissolving effect, and they were positively correlated with the content of available phosphorus (Fig. 2A) (Fierer et al. 2012). Some *Chloroflexi* members are autotrophic bacteria that can decompose organic matter and use 3-hydroxypropionic acid to fix CO_2 to produce energy. Therefore, they can survive in environments with different levels of nutrition. *Chloroflexi* members are more abundant under higher nutrient conditions (Boyle-Yarwood et al. 2008). These findings

show that Chloroflexi members are more inclined to live in an environment with adequate nutrition. This is similar to the results of a previous study (Zhao et al. 2020). At the genus level, T treatment increased the abundance of *Lysobacter*, *Holophaga*, *Candidatus-Koribacter*, *Povalibacter*, and *Pyrinomonas*. TS treatment increased the relative abundance of *Gemmatimonas*, *Geobacter*, and *Thiobacter*. T and TS decreased the abundance of *Granulicella*, *Bacillus*, *Raoultella*, *Lactococcus*, and *Klebsiella*, while increased the abundance of *Pseudomonas*. Gonzalez-Gil et al. (2016) reported that about 90% of the Pseudomonadacea of the selenite reducing granules belonged to the genus *Pseudomonas*. The presence of *Pseudomonas* is of particular interest because most of the members of this family are aerobic. *Pseudomonas* might be distinctly highly tolerant to Se and selenite (Ye et al. 2020). Overall, these results showed that Se fertilizer application to soil had a significant effect on the structure of the soil microbial community.

Until now, only a few bacterial genes involved in Se utilization had been identified. The most abundant gene sequences of soil microbes were mainly related to metabolism, environmental information processing, and genetic information processing. Among all flora involved in major metabolic functions, those involved in carbohydrate metabolism, amino acid metabolism, membrane transport, replication, and repair have significant advantages. The soil application of Se fertilizer significantly improved the abundance of bacteria involved in amino acid metabolism, replication and repair, and energy metabolism, which further confirmed that the soil application of Se fertilizer is beneficial for the metabolism of soil bacteria.

5 Conclusions

The topsoil application of organic Se fertilizer or the foliar spray of Se fertilizer increased the grain yield of oats and the Se content in the grains. On the whole, the foliar spray of Se fertilizer had no significant effect on soil nutrients and soil microbial communities. Topdressing of organic Se fertilizer is conducive to increasing the C and N nutrients in the soil by increasing the diversity and richness of the microbial communities of oat rhizosphere soil and the expression of related genes. The combined soil and foliar application of Se fertilizer proved more advantageous than the single soil or foliar application of Se for increasing yield and Se content in oat grains.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data supporting the findings of this study are available within the supplementary information file named "Supplementary Information of Figures"

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the study conception and design. Overall experimental design and field planning were performed by Yang Zhenping, Gao Zhiqiang, Guo Anna, and Li Junhui. Field sampling and survey indicators was performed by Li Junhui, Yang Wenping, Guo Anna, Yang Sheng, and Wang Kai. Data collection and analysis were performed by Li Junhui, Yang Wenping, Chen Jie, Qiao Yuejing, and Wang Jianwu. Language technology service: Sumera Anwar. The first draft was written by Li Junhui and all authors commented on previous versions of the manuscript. The review and editing of the previous versions of the manuscript was performed by Yang Zhenping. All authors read and approved the final manuscript.

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Figures

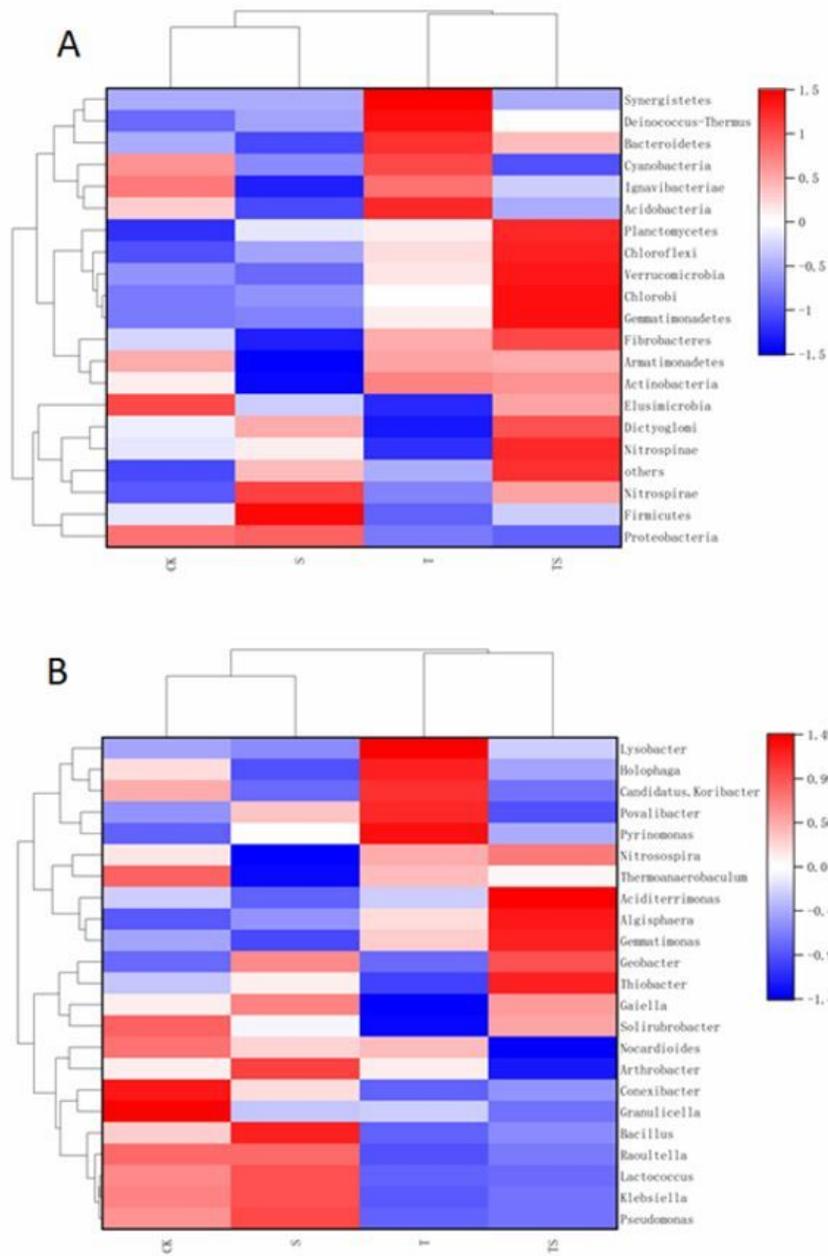


Figure 1

Relative abundance of the dominant bacterial phyla(A) and the dominant bacterial genera (B) under different selenium treatments. Note: CK: Control, T: soil application, TS: combined soil and foliar application, S: foliar application

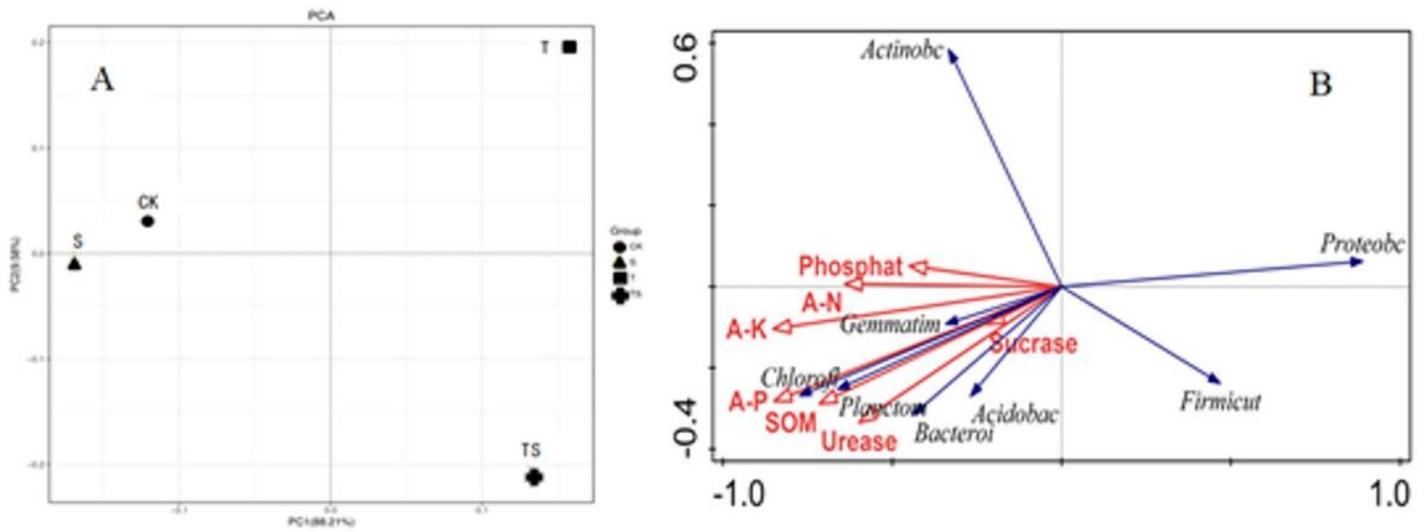


Figure 2

A: Principal component analysis of the soil microbial community under different selenium fertilizer treatments. 2B: Redundancy analysis of the soil bacterial community at the phyla level with soil nutrients, enzyme activities, and bacterial diversity indices. Note: CK: Control, T: soil application, TS: combined soil and foliar application, S: foliar application.

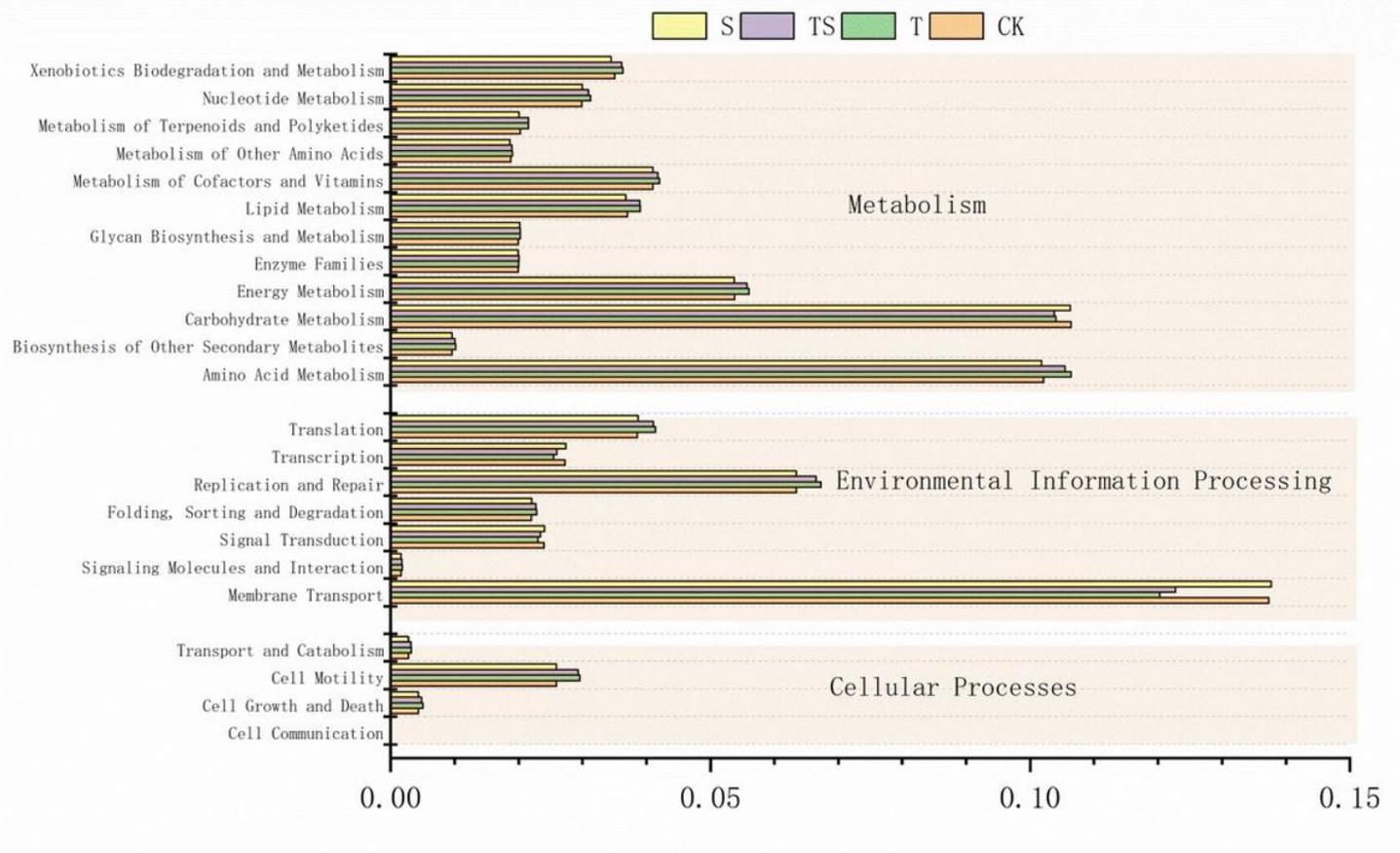


Figure 3

The relative abundance of KEGG functional genes under different selenium application treatments. Note: CK: Control, T: soil application, TS: combined soil and foliar application, S: foliar application.

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