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Effects of foliar selenium application on growth and rhizospheric soil micro-ecological environment of *Atractyloides macrocephala* Koidz

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**ABSTRACT**

*Atractyloides macrocephala* (*A. macrocephala*), a famous medicinal herb in China, is widely cultivated and consumed in China with various beneficial effects. Numerous studies have shown that selenium (Se) plays an important role in promoting plant growth, although Se has not been considered an essential element for higher plants. The objectives of this research were to determine the effects of foliar Se application (0, 2.5, 5.0, 10.0 and 20.0 mg m\(^{-2}\) Se in sodium selenite, sprayed monthly from May to August) on the growth and rhizospheric soil micro-ecological environment of *A. macrocephala*, and to explore the possible mechanisms underlying plant response to foliar Se application through a field experiment. The results were: The foliar application of 5.0 mg m\(^{-2}\) Se significantly increased the survival rate of *A. macrocephala* compared to the control. The yield of *A. macrocephala* was increased when the Se level maintained belowed 10.0 mg m\(^{-2}\) but decreased when Se level reached 20.0 mg m\(^{-2}\). The Se content in the rhizome of *A. macrocephala* showed a significant positive correlation with the Se level, while the insect attack rate was significantly negatively correlated with the Se level. However, foliar Se application hardly affected the concentration of bioactive compound atractylenolide in the rhizome of *A. macrocephala*. Notably, the application of foliar Se changed the content of partial soil nutrients, microbial diversity and composition in the rhizosphere soil of *A. macrocephala*. Bacterial diversity was positively correlated with *A. macrocephala* growth whereas fungal diversity was negatively correlated, suggesting that microbial diversity in the rhizosphere soils is closely related to plant growth. Moreover, correlation analysis showed that available potassium, *Burkholderia* and *Cupriavidus* in rhizospheric soil might be critical factors for promoting the growth of *A. macrocephala*. Overall, the foliar application of Se at moderate concentration was beneficial for the growth of *A. macrocephala*, and 5.0-10.0 mg m\(^{-2}\) Se level was the optimum. Our findings revealed novel insights into the response of *A. macrocephala* to foliar Se application from plant growth, rhizospheric soil nutrient and microbial community composition.

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**1. Introduction**

Selenium (Se) is arguably one of the most interesting micronutrients in biology due to its both essential and toxic properties for most species, with a very narrow window between deficiency and toxicity compared with that of other trace elements (Schiavon and Pilon-Smits, 2017; Stadtman, 1990; Zhu et al., 2009). Se intake at an adequate level, confers a variety of health benefits for humans, while Se intake at a low level is associated with considerable health disorders, including higher risk of cancer, heart disease and a weakened immune system (Rayman, 2009). Although Se is not an essential nutrient for higher plant (Yan and Gladyshev, 2009), Se is recognized as a beneficial element for promoting the growth and stress resistance of plants (Aghighi Shahverdi et al., 2018; da Silva et al., 2020; Hartikainen, 2005; Hussein et al., 2019; Pilon–Smits et al., 2009). Selenium and selenite are the two main chemical forms available in the soil and water-soluble Se fertilizers (Schiavon and Pilon–Smits, 2017). The bioavailability of Se fertilizers applied to soil is lower than that of those applied to leaves for some natural processes such as Fe-Mn oxide immobilization (Liu et al., 2015), redox reactions (Ros et al., 2016) and leaching (Wang et al., 2013). Therefore, soil application of Se fertilizer is considered disadvantageous for the Se biofortification of plants, and foliar Se spray usually has a higher bioavailability than Se fertilizers designed for soil application.

*Atractyloides macrocephala* is a medicinal herb, widely used in Chinese traditional medicine for approximately 2000 years. It is rich in...
bioactive compounds such as atractylenolide I, II, and III (Chen et al., 2009; Sun and Jian, 2014) and possesses various pharmacological properties, including antioxidant (Li et al., 2012), anti-inflammatory (Li et al., 2007), antimutation (Huang et al., 2006), and antitumor (Zhen et al., 2010) effects. Since the outbreak of novel coronavirus pneumonia (SARS-CoV-2) in China, A. macrocephala has played an important role in treating this disease. Notably, Se acts as an antioxidant and plays a protective role in the human immune system, indicating that Se has some similar effects to those of A. macrocephala. Moreover, Se biofortification may increase both the Se concentration and bioactive compound content in plants (Malagoli et al., 2015). Various studies have been reported on the Se biofortification of different crop plants (Zhao and McGrath, 2009). The Se biofortification of plants was mainly applied on grain crops, fruits and vegetables, such as rice (Deng et al., 2017), blueberry (Li et al., 2018), tomato (Zhu et al., 2018) and lentil (Ekanayake et al., 2015), and only a few studies have been conducted on medicinal plants. Recently, Zhu et al. (2017) reported that the application of Se remarkably increased the polysaccharide and total flavonoid contents in Codonopsis lanceolata, which enhanced its health benefits for human beings. Similar results were also observed in Lycium chinense (Dong et al., 2012) and Ganoderma lucidum (Gasecka et al., 2016). However, the Se biofortification of A. macrocephala has not been reported. Hence, it is necessary to determine whether Se application could improve the contents of atractylenolide and Se in A. macrocephala rhizome, which may enhance the medicinal effects and market competitiveness of A. macrocephala.

Bacteria and fungi are the most abundant and diverse groups of all soil organisms; as they dominate the soil ecosystem and are the key regulators of physical, chemical, and biological processes in terrestrial ecosystems (Van Der Heijden et al., 2008; Wu et al., 2008). In addition to their effect on plant growth and soil health, bacteria and fungi also have indispensable roles in soil formation, organic matter decomposition, and biogeochemical cycling (Kowalchuk and Stephen, 2001; Rillig and Mummen, 2006; Whitman et al., 1998). Rhizosphere soil refers to the part of soil directly affected by plant roots and their exudates, and it was first proposed by Lorentz hiltner in 1904 Hiltner (1904). After more than a century of research, it is concluded that rhizosphere soil is a microsite where interactions among roots, microorganisms and soil constituents (minerals and organic matter) take place (Lundberg et al., 2012). To date, few works have been reported on the plant Se biofortification induced changes in rhizospheric soil nutrients and microbial communities of plants. Hence, it is essential to understand the interactions between plant Se biofortification, rhizospheric soil nutrients and microbial communities in order to elucidate the effects of foliar Se application on plant growth and explore the possible mechanisms underlying plant response to foliar Se application.

In this study, we did integrative research on the Se biofortification of A. macrocephala to determine the effects of foliar Se application on the growth and rhizospheric soil micro-ecological environment of A. macrocephala. The results could provide critical information regarding the Se biofortification of A. macrocephala and promote the development of selenium-enriched medicinal plants.

2. Materials and methods

2.1. Test materials and site description

The seeds of A. macrocephala, a well-known medicinal plant in China, were purchased from local farmers and identified by Jinwen You, a researcher at the Institute of Chinese Herbal Medicines, Hubei Academy of Agricultural Sciences. The experiment was conducted in a field with the soil derived from grey sandy loam in Lizixi village (29°53′02″N, 108°54′54″E, 819 m above sea level), Xiaocun township, Xianfeng County, Hubei, China, from December 2017 to October 2018. The procedure described by Ramos et al. (2010) was used to estimate the total Se content of soil, and other physicochemical characteristics of the soil of the study site were determined according to the method of Bao (2000), with results as follows: pH= 4.71 (soil water ratio of 1:2.5), total Se content=0.38 mg kg⁻¹, organic matter content= 41.26 g kg⁻¹, total nitrogen (N)=2.16 g kg⁻¹, total phosphorus (P)=0.81 g kg⁻¹, total potassium (K)= 17.78 g kg⁻¹, available N (alkaline hydrolysis N)= 96.92 mg kg⁻¹, available P (sodium bicarbonate extractable P)= 94.75 mg kg⁻¹, available K (ammonium acetate extractable K)= 144.56 mg kg⁻¹, exchangeable calcium= 1.35 cmol kg⁻¹, exchangeable magnesium= 0.44 cmol kg⁻¹, available sulphur= 28.26 mg kg⁻¹.

2.2. Treatments and experimental design

The seeds of A. macrocephala were sown on the plot beside the experimental site and cultivated into seedlings. One-year-old seedlings of A. macrocephala of the same size were transplanted in December 2017. Five levels of Se (elemental selenium in sodium selenite) treatment, i.e. 0 (control), 2.5, 5.0, 10.0, and 20.0 mg m⁻² Se (200 mL Se sources m⁻² were sprayed at the concentration of 0, 12.5, 25.0, 50.0 and 100.0 mg L⁻¹, respectively) were sprayed monthly on the leaves of A. macrocephala from May to August in 2018, and were denoted as CK, T1, T2, T3, and T4, respectively. Fertilizers, including N, P and K as urea (360 kg N ha⁻¹), superphosphate (225 kg P₂O₅ ha⁻¹) and potassium sulfate (180 kg K₂O ha⁻¹), were applied based on the nutrient element absorption characteristics of A. macrocephala (Zhang et al., 2016a) and fertilization formula of A. macrocephala in Lizixi village (Chinese patents, No. ZL 20161105634-4). Both P and K were applied at sowing, but N was applied both at sowing and florescence with two equal split doses. The planting density was 80,000 plants ha⁻¹. Treatments were arranged in a randomized block design with three replicates. The plot size was 6 m² (1 × 6 m), including 16 rows and 3 columns. Twenty-five-centimeter trenches were prepared between each plot for drainage, and the plots were maintained under a conventional management model. During the growth period, the roots of A. macrocephala were irrigated twice with 50% thiophanate-methyl WP (× 1000) to cure root rot and 2.5% deltamethrin WP (× 800–1000) was applied as a foliar spray to prevent pests.

2.3. Measurements and sample preparation

Six months after transplanting, plant height, leaf characteristics (including insect bite area) and chlorophyll content were measured with a measuring tape, crop leaf morphometer (YMI-C, Zhejiang Top Cloud-agri Technology Co., Ltd., Zhejiang, China) and portable chlorophyll analyzer (TYS-A, Zhejiang Top Cloud-agri Technology Co., Ltd. Zhejiang, China), respectively. Ten months after transplanting, the plants were harvested (October 2018), the rhizomes of A. macrocephala were weighed in the field, and some of them were taken to laboratory, washed with tap water and rinsed with Milli-Q water (Millipore, USA). After drying the water with absorbent paper, the rhizomes were sliced, dried at 45 °C for 72 h, weighed and pulverized for determination of Se and atractylenolide. The rhizosphere soils of A. macrocephala were collected by shaking the roots and were separated into two parts. One part was homogenized by being passed through a 2 mm sieve and stored at −80 °C until further processing for the experiments. The other part was air-dried at room temperature for two weeks and passed through a 2 mm sieve for the determination of soil nutrients.

2.4. Determination of Se, atractylenolide I, II and III content

The total Se concentration in rhizomes of A. macrocephala was determined by the HNO₃ —HClO₄ digestion method described by Deng et al. (2017). The atractylenolide I, II and III concentrations in A.
macrophala were determined by the HPLC-wavelength switching method described by Yin et al. (2013). The atracylolidine concentration was calculated using the following equation:

\[
\text{Atracylolidine concentration (mg ml}^{-1} \times C \times W
\]

In the above equation, C denotes the atracylolidine concentration in rhizome (mg g\(^{-1}\)) and W denotes the dry weight of the rhizome (g).

2.5. Soil DNA extraction, library construction and high-throughput sequencing

DNA extraction and library construction were performed following the method described by Wei et al. (2018) with a slight modification. DNA from soil samples was extracted using the Mag-Bind Soil DNA Kit (OMEGA) following the manufacturer's instructions. The quantity and quality of extracted DNA were assessed by spectrophotometry (Eppendorf, Germany) and agarose gel (1%) electrophoresis, respectively. The V3–V4 hypervariable region of bacterial 16S rRNA genes was amplified using 16S 338F (5'-ACTCTACGCGGAGCACCA-3') and 16S 806R (5'-GGACTACHVGGGTWTCTAAT-3') as primers and genomic DNA as template. The PCR reaction was performed in a final volume of 50 \(\mu\)L of solution containing 4 \(\mu\)L of dNTP mixture, 5 \(\mu\)L of 10 \(\times\) PCR buffer (Mg\(^{2+}\) plus), 5 \(\mu\)L of template DNA, 1 \(\mu\)L of each primer, and 0.5 \(\mu\)L of Ex Taq, brought to the final volume using ddH\(_2\)O. The PCR conditions were as follows: 5 min at 95 °C for initial denaturation, 32 cycles at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 45 s and a final extension at 72 °C for 10 min. For fungal ITS1 regions, PCR amplification was performed using ITS5F (5'-GGAGATTAAAGCCTAACAAGG-3') and ITS1R (5'-GCTGCGTTCTTCATGATGC-3') as primers. The PCR system used for fungi was the same as that used for bacteria. The PCR conditions were 5 min at 95 °C for initial denaturation, followed by 35 cycles at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s and a final extension at 72 °C for 10 min. Both bacterial and fungal PCR products were stored at -20 °C and analyzed with a 1% gel electrophoresis. Then the PCR products were purified using a PCR Purification Kit (Axygen Bio, USA) and pooled in equimolar concentrations of 10 ng \(\mu\)L\(^{-1}\) for library construction. High-throughput sequencing was performed using an Illumina MiSeq platform with PE250 mode at Shanghai Personal Biotechnology Co., Ltd., Shanghai, China.

2.6. Determination of soil nutrients

Soil organic matter, available nitrogen, phosphorus and potassium were measured according to the Soil Physicochemical Analysis Handbook (Bao, 2000) with slight modifications. Briefly, soil available nitrogen was determined using the method of alkaline hydrolysis diffusion. Soil available phosphorus was determined using Olsen's method. Soil available potassium was extracted with 1.0 mol L\(^{-1}\) NH\(_4\)OAC and determined by flame photometry (FP6400, INESA, China).

2.7. Statistical analysis

After discarding low-quality (\(\leq 0.001\%\)) sequences, sequences were randomly resampled to the same depth (12769 sequences for 16S rRNA genes and 23886 sequences for ITS) for standardizing the sampling efforts, then the sequences were assigned to operational taxonomic units (OTUs) at a 97% nucleotide similarity (Edgar, 2010). Relative abundances of a given taxonomic group were calculated as the number of sequences affiliated to that group divided by the total number of sequences. The species richness estimators (Chao 1 and ACE) and alpha diversities indices (Shannon and Simpson) were calculated by using the R package phyloseq from the resampled OTU abundance matrices. A heatmap of the top 30 classified bacterial and fungal genera of all soil samples was made using RStudio. Non-metric multidimensional scaling (NMDS) based on weighted UniFrac metric matrices was performed to explore the differences in microbial community composition. Permutational multivariate analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM) based on weighted UniFrac metric matrices were also performed to evaluate the significant differences of microbial community composition according to different Se treatments. Redundancy analysis (RDA) was performed in the statistical software (Canoco 5.0) to test the relationship among frequencies of samples, OTUs, and measured soil variables.

One-way ANOVA with multicomparisons by Duncan's test was employed to compare the means among different treatments at \(P \leq 0.05\) in the SPSS 20.0 software. Pearson correlation was used to test the relationship between two response variables. All results were expressed as the means with corresponding standard deviations. Graphs were plotted using Origin 8.1.

2.8. Sequence accession numbers

All raw sequences have been deposited in the NCBI Sequence Read Archive (SRA) database under the BioProject accession number PRJNA563998.

3. Results and analysis

3.1. Morphological characteristics and chlorophyll content

Se treated A. macrophala plants showed significant differences in the morphological characteristics compared to the control (Table 1). The leaf area and leaf width under 10 mg m\(^{-2}\) Se level were significantly higher than that of the control by 15.08% and 15.79% (\(P < 0.05\)), respectively. In contrast, the 20.0 mg m\(^{-2}\) Se level slightly inhibited the growth of A. macrophala (\(P > 0.05\)). However, the foliar application of Se had no significant effect on the plant height or leaf length of A. macrophala compared to the control. All Se treatments remarkably increased the chlorophyll content of A. macrophala leaves (\(P < 0.05\)) (Table 1). Higher chlorophyll content was recorded under the 10 mg m\(^{-2}\) Se level, 1.2-fold of the control, and

| Se treatments | Plant height (cm) | Leaf area (mm\(^2\)) | Leaf length (mm) | Leaf width (mm) | SPAD |
|---------------|------------------|---------------------|-----------------|----------------|------|
| CK            | 46.8 ± 1.3 a     | 1535.6 ± 32.1 c     | 91.5 ± 1.7 a    | 27.1 ± 0.5 c   | 53.5 ± 2.6 b |
| T1            | 45.6 ± 1.6 a     | 1628.1 ± 15.7 b     | 93.2 ± 2.4 a    | 28.1 ± 1.0 bc  | 57.9 ± 4.0 b |
| T2            | 47.9 ± 2.8 a     | 1687.6 ± 33.4 a     | 89.7 ± 3.6 a    | 30.2 ± 2.5 ab  | 62.8 ± 2.9 a |
| T3            | 48.1 ± 1.7 a     | 1751.3 ± 26.0 a     | 89.2 ± 2.2 a    | 31.3 ± 1.1 a   | 64.3 ± 2.7 a |
| T4            | 46.4 ± 1.2 a     | 1767.2 ± 38.6 c     | 88.7 ± 1.6 a    | 28.5 ± 1.1 bc  | 53.6 ± 3.5 b |

The values are the mean ± SD (n = 3). Different letters in the same column indicate significant differences at the \(P < 0.05\) level. CK, T1, T2, T3 and T4 represent 0, 2.5, 5.0, 10.0 and 20.0 mg m\(^{-2}\) Se treatment, respectively.
were followed by 5.0 and 2.5 mg m\(^{-2}\) Se levels which were 1.17-fold and 1.08-fold of the control, respectively.

### 3.2. Survival rate and insect bite area

The 2.5 mg m\(^{-2}\) to 10.0 mg m\(^{-2}\) Se levels increased the survival rate of \(A.\) *macrocephala* by 5.4%-10.7%, but 20.0 mg m\(^{-2}\) Se level decreased the survival rate by 3.6% compared to the control, and showed an increment trend with increasing Se levels as shown in Fig. 2. In particular, Se contents were 42, 105, 193 and 417 times higher than that of the control, indicating that the survival rate was dependent on foliar Se level. All Se treatments decreased the insect bite area of \(A.\) *macrocephala* leaves by 21.8%-57.81% compared with the control (Fig. 1), indicating that foliar application of 2.5–20.0 mg m\(^{-2}\) Se could decrease the pest damage to \(A.\) *macrocephala* leaves. Moreover, the insect bite area was significantly (\(P < 0.05\)) negatively correlated with the foliar Se levels (Fig. S1).

### 3.3. Yield and Se content

Compared to the control, the yield of \(A.\) *macrocephala* was increased by 5.5%–13.5% under Se levels from 2.5 to 10 mg m\(^{-2}\), but decreased by 7.5% under 20.0 mg m\(^{-2}\) Se level (Fig. 2). This demonstrated that Se at a low dose (\(\leq 10.0\) mg m\(^{-2}\)) could increase the yield of \(A.\) *macrocephala*, while Se at high dose (=20.0 mg m\(^{-2}\)) had an opposite effect. All Se treated plants exhibited significantly higher Se content compared to the control, and showed an increment trend with increasing Se levels as shown in Fig. 2. In particular, Se contents in \(A.\) *macrocephala* under 2.5, 5.0, 10.0 and 20.0 mg m\(^{-2}\) Se levels were 42, 105, 193 and 417 times higher than that of the control, respectively. Meanwhile, the Se content of \(A.\) *macrocephala*, respectively, compared with the control. Meanwhile, The Se level of 5.0 mg m\(^{-2}\) increased the accumulation of atractylenolide III and total atractylenolide by 42.1% and 25.5%, respectively, compared with the control (Fig. 3). However, there were no significant differences in the concentration of atractylenolide I, II, III and total atractylenolide between the Se-treated \(A.\) *macrocephala* and control \(A.\) *macrocephala*.

In other words, higher accumulations of atractylenolide in 2.5 mg m\(^{-2}\) and 5.0 mg m\(^{-2}\) Se treatments were due to the increased yield, rather than through the accumulation of higher Se concentrations.

### 3.4. Atractylenolide concentration and accumulation

The Se level of 2.5 mg m\(^{-2}\) increased the accumulation of atractylenolide II, III and total atractylenolide by 45.9%, 38.5% and 28.0%, respectively, compared with the control. Meanwhile, The Se level of 5.0 mg m\(^{-2}\) increased the accumulation of atractylenolide II, III and total atractylenolide by 42.1% and 25.5%, respectively, compared with the control (Fig. 3). However, there were no significant differences in the concentration of atractylenolide I, II, III and total atractylenolide between the Se-treated \(A.\) *macrocephala* and control \(A.\) *macrocephala*.

In other words, higher accumulations of atractylenolide in 2.5 mg m\(^{-2}\) and 5.0 mg m\(^{-2}\) Se treatments were due to the increased yield, rather than through the accumulation of higher Se concentrations.

### 3.5. Soil microbial richness and diversity

Fungal and bacterial richness (Chao1 and ACE indices) and diversity (Shannon and Simpson indices) are summarized in Table 2. Compared to the control, significantly higher bacterial richness and diversity were observed under 10.0 mg m\(^{-2}\) Se treatment (\(P < 0.05\)). The bacterial richness and diversity were increased with increasing Se levels (\(\leq 10.0\) mg m\(^{-2}\)), but were suppressed by 20.0 mg m\(^{-2}\) Se treatment. In contrast, the rhizosphere soil under 10.0 mg m\(^{-2}\) Se treatment exhibited lower fungal richness and diversity (\(P < 0.05\)). The fungal richness and diversity showed decrement trends at Se levels \(\leq 5.0\) mg m\(^{-2}\) and then increased with increasing Se levels (Se \(\geq 10.0\) mg m\(^{-2}\)).

### 3.6. Soil microbial community composition

A total of 582456 V3V4 16S rRNA and 565377 ITS sequences were retained for analysis across the 15 soil samples after discarding low-quality sequences (Table S1). Based on the 97% similarity, a total of 8344 and 1391 OTUs were obtained for 16S rRNA genes and ITS, respectively across all samples. Bacterial sequences were classified into a total of 28 different phyla (Fig. 4), and Chloroflexi (32.13%), Proteobacteria (20.55%), Actinobacteria (14.79%), Acidobacteria (12.45%), WPS-2 (7.95%) and Planctomycetes (4.39%) were the most dominant phyla. Relatively, Proteobacteria, Acidobacteria, WPS-2 and Planctomycetes were more abundant in the Se treated (\(\geq 5.0\) mg m\(^{-2}\)) soil than in the control, while Chloroflexi and Actinobacteria were more...
Fig. 2. Effects of foliar Se application on the yield and Se content of *A. macrocephala*. The values are the mean ± SD (n = 3). Different letters indicate significant differences (P < 0.05) according to ANOVA test. CK, T1, T2, T3 and T4 represent 0, 2.5, 5.0, 10.0 and 20.0 mg m⁻² Se treatment, respectively.

Fig. 3. Effects of foliar Se application on the concentration and accumulation of atractylenolide I (A), II (B), III (C) and total atractylenolide (D) in *A. macrocephala*. The values are the mean ± SD (n = 3). Different letters indicate significant differences (P < 0.05) according to ANOVA test. CK, T1, T2, T3 and T4 represent 0, 2.5, 5.0, 10.0 and 20.0 mg m⁻² Se treatment, respectively.
Table 2
Effects of foliar Se application on the richness and diversity of bacteria and fungi from A. macrocephala rhizosphere soils.

| Se treatments | Chao1     | ACE       | Shannon   | Simpson   |
|---------------|-----------|-----------|-----------|-----------|
| Bacteria      |           |           |           |           |
| CK            | 3240.41 ± 158.23 b | 3358.43 ± 57.89 c | 9.24 ± 0.11 a | 0.997 ± 0.00 a |
| T1            | 3310.43 ± 130.59 ab | 3400.24 ± 116.78 bc | 9.38 ± 0.08 b | 0.997 ± 0.00 a |
| T2            | 3344.38 ± 161.06 ab | 3560.84 ± 92.15 ab | 9.71 ± 0.17 a | 0.997 ± 0.00 a |
| T3            | 3459.16 ± 89.77 a  | 3672.28 ± 108.15 a | 9.84 ± 0.05 a | 0.997 ± 0.00 a |
| T4            | 3149.60 ± 106.45 b | 3249.71 ± 138.56 c | 9.13 ± 0.16 c | 0.951 ± 0.02 b |
| Fungi         |           |           |           |           |
| CK            | 590.81 ± 49.59 a  | 596.23 ± 46.80 a  | 6.06 ± 0.05 a | 0.960 ± 0.00 a |
| T1            | 506.44 ± 6.08 b   | 507.75 ± 6.26 b   | 5.96 ± 0.11 b | 0.943 ± 0.01 a |
| T2            | 414.68 ± 30.32 c  | 423.61 ± 37.00 c  | 4.49 ± 0.17 e | 0.898 ± 0.01 b |
| T3            | 501.62 ± 19.90 b  | 498.91 ± 19.90 b  | 4.82 ± 0.16 d | 0.894 ± 0.01 b |
| T4            | 526.07 ± 59.84 b  | 534.20 ± 69.77 ab | 5.27 ± 0.06 c | 0.900 ± 0.01 b |

The values are the mean ± SD (n = 3). Different letters in the same column indicate significant differences at the P< 0.05 level. CK, T1, T2, T3 and T4 represent 0, 2.5, 5.0, 10.0 and 20.0 mg m⁻² Se treatment, respectively.

Fig. 4. Effects of foliar Se application on the average relative abundances of bacterial and fungal phyla in A. macrocephala rhizosphere soils. Others include phyla below 0.1% relative abundance and the unclassified phyla. CK, T1, T2, T3 and T4 represent 0, 2.5, 5.0, 10.0 and 20.0 mg m⁻² Se treatment, respectively.
prevalent in the soil of the control than that of the Se treatments (≥2.5 mg m⁻²). Fungal OTUs were predominantly associated with the phyla Ascomycota, Basidiomycota and Zygomycota, and these three phyla accounted for 90.04% of the total fungal sequences (Fig. 4). The abundance of Ascomycota under 2.5 mg m⁻² Se treatment was significantly higher than that of the control and other Se treatments. On the other hand, Basidiomycota, Zygomycota and Chytridiomycota were more abundant in soils treated with 10.0 mg m⁻² Se compared to the control, whereas Rozellomycota showed the opposite trends.

At a finer resolution, the comparison of top 30 classified genera revealed obvious differences between different Se treatments (Fig. 5). Based on the cluster analysis of the top 30 bacterial genera, soil samples of 2.5 and 20.0 mg m⁻² Se treatments were separated from soil samples of 5.0 and 10.0 mg m⁻² Se treatments and clustered in the same branch with the control (Fig. 5). The relative abundances of Burkholderia and Cupriavidus were significantly higher in the 5.0 and 10.0 mg m⁻² Se treatments than in the control. The cluster analysis of the top 30 fungal genera showed that soil samples of CK and 2.5 mg m⁻² Se treatment clustered in the same branch and were separated from soil samples of 5.0–20.0 mg m⁻² Se treatments (Fig. 5). The relative abundances of Trichoderma, Humicola and Guehomyces in the 5.0 mg m⁻² Se treatment; Pseudogymnoascus, Mortierella, Boothiomycetes and Cokeromyces in the 10.0 mg m⁻² Se treatment; Dictyocatenulata and Monascus in the 20.0 mg m⁻² Se treatment were significantly higher than in the control. Significant differences in microbial community composition according to different Se treatments were confirmed by PERMANOVA (bacteria, R=0.779, P=0.001; fungi, R=0.884, P=0.001). ANOSIM (bacteria, R=0.720, P=0.001; fungi, R=0.932, P=0.001) (Table S2) and NMDS ordinations (Fig. S3).

3.7 Soil nutrients in rhizosphere soil

In this study, levels of soil organic matter (OM), available nitrogen (AN), available phosphorus (AP) and available potassium (AK) showed significant variations in different Se treatments (Fig. 6). All Se treatments significantly increased the organic matter content in rhizosphere soil of A. macrocephala (Fig. 6A). Compared to the control, 2.5, 5.0, 10.0 and 20.0 mg m⁻² Se treatments increased the organic matter content by 9.0%, 13.9%, 13.6% and 14.1%, respectively. Se treatments at 10.0 mg m⁻² levels remarkably enhanced the available nitrogen content, and was 1.13 times of the control. (Fig. 6B). The available phosphorus content was stimulated by Se treatments of 10.0 mg m⁻² and peaked at 10.0 mg m⁻² Se level (Fig. 6C). Compared to the control, the available phosphorus content under 2.5, 5.0 and 10.0 mg m⁻² Se treatments was enhanced by 8.25%, 2.77% and 15.23%, respectively. However, the available phosphorus content was significantly decreased by 18.01% under the 20.0 mg m⁻² Se treatment. Meanwhile, Se treatments at 5.0 and 10.0 mg m⁻² levels

Fig. 5. Heatmap of the top 30 classified bacterial and fungal genera of all soil samples. CK, T1, T2, T3 and T4 represent 0, 2.5, 5.0, 10.0 and 20.0 mg m⁻² Se treatment, respectively.
significantly increased the available potassium content by 24.82% and
17.25% compared to the control, respectively (Fig. 6D). But, the avail-
able potassium content at 2.5 and 20.0 mg m$^{-2}$ Se levels were
remarkably lower than that of the control.

3.8. Relationship between plant growth, soil nutrients and microbial
community composition

The survival rate showed a significant positive correlation with
the relative abundances of *Burkholderia* and *Cupriavidus* (Table 3).
The insect bite area was significantly negatively correlated with the
Se levels, organic matter (OM) content and available nitrogen (AN)
content. The yield of *A. macrocephala* exhibited a significant positive
correlation with available phosphorus (AP) content and available
potassium (AK) content as well as the relative abundances of *Burkhol-
deria* and *Cupriavidus*. The Se content was significantly positively cor-
related with the Se levels and OM content while exhibited a
significant negative correlation with AP content. The total atractyle-
nolide was significantly negatively correlated with the AN content.
Besides, the *A. macrocephala* yield, as well as the relative abundances
of *Burkholderia* and *Cupriavidus*, were significantly positively corre-
lated with the AK content (Table S3).

As shown by the RDA of microbial OTUs and partial soil nutrients
(Fig. 7), the first two axes explained 71.63% and 27.98% of the total
variation of the bacterial communities and soil nutrients (Fig. 7A),
respectively. For the fungal communities and soil nutrients (Fig. 7B),
the first two axes explained 98.61% and 1.28% of the total variation,
respectively, as evidenced by both the RDA vectors and the highest
significant Mantel-based correlations (Table S4). In this study, the
OM was the most important soil nutrient shaping both bacterial and
fungal community composition.

4. Discussion

4.1. Effects of foliar Se application on the growth of *A. macrocephala*

Previous studies revealed that Se application can affect the mor-
phological, physiological and molecular characteristics of plants
(Ekanayake et al., 2015; Han et al., 2013; Schiavon and Pilon-
Smits, 2017). Generally, Se often exerts a dual effect on plant growth
(Han et al., 2013; Hawrylak-Nowak et al., 2015; Zhu et al., 2017). In
this experiment, Se potentially stimulated the growth of *A. macroce-
phala* at low levels but showed an inhibitory effect at high levels
(Table 1), which was consistent with the findings of previous studies
on *Nicotiana tabacum* L. (Han et al., 2013, 2015), cucumber (Hawry-
lak-Nowak et al., 2015) and *Codonopsis lanceolata* (Zhu et al., 2017).
The leaf area and the leaf width were significantly increased by 5.0
and 10.0 mg m$^{-2}$ Se treatments, but under 20.0 mg m$^{-2}$ Se level, the
leaf area was significantly decreased (Table 1), demonstrating the
dual effects of Se on *A. macrocephala* growth.

Chlorophyll is the basis for photosynthesis and carbon assimila-
tion in the metabolic process of plants. In this study, both 5.0 and
10.0 mg m$^{-2}$ Se levels significantly improved the chlorophyll content
of *A. macrocephala*, but showed a decrement trend at 20.0 mg m$^{-2}$ Se
level (Table 1), indicating that appropriate Se treatments could promote photosynthesis in *A. macrocephala*. Our result was consistent with previous findings on strawberry (Zahedi et al., 2019), *Ulva fasciata* (Zhong et al., 2015) and *Gracilaria lemaneiformis* (Wang et al., 2019). The reason might be that the suitable Se levels promoted the uptake of mineral elements which were related to the synthesis of chlorophyll (Lin et al., 2012) and thus increased the chlorophyll content.

Additionally, Se treatment at 5.0 mg m$^{-2}$ level significantly increased the survival rate of *A. macrocephala* compared with the control, indicating that reasonable Se levels were beneficial for the growth of *A. macrocephala* (Fig. 1). Interestingly, Se treatments (2.5–20.0 mg m$^{-2}$) could effectively reduce the insect bite area of *A. macrocephala* leaves (Fig. 1). In our opinion, the most likely reason was that selenium salts which were taken up by *A. macrocephala* would make the *A. macrocephala* so treated poisonous to certain pests that attacked them and thus reduced the incidence of pests (Use of Selenium for Pest Control. Nature, 1948, 162(4109), 176–176. doi:10.1038/162176a0).

4.2. Effects of foliar Se application on the yield, Se content and atractylenolide concentration of *A. macrocephala*

Previous studies revealed that yield was the best index for evaluating growth state of plants (Zhu et al., 2017). In this study, foliar Se application of $\leq$ 10.0 mg m$^{-2}$ level increased the yield of *A.

![Table 3](image-url)

**Table 3**

| Se level | Organic matter | Available nitrogen | Available phosphorus | Available potassium | Burkholderia | Cupriavidus |
|----------|----------------|--------------------|----------------------|---------------------|---------------|-------------|
| Survival rate | -0.074 | 0.443 | 0.205 | 0.284 | 0.474 | 0.577* | 0.603* |
| Insect bite area | -0.860* | -0.845* | -0.540* | 0.246 | -0.083 | -0.193 | -0.022 |
| Yield | -0.384 | 0.276 | 0.391 | 0.743* | 0.677* | 0.664* | 0.710* |
| Se content | 0.999* | 0.013* | 0.266 | -0.605* | -0.286 | -0.193 | -0.341 |
| Total atractylenolide | 0.231 | 0.280 | -0.516* | -0.441 | -0.363 | -0.019 | -0.057 |

* indicates significant correlations at the $P < 0.05$ level; ** indicates significant correlations at the $P < 0.01$ level.
macrocephala but decreased when Se level reached 20.0 mg m\(^{-2}\) (Fig. 2), indicating that Se was beneficial at low levels but harmful at high levels for plant growth (Han et al., 2015; Hawrylak-Nowak et al., 2015; Zhu et al., 2017). Our results were consistent with the previous studies, which reported that increasing the levels of Se applied to soil or as foliar spray could induce an increase of Se content in crop plants (Han et al., 2013; Hawrylak-Nowak et al., 2015; Hu et al., 2002). In our study, the total Se concentration in *A. macrocephala* increased in a dose-dependent manner after application of foliar Se (Fig. S2). Previous studies demonstrated that the Se application could significantly increase the quantity of bioactive compounds in *Codonopsis lanceolata* (Zhu et al., 2017), *Lycium chinense* (Dong et al., 2012) and *Ganoderma lucidum* (Gasecka et al., 2016). In this study, although 2.5 mg m\(^{-2}\) and 5.0 mg m\(^{-2}\) Se treatments could increase the accumulation of atracylolidine III and total atracylolidine, all the Se treatments had no significant effect on the concentration of atracylolidine I, II, III and total atracylolidine in *A. macrocephala* compared with the control (Fig. 3), which indicated that the metabolic process of atracylolidine in *A. macrocephala* had little relationship with foliar Se application.

4.3. Effects of foliar Se application on soil nutrients and microbial community composition

Plant and soil are important components of terrestrial ecosystems, they are dependent on each other in plant growth and soil development processes. Rhizosphere soil is an important environmental site for plants to absorb nutrients; changes of nutrient content in rhizosphere soil will directly affect plant nutrient absorption, and consequently affect the growth of plants (Zhang et al., 2016b). In this study, foliar application of 10.0 mg m\(^{-2}\) Se significantly increased the organic matter, available nitrogen, available phosphorus and available potassium content in the rhizosphere soil of *A. macrocephala* (Fig. 6). This result was similar to the previous study that foliar application of 5.0–15.0 mg m\(^{-2}\) Se (based on the application of organic manure) could increase the available potassium content in the rhizosphere soil of *A. macrocephala* (Zhou et al., 2020). Bacteria in the rhizosphere soil which can promote plant growth are defined as plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 2004). We presumed that the effects of foliar Se application on partial rhizospheric soil nutrients content of *A. macrocephala* might depend on the activities of PGPRs, since some PGPRs might fix nitrogen and activate minerals in plant rhizosphere soil (Kim et al., 2012) and enhance the formation of root exudates by improving plant photosynthesis (Timmusk et al., 2014), as is known that over 40% of the carbon fixed by photosynthesis is released with root exudates, and 11% of them is left in the rhizodeposition (Hütsch et al., 2002; Zang et al., 2015). *Burkholderia* which consists of many plant-associated microorganisms can improve the growth of plants (Dobritsa and Samadpour, 2016). *Cupriavidus* including the β-rhizobium *Cupriavidus taiwanensis*, has the symbiotic ability with legumes (Amadou et al., 2008). In this study, the relative abundances of *Burkholderia* and *Cupriavidus* were significantly positively correlated with the survival rate and yield of *A. macrocephala* (Table 3), indicating that *Burkholderia* and *Cupriavidus* might act as potential PGPRs in promoting *A. macrocephala* growth. It was noteworthy that the available potassium content was significantly positively correlated with *A. macrocephala* yield (Table 3) and the relative abundances of *Burkholderia* and *Cupriavidus* (Table S3), which indicated that the rhizospheric soil available potassium content might be a critical factor in the rhizome development of *A. macrocephala*.

It has been reported that plant growth and soil health are related to the diversity of soil microorganisms (Shen et al., 2018). In this study, we found that the yield and survival rate of *A. macrocephala* were positively correlated with soil bacterial diversity, but negatively correlated with soil fungal diversity (Table S5), indicating that bacterial diversity had a beneficial effect on the growth of *A. macrocephala*, whereas fungal diversity had the opposite effect. In this study, both 5.0 and 10.0 mg m\(^{-2}\) Se levels significantly increased bacterial diversity (ACE and Shannon indices) in the rhizosphere soil of *A. macrocephala*, but showed a decrement trend at 20.0 mg m\(^{-2}\) Se level. By contrast, the fungal diversity in these Se treatments showed opposite results (Table 2), suggesting that microbial diversity in rhizosphere soil is closely related to plant growth (Shen et al., 2018).

Generally, plants are known to determine microbial populations in the rhizosphere soil, and soil nutrient is a driving force that directly affects the microbial community composition and diversity (Cookson et al., 2008; Shen et al., 2018). In this study, we found that soil organic matter was the main environmental factor affecting both bacterial and fungal community composition in the rhizosphere soil of *A. macrocephala*. Moreover, we found that available nitrogen and available potassium were also important factors shaping fungal community composition (Table S4).

5. Conclusion

This study demonstrated the effects of foliar Se application on the growth of *A. macrocephala*. Application of 5.0–10.0 mg m\(^{-2}\) foliar Se (sprayed monthly from May to August) had a prominent positive effect on the growth of *A. macrocephala*. When the Se level increased,
the Se content in A. macrocephala was increased, while the insect attack rate was decreased. The application of foliar Se had no significant effect on the concentration of atracyleneolide in A. macrocephala, although the foliar application of 2.5–5.0 mg m⁻² Se could increase the accumulation of total atracyleneolide. Additionally, foliar application of specific Se level (e.g., 10.0 mg m⁻²) could change the measured soil nutrients and alter the microbial diversity and composition in the rhizosphere soil of A. macrocephala. We speculated that the available potassium, Burkholderia and Cupriavidus in rhizosphere soil might play a profound role in promoting the growth of A. macrocephala. Our work will be of great significance for the development of selenium-enriched medicinal plants and understanding the Se biofortification of A. macrocephala from plant growth, rhizospheric soil nutrient and microbial community composition.

Declaration of Competing Interest

We declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

We would like to thank the anonymous reviewers for their insightful comments and advice on this manuscript. This work was supported by the Technical Innovation Program of Hubei Province (2019YZYD064); and the Science and Technology Plan of Hubei Province (2019AKB092).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2020.09.032.

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