1 **Analysis of rhizosphere bacterial diversity of *Angelica***
2 ***dahurica* var. *formosana* based on different experimental**
3 **sites and varieties (strains)**

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Abstract

Only with good producing areas and good germplasm can good medicinal materials be produced. In this study, in order to explore the effects of soil environment and germplasm on yield and quality of *Angelica dahurica* var. *formosana* from microorganism level, Illumina MiSeq was used to study the rhizosphere bacterial diversity of *A. dahurica* var. *formosana* based on different origin and varieties (strains). The results showed that the bacterial community of *A. dahurica* var. *formosana* was stable and conserved to a certain extent, and the bacteria of *Proteobacteria* and *Firmicutes* might play an important role in improving the yield and quality of *A. dahurica* var. *formosana*. Soil variables were the key factors affecting the rhizosphere bacterial community composition of *A. dahurica* var. *formosana*. These results are of great significance for further understanding the growth promotion mechanism of beneficial rhizosphere bacteria, reducing fertilizer application, expanding planting area, improving soil environment, and improving yield and quality.

Importance

Rhizosphere microorganisms play an important role in plant growth and accumulation of secondary metabolites. In this study, it was found for the first time that the rhizosphere bacterial community of *A. dahurica* var. *formosana* was relatively stable and conserved which could adapt to different environmental changes and resist

the interference of external factors to a certain extent, providing a theoretical basis for distant planting and promotion of planting. The bacterial taxa of *Proteobacteria* and *Firmicutes* might play an important role in improving the yield and quality of *A. dahurica* var. *formosana*. This provided a theoretical basis for the exploitation and utilization of rhizosphere microbial resources of *A. dahurica* var. *formosana* to artificially control the soil environment and provided a new idea for increasing yield and quality of *A. dahurica* var. *formosana*.

Introduce

Rhizosphere is a specific microecosystem of soil-root-microorganism interaction, which is formed by different plant, soil and environmental conditions. Meanwhile, the rhizosphere microbial community is also considered to be the second genome of plants (3). Plant roots can customize their rhizosphere microbial community by producing secretions, which in turn can affect plant growth through mineral nutrient absorption, antagonism, nutrition and spatial site competition, soil improvement and plant induced system resistance generation (8). Meanwhile, the rhizosphere microbial community construction is affected by a variety of biotic and abiotic factors. The specific factor that plays a decisive role varies by the soil type and plant species. The stronger the environmental screening effect of which factor is, the greater the relative contribution to the microbial community construction may be (25). Therefore, understanding the key factors affecting the composition and construction of beneficial bacterial communities in the rhizosphere may be a sustainable and effective way to

actively manage soil microbial communities, improve soil conditions, reduce the application of pesticides and fertilizers, and promote plant growth (13).

Angelica dahurica var. *formosana* (known as Baizhi in Chinese) produced in Sichuan province is a famous Genuine Medicines, particularly in the Suining region of Sichuan province. It does not only serve for medicinal use in curing colds, headaches, nasal congestion, runny nose, toothache, but also possesses high commercial value for its wide adoption in the production of food, health care products, spices, skin care, beauty and other aspects. Modern pharmacological studies have found that coumarins, as the main index components of *A. dahurica* var. *formosana*, have antiinflammatory (12), anti-bacterial (33), vasodilator (39), anti-cancer (19), antiviral and antioxidant effects (2), and are regarded as one of the most important indicators to measure the quality of *A. dahurica* var. *formosana*. The *A. dahurica* var. *formosana* produced in Suining of Sichuan Province has a long history of cultivation with high yield and quality. However, some conditions during planting *A. dahurica* var. *formosana*, like early bolting, shrinking production areas, and abuse of pesticides and fertilizers are leading to its decline of the total yield of in recent years.

In order to solve the problem of production decline while ensuring quality, we could look for methods from a completely new angle, the beneficial bacterial community in the rhizosphere. Explore the secret of high yield and quality from rhizosphere from good origin to excavate the beneficial bacterial community structure. Meanwhile, plants with better germplasm will recruit more suitable microbe to promote their own growth. The development and utilization of rhizosphere bacteria

resources of *A. dahurica* var. *formosana* will be a good method to improve the soil environment, reduce the application of fertilizer to a certain extent, and expand the planting region.

Therefore, this study aims to characterize bacterial communities in the rhizospheric soils of *A. dahurica* var. *formosana* from different experimental sites or varieties (strains) under the same culture management method by using molecular methods. This study may be a cornerstone of further research on biological factors to improve the quality of *A. dahurica* var. *formosana*. The objects of the present study are (i) to characterize the rhizospheric bacterial community in the samples of *A. dahurica* var. *formosana* from different sites or varieties (strains); (ii) to predict the related bacterial community closely related to yield and quality of *A. dahurica* var. *formosana*; (iii) to determine the contribution of experimental sites and its own varieties (strains) differences to the construction of beneficial bacterial community, and confirm the importance of good origins and good germplasm resources in cultivation from the point of rhizospheric bacteria.

MATERIALS AND METHODS

Experimental design. One of the main producing region of *A. dahurica* var. *formosana* is Suining in Sichuan province, China. In previous experiments, our research group has bred several varieties (strains) of *A. dahurica* var. *formosana* which were named as BZA001, BZA002, BZA003, BZA004, BZB002 and BZB003.

In order to explore the effects of different planting sites of the same varieties

(strains) on rhizosphere bacteria of *A. dahurica* var. *formosana*, BZA001 was used as the test material, four different *A. dahurica* var. *formosana* planting sites in Suining and one in Chongzhou as the control with the same cultivation and management methods were investigated. The samples of five different experimental sites were named as XA (Shunjiang, Suining), XB (Shunhe, Suining), XC (Sangshulin, Suining), XD (Yongyi, Suining), XJ (Chongzhou, Chengdu). Meanwhile, in order to explore the rhizospheric bacteria of different varieties (strains) in the same site, six varieties (strains) of *A. dahurica* var. *formosana* were also sampled in one of sites in Suining (Yongyi, Suining), which were named as XD(BZA001), XE(BZA002), XF(BZA003), XG(BZA004), XH(BZB002) and XI(BZB003), respectively. All the *A. dahurica* var. *formosana* experimental materials were sown in late September, 2018. 750 kg/hm of superphosphate and 120 kg/hm of potassium phosphate were applied as base fertilizer. In December, the first topdressing application of urea was 66 kg/hm. The second topdressing application of urea 165 kg/hm was carried out in February, 2019. The third topdressing was carried out in March, 2019, applying urea 99 kg/hm, superphosphate 750 kg/hm and potassium sulfate 8 kg/hm. Plots within the field were established in randomized blocks design with each plot consisting of four rows (5 m long, 3 m wide) and three replicated plots per treatment.

All *A. dahurica* var. *formosana* samples were collected in harvest time (sown in September, 2018 and harvested in July, 2019). the five-point sampling method was used, representative and robust *A. dahurica* var. *formosana* were selected, the excess bulk soils were shaken off, and the soils that remained attached to the roots were

considered to be rhizospheric soils (30). Soil samples were respectively preserved at -80 °C and 4 °C before used. Meanwhile, the roots of *A. dahurica* var. *formosana* were brought back after sampling the soil.

Soil physicochemical properties. Soil samples were collected from the rhizosphere of *A. dahurica* var. *formosana* from different experimental sites or varieties (strains) for the determination of physicochemical properties. The soil pH was measured with a pHB-8 pen type acidity meter in a 1:2.5 soil/water (W/V) suspension. The OM was measured by potassium dichromate volumetric method. Soil TN was determined by Kjeldahl method. Soil TP was determined by sodium hydroxide solution-Mo-Sb anti spectrophotometric method. Soil TK was measured by acid fusion-flame photometric method. The HN in the soil was determined by alkali hydrolysis diffusion method. The AP was obtained by using the sodium hydrogen carbonate solution-Mo-Sb anti spectrophotometric method. The available potassium was obtained by ammonium acetate extraction - flame photometric method.

Yield and active component contents of *A. dahurica* var. *formosana*. Yield was estimated by the ratio of total fresh weight of root to area in each plot. Selected the representative root of *A. dahurica* var. *formosana* with the same growth trend, washed and water-removing at 105°C for 15 minutes, then dried at 55°C to a constant weight. The active components of *A. dahurica* var. *formosana* were coumarins. The contents of imperatorin and isoimperatorin of *A. dahurica* var. *formosana* were determined respectively according to the Chinese Pharmacopoeia (2020 edition, part I) (6).

Soil DNA extraction and MiSeq sequencing.

Genomic DNA of soil samples were extracted by method of CTAB or SDS. The DNA concentration and purity were detected by agarose gel electrophoresis, and then an appropriate amount of samples were diluted with sterile water to 1ng/μL. The diluted genomic DNA was used as the template. The primers with Barcode for the 16S V3-V4 region primers were 341F: CCTAYGGGRBGCASCAG, and 806R: GGACTACNNGGGTATCTAAT. PCR amplification was carried out using Phusion ® High-Fidelity PCR Master Mix with GC Buffer of New England Biolabs and high-fidelity enzyme to ensure the amplification efficiency and accuracy.

PCR products were detected by electrophoresis with 2% agarose gel. PCR products were mixed in equal amounts according to concentration. PCR products were purified by agarose gel electrophoresis with 1×TAE concentration of 2%, and the target bands were cut and recovered. The product purification kit USES GeneJET gel recovery kit to recover the product from Thermo Scientific company. Ion Plus Fragment Library Kit 48 rxns from Thermofisher was used for Library construction. After Qubit quantification and Library detection, Thermofisher Ion S5TMXL was used for computer sequencing. Raw reads were obtained from the reads by using Cutadapt (1) to shear low quality sequences and preliminary quality control. Raw Reads were detected by comparison with the species annotation database and chimeric sequences removal (26) and filtered to obtain Effective Tages.

Statistical analysis.

Sequences of clean reads with ≥97% similarity were assigned to the same OTUs (Operational Taxonomic Units). According to the

algorithm, the sequence with the highest frequency was selected as the OTU representative sequence. According to the algorithm, the sequence with the highest frequency was selected as the OTU representative sequence. Species annotation analysis was carried out by Mothur method and SSUrRNA database (the threshold value was set as 0.8~1), acquire information taxonomy and respectively in each classification level: kingdom, phylum, class, order, family, genus, species counted the community composition of each sample (9, 10, 40). The samples were homogenized with the least amount of data as the standard. The Observed-OTUs, Chao1, Shannon, Simpson, ACE, Goods-coverage, PD whole tree were calculated by QIIME (Version 1.9.1). R (Version 2.15.3) was used to analyze the differences between groups of alpha diversity index and draw PCoA chart. In the analysis of species differences between groups, LEFSE software was used for LEFSE analysis. The filter value of LDA Score was set as 4 by default, and software R was used for T-test. In Spearman correlation analysis, Corr.test function in R was firstly used to calculate Spearman correlation values of species and environmental factors and test its significance, and then heatmap function was used for visualization.

TABLE 1 The physicochemical properties in the rhizospheric soil of *A. dahurica* var. *formosana* from different experimental sites and varieties (strains).

sample name	Abbreviation	pH ^a	OM (g/kg) ^a	TN (g/kg) ^a	TP (g/kg) ^a	TK (g/kg) ^a	HN (mg/kg) _a	AP (mg/kg) ^a	AK (mg/kg) ^a
Rhizosphere of BZA001 from Chongzhou, Chengdu	XJ	7.1±0.06c	22.70±1.19 a	1.48±0.02a	0.52±0.03c	14.59±0.19b	80.50±0.00a	17.69±1.89a	195.62±4.66 a
Rhizosphere of BZA001 from Shunjiang, Suining	XA	7.8±0.15b	7.11±0.40b c	0.65±0.01b	0.98±0.01a	15.45±0.00a b	32.67±1.01c	13.71±1.01b	122.45±1.71 b
Rhizosphere of BZA001 from Shunhe, Suining	XB	7.9±0.00ab	7.34±1.05b	0.61±0.02c	0.96±0.05a	16.15±0.61a	42.00±1.75b	14.89±1.65b	30.24±2.24d
Rhizosphere of BZA001 from Sangshulin, Suining	XC	8.0±0.12a	5.04±1.05d	0.51±0.02d	0.73±0.03b	14.91±0.67b	29.75±3.03c	6.03±0.76c	14.18±1.71e
Rhizosphere of BZA001 from Yongyi, Suining	XD	8.0±0.06a	5.50±0.69c d	0.33±0.22e	0.58±0.05c	13.41±0.49c	17.50±1.75d	4.42±0.25c	79.89±3.42c
Rhizosphere of BZA001 from Yongyi, Suining	XD	8.0±0.06b	5.50±0.69a	0.33±0.02a b	0.58±0.05a	13.41±0.49b	17.50±1.75d	4.42±0.246b c	79.89±3.42b
Rhizosphere of BZA002 from Yongyi, Suining	XE	8.2±0.10a	5.27±1.05a	0.23±0.03b	0.55±0.01a b	14.48±0.00a	28.00±1.75b	5.28±0.61a	123.57±2.82 a
Rhizosphere of BZA003 from Yongyi, Suining	XF	7.9±0.06b	5.50±0.69a	0.41±0.10a	0.57±0.03a	14.54±0.09a	32.08±2.67a	4.74±0.406a b	78.77±2.33b
Rhizosphere of BZA004 from Yongyi, Suining	XG	8.1±0.00a	2.06±0.00c	0.28±0.03a b	0.49±0.03b	14.38±0.19a	21.58±1.01c	3.83±0.246c	31.73±2.59d
Rhizosphere of BZB002 from Yongyi, Suining	XH	8.1±0.12a	3.67±0.79b	0.32±0.04a b	0.52±0.04a b	14.38±0.09a	13.42±1.01e	5.22±0.406a	75.78±2.33b c
Rhizosphere of BZB003 from Yongyi, Suining	XI	8.2±0.06a	4.36±0.40a b	0.26±0.12b	0.42±0.03c	13.84±0.32b	15.17±1.01d e	4.58±0.186a b	70.93±3.42c

^a Different letters in the same column indicate significant differences (P<0.05) among treatments based on Duncan's mean test.

TABLE 2 The yields and active component contents of *A. dahurica* var. *formosana* from different experimental sites and varieties (strains)

sample name	Abbreviation	Yield (kg/hm) ^a	Imperatorin (mg/g) ^a	Isoimperatorin (mg/g) ^a
Rhizosphere of BZA001 from Chongzhou, Chengdu	XJ	9171.25±11 7.91d	2.83±0.31a	1.03±0.22ab
Rhizosphere of BZA001 from Shunjiang, Suining	XA	30268.46±8 74.37b	2.35±0.20b	1.34±0.30a
Rhizosphere of BZA001 from Shunhe, Suining	XB	25809.57±3 89.84c	2.23±0.17b	0.98±0.13b
Rhizosphere of BZA001 from Sangshulin, Suining	XC	29808.23±9 28.05b	1.63±0.16c	0.94±0.07b
Rhizosphere of BZA001 from Yongyi, Suining	XD	33603.46±5 60.42a	1.50±0.10c	0.85±0.09b
Rhizosphere of BZA001 from Yongyi, Suining	XD	33603.46±5 60.42e	1.50±0.11b	0.85±0.10abc
Rhizosphere of BZA002 from Yongyi, Suining	XE	43064.86±2 13.23a	1.86±0.23ab	0.89±0.10ab
Rhizosphere of BZA003 from Yongyi, Suining	XF	39646.48±7 68.77b	1.63±0.32b	0.93±0.13ab
Rhizosphere of BZA004 from Yongyi, Suining	XG	30478.57±4 25.13f	2.22±0.24a	1.08±0.12a
Rhizosphere of BZB002 from Yongyi, Suining	XH	37685.50±6 56.50c	1.69±0.32ab	0.63±0.12c
Rhizosphere of BZB003 from Yongyi, Suining	XI	36231.44±3 11.96d	1.58±0.50b	0.83±0.16bc

^a Different letters in the same column indicate significant differences (P<0.05) among treatments based on Duncan's mean test.

RESULTS

The physicochemical properties of rhizospheric soil of *A. dahurica* var. *formosana*. Overall, shown in Table 1, the rhizospheric soil nutrients in Chongzhou were significantly higher than that in Suining from different experimental sites. The rhizospheric soil pH value of Chongzhou was neutral (7.1), and the others from

200 Suining were alkaline (pH value from 7.8-8.0). Previous results showed that the pH of
 201 *A. dahurica* var. *formosana* soil in Suining producing area was about 8.49-8.88 (43),
 202 indicating that the rhizosphere soil of *A. dahurica* var. *formosana* was acidified, and
 203 the pH of soil was about 0.49-1.08 lower than that of non-rhizosphere soil. The
 204 organic matter (OM) content of Chongzhou rhizospheric soil is triple to tenfold that of
 205 other rhizospheric soils in Suining, up to 22.7 g/kg. OM of Suining's rhizosphere soil
 206 varied from 5.04 to 7.34 g/kg. The rhizosphere soil total nitrogen (TN) value varied
 207 greatly, showing a 4.48-fold change (0.33-1.48 g/kg). Chongzhou had the highest TN
 208 (1.48 g/kg), while Yongyi of Suining had the lowest TN (0.23 g/kg). The rhizosphere
 209 soil total phosphorus (TP) value varied from 0.52 to 0.98g/kg, with the highest in
 210 Shunjiang (0.98 g/kg) and Shunhe (0.96 g/kg), and the lowest (0.52 g/kg) in
 211 Chongzhou. Rhizosphere soil total potassium (TK) had a small variation range
 212 (13.41-16.15 g/kg), with the highest (16.15 g/kg) in Shunhe and the lowest (13.41
 213 g/kg) in Yongyi. The rhizosphere soil hydrolysable nitrogen (HN) value changed
 214 greatly, showing a 4.60-fold change (17.5-80.50 mg/kg). The HN of rhizospheric soil
 215 of *A. dahurica* var. *formosana* from Chongzhou was the highest (80.50 mg/kg), while
 216 that of Yongyi was the lowest (17.50 mg/kg). The rhizosphere soil available
 217 phosphorus (AP) value changed greatly, showing a 4.00-fold change (4.42-17.69
 218 mg/kg), with the highest from Chongzhou (17.69 mg/kg), and the lowest from Yongyi
 219 (4.42 mg/kg). The rhizospheric soil available potassium (AK) value changed greatly
 220 from 14.18 to 195.62mg/kg with the highest from Chongzhou (195.62 mg/kg) and the
 221 lowest from Sangshulin (14.18 mg/kg).

Among all varieties (strains), overall, BZA002 had the highest rhizosphere nutrients of *A. dahurica* var. *formosana*, while BZA004 had the lowest. The pH value changed little (7.9-8.2). The OM of Yongyi varied from 2.06-5.50 g/kg, among which BZA004 had the lowest (2.06 g/kg). The TN value changed little (0.23-0.41 g/kg). The TP value varied from 0.42 to 0.58g/kg, of which BZB003 was the lowest (0.42 g/kg). TK had a small variation range (13.41-14.54 g/kg). The HN value of rhizospheric soil was low (13.42-32.08 mg/kg), of which BZA003 was the highest (32.08 mg/kg) and BZB002 was the lowest (13.42 mg/kg). The AP value was low (3.83-5.28 mg/kg), too. The rhizospheric soil AP of BZA002 was the highest (5.28 mg/kg), while that of BZA004 was the lowest (3.83 mg/kg). The AK value changed greatly from 31.73 to 123.5 mg/kg, of which BZA002 was the highest (123.57 mg/kg) and BZA004 was the lowest (31.73 mg/kg).

The yields and active component contents of *A. dahurica* var. *formosana* from different experimental sites and varieties (strains). For different experimental sites of the same varieties (strains) of *A. dahurica* var. *formosana*, the yield in Suining was about 3-4 times that of Chongzhou, although the active component contents of *A. dahurica* var. *formosana* of Suining were not as high as that of Chongzhou (Table 2). To be specific, the yield of *A. dahurica* var. *formosana* from Yongyi was the highest (33603.46kg/hm), and that from Chongzhou was the lowest (9171.25kg/hm). The imperatorin content of *A. dahurica* var. *formosana* in Chongzhou was the highest (2.83 mg/g), and that of Yongyi was the lowest (1.50 mg/g). The isoimperatorin content in Shunjiang was the highest (1.34 mg/g), followed by Chongzhou (1.03

mg/g), and Yongyi was the lowest (0.85 mg/g).

For different varieties (strains) of *A. dahurica* var. *formosana* from the same experimental sites, the yield of BZA002 was the highest (43064.86 kg/hm) and that of BZA004 was the lowest (30478.57 kg/hm). The imperatorin content of BZA004 was the highest (2.22 mg/g), and that of BZA001 was the lowest (1.50 mg/g). The isoimperatorin content of BZA004 was the highest (1.08 mg/g) and BZB002 the lowest (0.63 mg/g).

Bacterial diversity in the rhizospheric soil of *A. dahurica* var. *formosana*.

Through comparison with database SILVA132, species annotation and statistics of different classification levels, a total of 16,557 OTUs were found, of which 16,557 (100.00%) could be annotated to the database. As shown in Table 3, for the same varieties (strains), the rhizosphere bacterial diversity of *A. dahurica* var. *formosana* in Chongzhou experimental site with high nutrients and low yield was the lowest, while the other four experimental sites in Suining were significantly higher. Among them, the rhizosphere bacterial diversity from Yongyi with the lowest nutrients was the highest. For different varieties (strains) of *A. dahurica* var. *formosana*, the rhizospheric bacterial diversity of BZA002 with high nutrients and yield was less than that of the others from Yongyi, while the bacterial diversity of BZB003 was the highest. Spearman correlation analysis with soil nutrients showed that, except for AK, the rhizosphere diversity of *A. dahurica* var. *formosana* was significantly negatively correlated with soil nutrients (Fig S1).

266 TABLE 3 Diversity indices of the bacterial community in rhizospheric soil samples of *A. dahurica* var. *formosana* from different experimental
267 sites and varieties (strains).

sample name	Abbreviation	OTU (97%)	observed_species	shannon	chao1	ACE	goods_coverage	PD_whole_tree
Rhizosphere of BZA001 from Chongzhou, Chengdu	XJ	4991	4401	10.376	4937.15 9	5000.70 3	0.984	349.038
Rhizosphere of BZA001 from Shunjiang, Suining	XA	6494	5631	10.876	6395.73 1	6510.05 4	0.977	450.13
Rhizosphere of BZA001 from Shunhe, Suining	XB	6509	5638	10.878	6404.07 3	6547.94	0.978	440.314
Rhizosphere of BZA001 from Sangshulin, Suining	XC	6277	5491	10.792	6212.51 8	6325.26 6	0.978	438.82
Rhizosphere of BZA001 from Yongyi, Suining	XD	7190	6191	10.963	7123.93 7	7311.16 9	0.973	489.036
Rhizosphere of BZA001 from Yongyi, Suining	XD	7190	6191	10.963	7123.93 7	7311.16 9	0.973	489.036
Rhizosphere of BZA002 from Yongyi, Suining	XE	6596	5651	10.636	6520.35 9	6721.56 6	0.975	445.364
Rhizosphere of BZA003 from Yongyi, Suining	XF	7331	6306	11.115	7218.09 2	7424.80 7	0.973	489.384
Rhizosphere of BZA004 from Yongyi, Suining	XG	7138	6097	10.67	7048.73 7	7231.26 5	0.973	476.472
Rhizosphere of BZB002 from Yongyi, Suining	XH	7066	6062	10.939	7017.95 1	7177.50 8	0.973	472.764
Rhizosphere of BZB003 from Yongyi, Suining	XI	7744	6630	11.178	7600.35	7791.42 5	0.971	502.896

Relative abundances of rhizospheric bacterial communities in *A. dahurica*

var. formosana. In the rhizosphere soil of *A. dahurica var. formosana*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* were the dominant phylum (Fig 1). For different experimental sites, sample from Yongyi (XD) had the highest levels of *Proteobacteria* sequences(49.1%) and the lowest *Acidobacteria*(6.6%) and *Rokubacteria* sequences(1.1%). The proportions of *Firmicutes* and *Actinobacteria* in the rhizosphere soil samples of *A. dahurica var. formosana* from Chongzhou were significantly higher than those in other Suining sites, showing ratios of 7.2% and 8.1%. For different varieties(strains) of *A. dahurica var. formosana* from Suining, the proportion of *Proteobacteria* was highest in the rhizosphere of BZA002 and lowest in the rhizosphere of BZA004. The proportion of *Acidobacteria* was highest in the rhizosphere of BZA002 and lowest in the rhizosphere of BZB003. The proportion of *Actinobacteria* was lowest in the rhizosphere of BZA001. The proportion of *Verrucomicrobia* was lowest in the rhizosphere of BZA004.

At the genus level, we selected the 35 of the most abundant genera to generate a mapping image (Fig. 2). The abundance of the sorting results, ordered from high to low, were *Bacteroides*, *Flavobacterium*, unidentified *Clostridiales*, *Anaeromyxobacter*, *Sphingomonas*, *Faecalibacterium*, *Polycyclovorans*, unidentified *Acidobacteria*, *Haliangium*, *Terrimonas*, etc. *Anaeromyxobacter*, *Sphingomonas*, *Polycyclovorans* and *Haliangium* belonged to the phylum *Proteobacteria*. A higher proportion of the genera *Haliangium*, *Anaeromyxobacter* and unidentified *Clostridiales* was detected in the rhizospheric bacteria of BZA001 from Chongzhou (XJ) with the highest nutrients.

290 *Sphingomonas*, *Flavobacterium* and *Terrimonas* accounted for a higher proportion
 291 among the high yield BZA002 rhizosphere bacteria (XE). *Faecalibacterium* and
 292 *Bacteroides* accounted for a higher proportion among the high yield BZA004
 293 rhizosphere bacteria (XG).

294
 295 FIG. 1 Relative species abundance at phylum level in bacterial communities (A) from different
 296 sites and (B) from different varieties (strains). Based on the species annotation results, we selected
 297 to examine the relative abundance distribution of the species among the top-ranked phyla, the
 298 relative abundance distribution of the top ten species is shown.

299 **PCoA cluster analysis in the rhizospheric soil bacteria of *A. dahurica* var.**
 300 ***formosana*.** Comparison of PCoA among treatment groups was conducted to explore
 301 the similarity and difference of community composition among different groups. The
 302 results showed that there were significant differences among all groups (Fig 3, Table
 303 S1). For different experimental sites of the same varieties (strains) of *A. dahurica* var.

304 *formosana*, the explanatory variances of principal component 1 and principal
305 component 2 were 27.85% and 15.79% respectively. The rhizosphere bacterial
306 community of *A. dahurica* var. *formosana* in Chongzhou (XJ) had the largest
307 difference, followed by that in Yongyi of Suining (XD). The rhizosphere bacterial
308 community of Shunjiang (XA), Shunhe (XB) and Sangshulin (XC) experimental sites
309 were similar. For different varieties (strains) of *A. dahurica* var. *formosana* from the
310 same experimental sites, the explanatory variances of principal component 1 and
311 principal component 2 were 10.57% and 8.56%, respectively. The rhizospheric
312 bacterial communities of BZA002 and BZA004 were the most different from those of
313 other varieties (strains). The results indicated that there were significant differences
314 among the experimental groups in the selected experimental sites and the background
315 of varieties (strains). Moreover, there were significant differences between Suining
316 and Chongzhou. Among them, the dominant factors of experimental sites affecting the
317 rhizosphere bacterial community structure of *A. dahurica* var. *formosana* were more
318 obvious.

319

320

321 FIG. 2 Generate a mapping image from different sites and from different strains based on
322 abundance in formation. To examine the abundance information available, we selected genera
323 represented by the 35 most abundant sequences to draw a species abundance cluster heatmap.
324 Abscissae indicate species annotation information, and ordinates indicate sample information.
325 Clustering tree on the left side of the figure is a sample cluster tree. Above the figure is a species
326 tree clustering and the results of the significance test of the species in different groups.

FIG 3 Principal co-ordinates analysis (PCoA). The main elements and structures were extracted from the multi-dimensional data by eigenvalue and eigenvector sorting, and the main coordinate combination with the largest contribution rate was selected for drawing and display.

FIG 4 Linear discriminant analysis effect size (LEfSe) method identifies the significantly different abundant taxa (a) from different experimental sites and (b) from different varieties (strains) in all of the rhizospheric soil samples of *A. dahurica* var. *formosana*. The groups with significant differences in relative abundance among treatments were represented by colored dots, which represented the level of boundary, phylum, class, order, family and genus from the center outward. The size of the colored dots was proportional to the relative abundance. The biomarker of different species followed the group for staining, represented by "a:", "b:", "c:", etc.

FIG 5 The LDA scores of the soil from different experimental sites(a) and different varieties (strains) (b) in each treatment from the LEfSe analysis. The figure shows species with significant difference in different (LDA value greater than 4.0 species with difference), and the length of the bar chart represents the impact of significantly different species.

LEfSe analysis in the rhizospheric soil bacteria of *A. dahurica* var. *formosana*. The biomarkers with significant differences in the rhizosphere samples of *A. dahurica* var. *formosana* from different sites or different varieties (strains) through LEfSe analysis were revealed (Fig 4), and linear discrimination analysis (LDA) values were given in Fig 5. The results showed that at the phylum level, for different experimental sites, enrichment of *Planctomycetes* were significant in the rhizospheric soil of BZA001 from Shunjiang (XA); enrichment of *Acidobacteriia* were significant in the sample from Shunhe (XB); enrichment of *Roteobacteria* were significant in the sample from Sangshulin (XC); *Proteobacteria* contributed the most in the sample from Yongyi (XD) with the highest yield of *A. dahurica* var. *formosana*, while *Firmicutes* and *Actinobacteria* contributed the most in the sample from Chongzhou (XJ) with the highest active component content of *A. dahurica* var.

formosana. There were 21 biomarkers with an LDA score >4, including *Gammaproteobacteria*, *Anaeromyxobacter*, *Sphingomonadales*, *Rokubacteria*, *Longimicrobiales*, *Firmicutes*, etc (Fig 4A , 5A). These biomarkers could be considered to represent the key bacterial taxa shaping the rhizosphere bacterial community of *A. dahurica* var. *formosana*.

From the same experimental sites, the major divergent species of different varieties (strains) of *A. dahurica* var. *formosana* rhizospheric soil were also differed. For example, *Gemmatimonadetes* contributed the most in the bacterial community of BZA001 rhizospheric soil (XD); *Proteobacteria* contributed the most in the bacterial community of BZA002 rhizospheric soil (XE) with the highest yield of *A. dahurica* var. *formosana*; *Firmicutes* contributed the most in the bacterial community of BZA004 rhizospheric soil (XG) with the highest active component content of *A. dahurica* var. *formosana*; *Actinobacteria* contributed the most in the bacterial community of BZB003 rhizospheric soil (XI). There were 8 biomarkers with an LDA score>4, including *Sphingomonadales*, *Firmicutes*, *Alphaproteobacteria*, *Longimicrobiaceae*, *Actinobacteria*, *Proteobacteria*, etc (Fig 4b, 5b).

Combined with the results of BZA001 rhizospheric bacterial from five experimental sites, it was found that *Proteobacteria* had a significant influence on the rhizosphere bacterial community of the samples with higher yield, and *Firmicutes* had a significant influence on the rhizosphere bacterial community of the samples with higher content of active components, regardless of whether the influencing factors were from the differences of experimental sites or varieties (strains). In addition to the

379 phylum level, Sphingomonadaceae was found to be significantly enriched in soils
380 with high nutrients and low α diversity.

381

382

383 FIG 6 Spearman correlation heatmap analysis of the correlation between soil factors and species
384 abundance of bacterial communities in rhizospheric soil of *A. dahurica* var. *formosana* at phylum
385 level. The vertical is environmental factor information, and the horizontal is species abundance
386 information. The value corresponding to the middle heat map is Spearman correlation coefficient r ,
387 which is between -1 and 1. “*” indicates significant difference ($P < 0.05$).

388

389 **Correlation analysis between environmental physicochemical factors and**
390 **bacterial taxa.** The results of spearman correlation heatmap analysis showed that, in
391 general, the physicochemical properties of rhizospheric soil (except for AK) were
392 closely related to bacterial taxa, especially AP and TN exerted the greatest impacts
393 upon microbial community composition (Fig 6). The abundance of *Acidobacteria*
394 ($r=0.5694$) and *Rokubacteria* ($r=0.6158$) were most closely related to AP, and that of

Acidobacteria ($r=0.6209$) and *Rokubacteria* ($r=0.6653$) were also most closely related to TN. Moreover, the abundance of *Proteobacteria* was significantly negatively correlated with AP($r=-0.4811$) and TN ($r=-0.5790$), and abundance of *Firmicutes* was significantly positively correlated with HN ($r=0.4895$) and TN ($r=0.4927$). The abundance of *Actinobacteria* was only negatively correlated with TP ($r=-0.3783$), while the abundance of *Verrucomicrobia* was positively correlated with AK ($r=0.4758$).

Discussion

Analysis of microbial diversity and community composition using high-throughput sequencing technology is becoming more and more common (24). To our knowledge, more and more studies had been conducted on the interaction between medicinal plants and their rhizosphere microorganisms, especially those that could improve the yield and quality of medicinal plants (16, 17, 18, 34, 41). Thus, this new sequencing technique was used for the first time to analyze the bacterial community structure in the rhizosphere soil of *A. dahurica* var. *formosana*.

It was possible that *A. dahurica* var. *formosana* recruited less beneficial bacteria when the soil was rich in nutrients and more bacteria when it was hungry based on the correlation analysis of bacterial diversity and physicochemical properties of rhizosphere samples. In this study, the content of active components of *A. dahurica* var. *formosana* in each group was far higher than the industry standard. The yield of *A. dahurica* var. *formosana* in Suining is 3-4 times that of Chongzhou, although the

content of active components in *A. dahurica* var. *formosana* in Suining is lower than that in Chongzhou. By comparison, *A. dahurica* var. *formosana* from Suining, the traditional main producing area, is still the best, while its rhizosphere nutrient content is lower. Mutualism exists between plants and a variety of microorganisms and provides more than half of a plant's nutritional needs (14). In this experiment, except AK, soil physicochemical properties were negatively correlated with bacterial diversity. Chaudhary found that rhizospheric bacteria could significantly improve the utilization rate of soil N, P and K, replacing the deficiency of soil nutrients to a certain extent (5). To our knowledge, when the phosphorus content in soil was too low, legumes would stimulate the enrichment of AMF in the rhizosphere by secreting isoflavones to promote nutrient absorption (23). Meanwhile, when soil phosphorus content is too high, plants can obtain the optimal nutrient supply without AMF symbiosis instead of preferring symbiosis (11). Therefore, ensuring bacterial diversity in the rhizosphere of *A. dahurica* var. *formosana* may be a new idea for fertilizer reduction.

There may be some different secretions in the root of BZA002, which activated the nutrient availability of rhizospheric soil and promotes the growth of *A. dahurica* var. *formosana* more than other varieties (strains). At the same experimental site, the soil nutrient availability was the highest in the rhizosphere of BZA002 with highest yield, and the lowest in the rhizosphere of BZA004 with lowest yield. Such differences in rhizosphere soil nutrients are caused by differences in germplasm. Different varieties secrete different root exudates, which lead to differences in

recruitment of beneficial bacteria and nutrient uptake by the root system (44). Yang found that there were significant differences in the activation ability of soil K by tobacco root exudates with different genotypes (42). Li pointed out that compared with the general genotype, the root exudates of grain amaranth with potassium-enrichment genotype had higher organic acid content, which could significantly activate the mineral potassium in the soil (21). Shen found that kidney beans with different genotypes could secrete plenty of organic acids to activate Al-P in the rhizosphere under P deficiency conditions (15).

Imperatorin might be one of the reasons for the decrease of rhizosphere microbial diversity of the highly active ingredient *A. dahurica* var. *formosana*. The main active components in *A. dahurica* var. *formosana* were coumarins and essential oils, and coumarins mainly include imperatorin and isoimperatorin. It was found that regardless of the difference of origin or germplasm, the variation trend of imperatorin was inverse direction to that of bacterial diversity. To our knowledge, Coumarins in *A. dahurica* var. *formosana* had antibacterial activity, which could recruit more phosphate-solubilizing microorganisms (32) and had inhibitory effect on a variety of pathogens (27).

Proteobacteria, *Firmicutes* and *Bacteroidetes* were dominant phylum of *A. dahurica* var. *formosana*, accounting for 47.6%-66.4% of all bacterial DNA sequences. These eutrophic bacteria can mineralize soil nutrients, enhance the absorption of plant nutrients, and promote plant growth (36). This is similar to the dominant rhizospheres of healthy *Panax notoginseng* and licorice (35, 45). Regardless of the difference of

experimental tests or varieties (strains), *Proteobacteria* were all the dominant phyla in the rhizosphere of *A. dahurica* var. *formosana*, and the dominant species at the genus level were also similar. The results indicated that the rhizosphere bacterial community of *A. dahurica* var. *formosana* was stable and conserved, and could resist some external disturbances to a certain extent, which provided a theoretical basis for remote planting and expanding the planting.

Acidobacteria and *Rokubacteria* were prevalent in the rhizosphere of *A. dahurica* var. *formosana*. There were a variety of gene clusters for the synthesis of antibiotics, antifungins, ferritin and immunosuppressants in these bacteria (7). In the similar environment of the four experimental sites in Suining, the bacterial diversity (XD) was high when the content of *Acidobacteria* and *Rokubacteria* in the rhizosphere was low, suggesting that these bacteria with antibacterial activity might inhibit the colonization of other bacteria in the rhizosphere of *A. dahurica* var. *formosana*. Due to continuous cropping obstacles, the rhizosphere microflora of *P. notoginseng* and other peanut species changed from “bacterial-type” to “fungal-type” during continuous cropping period, and the bacterial community of *Proteobacteria* and other bacteria showed a downward trend (22, 35). No significant continuous cropping obstacles were found in *A. dahurica* var. *formosana*, which might be due to its antibacterial active components and bacterial community, which inhibited the occurrence of soil-borne diseases.

Proteobacteria and *Firmicutes* might play a role in increasing yield and active ingredient content. By LEfSe analysis of five experimental sites (XA-XB-XC-XD-XJ),

we found that there were 21 taxa of differential distributions with LDA score >4. Among these biomarkers, *Proteobacteria* enrichment in the *A. dahurica* var. *formosana* rhizospheric soil of high-yield site (XD) was significant, while *Firmicutes* and *Actinobacteria* enrichment in the rhizospheric soil of high-active components of *A. dahurica* var. *formosana* (XJ). Therefore, *Proteobacteria*, *Firmicutes* and *Actinobacteria* might be closely related to yield and content in terms of origin factors. By LEfSe analysis of six varieties (strains) of *A. dahurica* var. *formosana* (XD-XE-XF-XG-XH-XI), there were 8 taxa of differential distributions with LDA score >4. *Proteobacteria* is significantly enriched in the rhizosphere of high-yield BZA002. *Firmicutes* was significantly enriched in the rhizosphere with high content of active components of BZA004. *Actinobacteria* was significantly enriched in the rhizosphere with low-active components of BZB003. In general, *Proteobacteria* were always significantly enriched in the rhizosphere of the high-yield samples, while *Firmicutes* were always significantly enriched in the rhizosphere of the high-active component of *A. dahurica* var. *formosana*, regardless of the difference of experimental tests or varieties (strains).

Interestingly, *Sphingomonadaceae* were found in differential taxas with LDA score>4 for soil environmental differences or genotypic differences. *Sphingomonadaceae* was significantly enriched in the samples with the highest soil nutrients and the lowest bacterial diversity. Many strains of *Sphingomonadaceae* can degrade polycyclic or monocyclic aromatic compounds, such as benzoic acid salicylic acid, and use these aromatic compounds as carbon sources for growth (31). Lin

believed that allelopathic rice could secrete more aromatic compounds to induce the enrichment of these bacteria under chemical chemotaxis (20). *A. dahurica* var. *formosana* contained a large number of aromatic compounds. Similarly, a similar allelopathy may exist in *A. dahurica* var. *formosana*. The increasing of active components in *A. dahurica* var. *formosana* caused more aromatic compounds to be secreted from the root, recruiting more *Sphingomonadaceae* and anti-bacteria. Further studies on root exudates of *A. dahurica* var. *formosana* should be carried out to verify this hypothesis.

Understanding the key factors affecting the rhizosphere bacterial construction of *A. dahurica* var. *formosana* is an effective method to actively regulate the establishment of beneficial rhizosphere bacterial community, improve the rhizosphere environment and promote plant growth. As we know, in addition to suitable cultivation and management methods, good germplasm and suitable environment were crucial influencing factors for good medicinal plants. The rhizosphere bacterial communities of plants were also affected by these factors. Assembly of the rhizosphere microbial community was controlled by a complex interaction between microorganisms, plant hosts, and the environment (37). Among these factors, the stronger the environmental screening effect of which factor is, the greater the relative contribution to microbial community construction is likely to be (4).

The suitable origin and germplasm did have significant influence on the construction of rhizosphere bacterial community of *A. dahurica* var. *formosana*. PCoA indicated that the rhizosphere bacterial community of *A. dahurica* var.

formosana showed significant differences among different experimental sites and varieties (strains). This confirmed the importance of suitable origin and germplasm for cultivation of medicinal plants from the perspective of rhizosphere bacteria, which was of great significance for improving the yield and quality of *A. dahurica* var. *formosana*.

Compared with germplasm resources, suitable planting site was the key factor for the construction of rhizosphere microbial community of *A. dahurica* var. *formosan*, which was of great significance for future development and application to improve the yield and quality. The four sites of Suining were all located along the coast of the Fujiang River and shared the similar climate. Excepted for the Chongzhou test site (XJ) with different climate, the differences among experimental sites were still slightly greater than the differences among varieties (strains). The results indicated that the rhizosphere bacterial community of *A. dahurica* var. *formosana* was more affected by soil in this study. Previous studies had shown that for many plants, genotype had a significant influence on the bacterial community in the aboveground part, especially perennial plants, while the rhizospheric bacterial community is mostly affected by soil factors (38). The difference of sorghum varieties had little effect on the rhizosphere bacteria, while the soil was the main factor (29). Automated ribosomal intergenic spacer analysis (ARISA) was used to investigate 9 rice varieties treated with different nitrogen levels. It was found that the varieties had a significant effect on the bacterial community of the stem and bud of rice, while the bacterial community of the root was mainly affected by the nitrogen level in the paddy field (28). Similarly,

the rhizosphere bacterial community of *A. dahurica* var. *formosana* was significantly affected by soil of origin.

In addition to differences in soil nutrients, the original soil bacterial community might be another soil factor that affected the rhizosphere bacteria of *A. dahurica* var. *formosana*. When De Ridder-Duine studied the composition of rhizosphere and non-rhizosphere bacteria in different soils, he found that the structure of rhizosphere bacterial community was largely determined by the non-rhizosphere soil bacterial community (25). In other words, the original soil bacterial community played an important role in the construction of plant rhizosphere bacteria.

In general, this research results would be helpful in improving the cultivation and management of *A. dahurica* var. *formosana*. The results showed that the bacterial community of *A. dahurica* var. *formosana* was stable and conserved to a certain extent, and the bacteria of *Proteobacteria* and *Firmicutes* might play an important role in improving the yield and quality of *A. dahurica* var. *formosana*. The development and utilization of the rhizosphere microbial resources of *A. dahurica* var. *formosana* provided a new idea for further increasing production and quality and reducing the application of pesticide and fertilizer. The importance of germplasm resources and origin for the cultivation of *A. dahurica* var. *formosana* was discussed from the perspective of rhizosphere bacteria for the first time, among which the origin was the key factor affecting the composition of rhizosphere bacterial community. Soil conditions of origins would become a crucial factor to construct beneficial bacterial community structure in the cultivation of *A. dahurica* var. *formosana*, which laid a

theoretical foundation for expanding the planting area in the future. However, further experiments were needed to explore the key influencing factors of soil factors, such as soil fertility, soil original microbiome, etc.

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Appendixes

TABLE S1 Analysis of molecular variance of rhizosphere bacterial community of *A. dahurica* var. *formosana*

	SS	df	MS	Fs	P-value
weighted_unif					
rac_amova	1.0465(0.554279) ^a	9(20) ^a	0.116278(0.027714) ^a	4.19563	<0.001**
unweighted_u					
nifrac_amova	1.7329(1.48762) ^a	9(20) ^a	0.192544(0.0743811) ^a	2.58861	<0.001**

^a In parentheses were the corresponding values of the residuals.

** indicates extremely significant difference between groups ($P < 0.01$).

585

586 FIG S1 Spearman correlation heatmap analysis of the correlation between soil factors and
587 diversity indices of bacterial communities in rhizospheric soil of *A. dahurica* var. *formosana*. The
588 vertical is environmental factor information, and the horizontal is diversity index. The value
589 corresponding to the middle heat map is Spearman correlation coefficient r , which is between -1
590 and 1. “*” indicates significant difference ($P < 0.05$).

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592 References

- 593 1. Aßhauer K, Bernd W, Rolf D, Peter M. 2015. Tax4Fun: predicting functional profiles from
594 metagenomic 16S rRNA data. J Bioinform 31: 2882-2884.
- 595 2. Bai Y, Li D, Zhou T, Qin N, Li Z, Yu Z, Hua H. 2016. Coumarins from the roots of *Angelica*
596 *dahurica* with antioxidant and antiproliferative activities. J Funct Foods 20: 453-462.
- 597 3. Berendsen RL, Pieterse C, Bakker P. 2012. The rhizosphere microbiome and plant health.
598 Trends Plant Sci 17: 478-486.
- 599 4. Chaparro JM, Badri DV, Vivanco JM. 2014. Rhizosphere microbiome assemblage is affected
600 by plant development. ISME J 8: 790-803.
- 601 5. Chaudhary DR, Rathore AP, Sharma S. 2019. Effect of halotolerant plant growth promoting

rhizobacteria inoculation on soil microbial community structure and nutrients. Appl Soil Ecol 150: 103461.

6. Chinese Pharmacopoeia Commission. 2020. Chinese Pharmacopoeia (2020 edition, part I). China Medical Science Press 109-110.

7. Crits-Christoph, Alexander, Diamond, Spencer, Butterfield, Cristina N., Thomas, Brian C. 2018. Novel soil bacteria possess diverse genes for secondary metabolite biosynthesis. Nature 558.

8. Dutta S, Podile AR. 2010. Plant growth promoting rhizobacteria (PGPR): the bugs to debug the root zone. Crit Rev Microbiol 36: 232-244.

9. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 5: 1792-1797.

10. Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10: 996-998.

11. Ferrol N, Azcón-Aguilar C., Pérez-Tienda J. 2018. Review: Arbuscular mycorrhizas as key players in sustainable plant phosphorus acquisition: An overview on the mechanisms involved. Plant Sci 280: 441-447.

12. Garcíaargáez A, Apan T, Delgado HP, Velázquez G, Martínezvázquez M. 2000. Anti-inflammatory activity of coumarins from *Decatropis bicolor* on TPA ear mice model. Planta Med 66: 279-281.

13. Glick BR. 2010. Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28: 367-374.

14. Heijden M, Bruin S, Luckerhoff L, Logtestijn RSP, Schlaeppi K. 2016. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling

624 recruitment. ISME J 10: 389-399.

625 15. Hong S, Yan X, Ming Z, Zheng S, Wang X. 2002. Exudation of organic acids in common
626 bean as related to mobilization of aluminum- and iron-bound phosphates. Environ Exp Bot 48:
627 1-9.

628 16. Horinouchi S. 2009. Combinatorial biosynthesis of plant medicinal polyketides by
629 microorganisms. Curr Opin Chem Biol 13: 197-204.

630 17. Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R,
631 Panneerselvam R. 2007. *Pseudomonas fluorescens* enhances biomass yield and ajmalicine
632 production in *Catharanthus roseus* under water deficit stress. Colloids Surf B Biointerfaces 60:
633 7-11.

634 18. Karthikeyan B, Jaleel CA, Lakshmanan GM, Deiveekasundaram M. 2008. Studies on
635 rhizosphere microbial diversity of some commercially important medicinal plants. Colloids Surf B
636 Biointerfaces 62: 143-145.

637 19. Kim YK, Kim YS, Shi YR. 2010. Antiproliferative effect of furanocoumarins from the root of
638 *Angelica dahurica* on cultured human tumor cell lines. Phytother Res 21: 288-290.

639 20. Lin HF. 2010. Analysis on function microorganism in the rhizosphere of allelopathic rice
640 under different water conditions. Fu Jian Nong Ling Da Xu.

641 21. Li TX, Ma GR, Zhang XZ. 2006. Root exudates of potassium-enrichment genotype grain
642 Amaranth and their activation on soil mineral potassium. Ying Yong Sheng Tai Xue Bao 03:
643 3368-3372.

644 22. Li XG, Ding CF, Zhang TL, Wang XX. 2014. Fungal pathogen accumulation at the expense of
645 plant-beneficial fungi as a consequence of consecutive peanut monoculturing. Soil Biol Biochem.

646 72: 11-18.

647 23. Mathesius HU. 2012. The role of flavonoids in root-rhizosphere signalling: opportunities and
648 challenges for improving plant-microbe interactions. J Exp Bot 63: 3429-3444.

649 24. Monard, Gantner, and Stenlid 2013. Utilizing ITS1 and ITS2 to study environmental fungal
650 diversity using pyrosequencing. FEMS Microbiol Ecol 84: 165-175.

651 25. Ridder-Duine A, Kowalchuk GA, Gunnewiek P, Smant W, Veen J, Boer WD. 2005.
652 Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is
653 determined by bulk soil community composition. Soil Biol Biochem 37: 349-357.

654 26. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source
655 tool for metagenomics. Peer J 4: e2584.

656 27. Rosselli S, Maggio A, Bellone G, Formisano C, Bruno M. 2006. Antibacterial and
657 anticoagulant activities of coumarins isolated from the flowers of *Magydaris tomentosa*. Planta
658 Med. 73: 116-120.

659 28. Sasaki K, Ikeda S, Ohkubo T, Kisara C, Sato T, Minamisawa K. 2013. Effects of plant
660 genotype and nitrogen level on bacterial communities in rice shoots and roots. Microbes
661 Environments 28: 391-395.

662 29. Schlemper TR, Leitev M, Lucheta AR, ShNat Rev Microbiolmels M, Kuramae EE. 2017.
663 Rhizobacterial community structure differences among sorghum cultivars in different growth
664 stages and soils. FEMS Microbiol Ecol 93: 1-11.

665 30. Smalla K, Cresswell N, Mendonca-Hagler LC, Wolters A, Elsas J. 1993. Rapid DNA
666 extraction protocol from soil for polymerase chain reaction-mediated amplification. J Appl
667 Bacteriol 74: 78-85.

668 31. Story SP, Kline EL, Hughes TA, Riley MB, Hayasaka SS. 2004. Degradation of aromatic
669 hydrocarbons by *Sphingomonas paucimobilis* strain EPA505. Arch Environ Contam Toxicol 47:
670 168-176.

671 32. Stringlis IA, Ronnie D, Pieterse C. 2019. The age of coumarins in plant–microbe interactions.
672 Plant Cell Physiol 7: 1405-1419.

673 33. Suleimenov EM. 2009. Components of *Peusedanum morisonii* and their antimicrobial and
674 cytotoxic activity. Chem Nat Compd 45: 710-711.

675 34. Sutthinan K, Akira Y, Saisamorn L. 2009. L-Asparaginase production by actinomycetes
676 isolated from some Thai medicinal plant rhizosphere soils. Int J Integr Biol 6: 22-26.

677 35. Tan Y, Cui Y, Li H, Kuang A, Li X, Wei Y, Ji X. 2017. Diversity and composition of
678 rhizospheric soil and root endogenous bacteria in *Panax notoginseng* during continuous cropping
679 practices. J Basic Microbiol. 57: 337-344.

680 36. Tian P, Razavi BS, Zhang X, Wang Q, Blagodatskaya E. 2019. Microbial growth and enzyme
681 kinetics in rhizosphere hotspots are modulated by soil organics and nutrient availability. Soil Biol
682 Biochem 141: 107662.

683 37. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant-microbiome interactions: from
684 community assembly to plant health. Nat Rev Microbiol 18: 607-621.

685 38. Wagner MR, Lundberg DS, Rio TG, Tringe SG, Dangl JL, Mitchell-Olds T. 2016. Host
686 genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat Commun 7:
687 12151.

688 39. Wang C, Wang T, Huang L, Lu W, Zhang J, He H. 2016. Synthesis and fluorescent study of
689 5-phenyl furocoumarin derivatives as vasodilatory agents. Bioorg Med Chem Lett 26: 640-644.

690 40. Wang Q. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the
691 new bacterial taxonomy. Appl Environ Microbiol 73: 5261-5267.

692 41. Wu L, Wang H, Zhang Z, Lin R, Zhang Z, Lin W,Anthony GJ. 2011. Comparative
693 metaproteomic analysis on consecutively rehmannia glutinosa-monocultured rhizosphere Soil.
694 PLoS One 6: e20611.

695 42. Yang T. 2009. K nutrition in rhizosphere and characteristics of roots of flue-cured tobaccos
696 different in genotype. Tu Rang Xue Bao 280: 441-447.

697 43. Zhai JY Wu W, Liao K, Zhang X, Hou K. 2010. Effects of soil factors on yield and quality of
698 *Angelica dahurica* var. *formosana*. Zhong Cao Yao 41: 984-988.

699 44. Zhang R, Vivanco JM, Shen Q. 2017. The unseen rhizosphere root-soil-microbe interactions
700 for crop production. Curr Opin Microbiol 37: 8-14.

701 45. Zhang ZZ, Ma M, Zhang XL. 2017. Effect of subsoiling on soil nutrient and microbe
702 functional diversity of *Glycyrrhiza uralensis* rhizospheres. Sheng Tai Xue Bao 15: 218-226.