A comparative study of bacterial diversity based on effects of three different shade shed types in the rhizosphere of *Panax quiquefolium* L.

Xianchang Wang[1,*], Xu Guo[1,*], Lijuan Hou[2], Jiaohong Zhang[1], Jing Hu[2], Feng Zhang[1], Jilei Mao[2], Zhifen Wang[1], Congjing Zhang[1], Jinlong Han[1], Yanwei Zhu[1], Chao Liu[1], Jinyue Sun[1], Chenggang Shan[1]

[1] Key Laboratory of Novel Food Resources Processing, Ministry of Agriculture and Rural Affairs/Research Center of Medicinal Plant, Shandong Academy of Agricultural Sciences/Institute of Agro-Food Science and Technology, Shandong Academy of Agricultural Sciences, Jinan, Shandong, PR China
[2] Weihai Academy of Agricultural Sciences, Weihai, Shandong, PR China

[*] These authors contributed equally to this work.

Corresponding Author:
Chenggang Shan[1]
202 Gongye North Road, Jinan, Shandong, 250100, PR China
Email address: shanchenggang@126.com

Abstract

Background. Shading treatment is an important factor affecting the cultivation of American ginseng（*Panax quiquefolium* L.）, and has a great influence on its quality and yield. Rhizosphere microorganisms is also one of the important factors for plant normal growth and development. It is not clear whether different shade shades can cause significant changes in rhizosphere microorganisms of American ginseng.

Methods. In this study, the rhizosphere soil samples of American ginseng under traditional shade shed, high flag shade shed and high arch shade shed were selected, respectively. The communities change of rhizosphere bacteria were studied by high-throughput 16S rRNA gene sequencing.

Results Under different shading conditions, the microbial diversity in rhizosphere soil of American ginseng was changed significantly. The bacteria diversity in high arch shed was more abundant than that in flat shed and traditional shed. A number of bacteria showed significantly different abundance in genera, including *Bradyrhizobium*, *Rhizobium*, *Sphingomonas*, *Streptomyces* and *Nitrospira*. Different shading conditions also cause the changes of microbial metabolic function in rhizosphere soil of American ginseng. The three kinds of shade sheds have their own specific functional groups. Functional enrichment analysis of bacterial microbiota revealed consistently increased abundance of ATP-binding cassette (ABC) transporters. The results are helpful to understand the influence of shading system on rhizosphere microecology of American ginseng, contribute to the cultivation of American ginseng, and to promote the sustainable development of medicinal plant industry.

Introduction

American ginseng (*Panax quinquefolius* L.) is a perennial understory herb belonging to the Araliaceae family *(Cruse-Sanders & Hamrick 2004)*. It has been used to improve the overall health of human beings by boosting vitality, improving the immune system and protecting against stress. It also used as a traditional medicine for a multitude pharmacological functions such as anti-inflammatory, anti-cancer activities, anti-diabetes, obesity treatment, and enhancement of the cardiocerebral vascular system and central nervous system *(Izzo 2009; Li et al. 2010; Poddar et al. 2011; Tsao & Liu 2007; Wang et al. 2007)*. American ginseng originated in the eastern part of North America *(Nadeau & Olivier 2003)*, and it was introduced to China in the 1980s *(Qin et al. 2018)*. At present, the American ginseng planting area has exceeded 10,000 ha in northern China *(Jiao et al. 2019)*.

In native areas, American ginseng grows as an understory plant in deciduous and mixed forests *(Punja 2011)*. American ginseng prefers mild and humid climate conditions. The latitude of origin of American ginseng is similar to that of many areas in China, but there are differences in rainfall and altitude. Generally, higher air humidity is conducive to the cultivation of American ginseng. The planting site should be well ventilated, loose and beneficial to water, and avoid water logging. Temperature has different effects on the growth stages of American ginseng. The temperature of the whole growth period of American ginseng is between 10-28℃, otherwise, the yield and quality of American ginseng will be affected *(Nadeau & Olivier 2003)*. As a typical shade plant, American ginseng is very strict with light. It likes oblique light, scattered light and fears strong light. Direct light around noon can lead to photoinhibition of photosynthesis, photobleaching, and leaf death *(Proctor & Palmer 2017)*. Because of its ecological habit of loving shade, it is necessary to set up artificial shade to adapt the planting conditions *(Hongpeng et al. 2018)*. The types of shade shed are variety. According to permeability, it is divided into total shade shed, single transparent shed and double transparent shed. By height, it contains high shed and low shed. The roof structures mainly involve arch, slope, flat and arch. Types of supporting materials include wood, metal, cement and bamboo pole. Shading materials are mainly straw cover, wood board, cloth, reed curtain and sunshade net. Compared with the simplex structure shed, duplex structure shed appears.

Rhizosphere refers to the narrow region in the soil that is in contact with and directly affected by plant roots. The exchange of inorganic and organic matter between roots and soil occurs through rhizosphere, which is the key to the normal plants growth and development *(Broeckling et al. 2008; Schmidt et al. 2019)*. Plants also alter the rhizosphere to better adapt to the changing environment *(Ryan et al. 2001)*. Large numbers of microorganisms live in the rhizosphere, forming a complex community of plant-associated microorganisms, which is considered to be closely related to the plant health *(Berendsen et al. 2012)*. Microorganisms not only provide many functional activities for plants to ensure their productivity and health, but also cause plant diseases as pathogens *(Chisholm et al. 2006)*. Plant-associated microorganisms, known as the second genome of plants, have attracted extensive attention in recent years *(Berg et al. 2014; Turner et al. 2013)*. In cultivation of American ginseng as a main development trend at present, shading treatment is a main factor for ginseng growth and development, playing a decisive role in economic yield and biological yield of American ginseng. This raises the question of whether different shade sheds cause the changes in rhizosphere microorganism of American ginseng.

In this research, the 16S rRNA sequences were analyzed in bacteria to estimate the diversity of American ginseng root rhizosphere under the effects of three different shade shed types. A large number of bacterial genera have been found in rhizosphere soil, and the abundance of bacteria in rhizosphere soil under three shade shed types has some different. The obtained results should be useful for understanding the effects of shading systems on rhizosphere microecology, contribute to the cultivation of American ginseng, and improve the sustainable development of the medicinal industry.

Materials & Methods

General situation of this experiment

The experiment was carried out in farmland ginseng experimental area of Hushan Township, Weihai City, Shandong Province, China (122°27′ E, 36°96′ N). Weihai is one of the main production areas of American ginseng in China. It belongs to the temperate continental monsoon climate and is also affected by the marine climate. Four distinct seasons, mild in winter and cool in summer are the remarkable climatic characteristics of Weihai city. The highest monthly average temperature is 27.2 ℃ and occurs in August． With global warming, in recent 55 years, the annual average temperature of Weihai has shown a significant increasing trend, with a climate tendency rate of 0.347 ℃/10A. Extreme high temperature weather began to appear in summer in this area, which seriously affected the cultivation of American ginseng. Therefore, farmers began to use shade shed to ensure the yield and quality of American ginseng. The shade sheds adopted in this study are traditional shade sheds (ctp), high flag shade sheds (pp) and high arch shade sheds (gp) respectively (Fig. 1). The traditional shed is a flat shed with wooden structure, shed height of 1.6m. On the basis of the traditional shed, the flat shed and the high arch shed have been built. The high flat shed is also a flat shed, but the supporting materials is alloy structure, with a height of 2.2m. The high arch shed is arch shed, alloy structure, shed height of 2.6m. The shading materials of the three sheds are all double-layer shading nets. The experiment took place between 2016 and 2018. Temperature and humidity in the shade shed and soil from April to October in 2018 (Table S1)，characteristics of these soil samples (Table S2) and yield and disease status of American ginseng (Table S3) are shown in Supplement file.

Soil collection and DNA extraction

At the end of October 2018, while ginseng root was manually harvested, surface soil of

0-10 cm was sampled. Under each type of shed, multiple sampling locations were selected and collected. One part of each sample was stored at -80°C for subsequent DNA extraction. Another portion were homogenized and sieved with a 2-mm mesh sieve to remove large pieces of debris, air-dried and used for chemical analysis.

Total DNA was extracted from 0.5g of well-mixed soil foreach sample using the Z.N.ATM Mag-Bind Soil DNA Kit (Omega Bio-Tek) following the manufacturer's instruction. DNA was separated and visually tested for quality by 0.8% (w/v) agarose gel electrophoresis. The concentration of DNA was measured with a NanoDrop^{TM} 2000 spectrophotometer (Thermo Scientific). DNA extracts were preserved at -20°C used for PCR.

PCR amplification and sequencing

Bacterial amplicon library was obtained for Illumina sequencing using the primer combination 338F (5'-ACTCCTACGGGAGGCAGCA-3') *(Wu et al. 2016)* and 806R (5'-GGACTACHVGGGTWTCTAAT-3') *(Wu et al. 2016)*, which target the V3-V4 region of the 16S rRNA gene. Each 25μl PCR reaction mixture contained 0.25μl Q5 High-Fidelity DNA Polymerase (New England Biolabs, USA), 5μl 5×Q5 Reaction Buffer, 0.5μl 10mM dNTP, with each primer in the reaction mixture being supplied at a concentration of 0.5μM. DNA was added depending on concentrations as inferred from gel electrophoresis and was modified accordingly with ddH$_2$O to reach the final volume. Cycling conditions included initial denaturation at 98°C for 1 min; followed by 30 cycles of denaturation at 98°C, each cycle lasting 15s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; a final extension phase was performed at 72°C for 5 min. The PCR products were separated on a 2%(w/v) agarose gel, purified using the AxyPrep^{TM} DNA Gel Extraction Kit (Axygen, USA), and quantifed using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA).

The amplicon libraries were constructed using TruSeq Nano DNA LT Library Prep Kit (Illumina) according to the manufacturer's protocol. The qualified libraries were sequenced on an Illumina MiSeq platform and 300 bp paired-end reads were generated. The data of raw reads have been deposited into the NCBI Sequence Read Archive under accession number PRJNA662686.

Sequencing data analysis, OTU production and annotation

The original paired-end reads, cutting off the barcode and primer sequences, were merged using FLASH V1.2.7 *(Magoč & Salzberg 2011)*. Split reads were sorted into each sample by the unique barcodes using QIIME V.1.8.0 *(Bokulich et al. 2013; Caporaso et al. 2010)*. Use USEARCH (v5.2.236, http://www.drive5.com/usearch/) to detect and remove the chimera sequences.

The effective tags with ≥97% similarity were assigned to the same operational taxonomic units (OTUs) using Uparse (v8.1.1861), and the sequence with the highest frequency of occurrence in each OTU was selected as the representative sequence for further annotation *(Edgar 2013)*. For each representative sequence, the annotation was performed using the UCLUST method *(Edgar 2010)* and the Greengenes database (Release 13.8， http://greengenes.secondgenome.com/) *(DeSantis et al. 2006)* to the level of kingdom, phylum, class, order, family, genus and species, to determine the community composition of each sample. Rare OTUs (singletons to tripletons), which could potentially originate from artificial sequences, were removed. The read counts were normalized with respect to the smallest read number per sample.

Statistical analysis

All statistical analyses were calculated with QIIME (Version 1.8.0) and displayed using R software (Version 2.15.3). All data were analyzed using analysis of variance (ANOVA) with SPSS19.0 (SPSS Inc., Chicago, USA). Differences between groups were compared by Fisher's least significant difference test. One-way ANOVA was performed with a P value<0.05.

Alpha diversity was applied to analyze complexity of species diversity for a community through four indices, including abundance-based coverage (ACE) estimator, Chao 1, Shannon diversity index and Inverse Simpson index. The ACE estimator and Chao 1 were selected to determine community richness, while the Shannon index and Inverse Simpson index was used to estimate community diversity.

For beta diversity analysis, principal component analysis (PCA) was applied to reduce the dimension of the original variables. Unweighted Pair-Group Method with Arithmetic Means (UPGMA) Clustering was performed as a type of hierarchical clustering method to interpret the distance matrix using average linkage.

Correlation analysis (using Spearman's rank correlation analysis) was performed based on the changes in species abundances in different communities, using Mothu *(Schloss et al. 2009)*. The first 50 groups were displayed by Cytoscape (http://www.cytoscape.org/). To analyze the function of the microbiota, PICRUSt (http://huttenhower.sph.harvard.edu/galaxy/tool_runner?tool_id=PICRUSt_normalize) was used to determine the Kyoto Encyclopedia of Genes and Genomes (KEGG) estimate. The variation analysis was carried out based on the functional abundance of the samples.

Results

Sequencing statistic

The sequencing of the V3-V4 region of the 16S rRNA gene from all samples yielded 660,115 total tags. After trimming and filtering, 430,843 high-quality reads were obtained, with an average reading length of 415 bp. OTU clustering (97% similarity) of sequencing reads resulted in a total of 33,858 OTUs from 9 samples. Of these sequences, 99.97% was classified at the phylum level. All the rarefaction curves indicated that the sampling effort was sufficient to capture each sample's overall composition (Fig. S4).

Taxonomic composition of bacterial assemblages in rhizosphere soil

To obtain an overall idea of the bacterial assemblages and their variability across American ginseng rhizosphere soil under three different shade sheds, we analyzed the taxonomy of 16S rRNA gene amplicons and plotted the relative abundance of the top twenty taxa (Fig. S5). At the phyla level, the most prominent taxon was Proteobacteria, which represents approximately 30.5% of the total bacterial community, followed by Chloroflexi (17.2%), Acidobacteria (15.4%), Actinobacteria (15.3%), and Gemmatimonadetes (5.8%) (Fig. S5A). The dominant class across all samples were Alphaproteobacteria, Acidobacteriia, Gammaproteobacteria, KD4-96, Gemmatimonadetes, Bacteroidia, Actinobacteria, Thermoleophilia, Deltaproteobacteria, Subgroup_6, Saccharimonadia, Ktedonobacteria, Anaerolineae, Acidimicrobiia, Clostridia, AD3, Verrucomicrobiae, Planctomycetacia, Blastocatellia (Subgroup_4) and Parcubacteria (relative abundance >1% across all samples) (Fig. S5B). We also investigated the ten most-abundant items with respect to different taxonomic levels of order, family and genus. The extent of bacteria variation increased from phylum to genus (Fig. S6C–S6E).

Alpha and beta diversity analysis of samples

To quantify alpha diversity, the diversities of bacteria in each sample were analyzed based on the diversity indices of ACE, Chao 1, Shannon diversity index and Inverse Simpson index. The ACE and Chao 1 in pp were significantly lower than gp and ctp (Fig. 2), demonstrating that shading had an effect on bacterial richness of the rhizosphere. The Shannon diversity index and Inverse Simpson index were used to estimated bacterial diversity, but have no significant differences diversity among the three kinds of shade sheds.

For beta diversity, PCA analysis was carried out to cluster bacterial in rhizosphere soil according to the different samples. At the OTU level, PC1 explained 60.77% and PC2 13.07% of the total variation in bacteria, and the community structure of soil bacteria under three types of shed was clearly distinguishable from each other (Fig. 3A). We also performed UPGMA cluster analysis and built cluster trees for the samples. Using this approach, all the samples collected from the rhizosphere were distinguishable (Fig. 3B).

Variations in the bacterial in the American ginseng rhizosphere soil under three different shade sheds

To take a closer look at the variation in the bacterial of the American ginseng rhizosphere soil under three different shade sheds, the data from phylum to genus in three sample communities were compared by LDA effect size taxonomic cladogram. The relative abundances of bacterial phyla showed some differences across the three different shading rhizosphere soil (Fig. 4). Relative abundance of Proteobacteria, Nitrospirae and WS2 in gp were significantly higher than that in pp and ctp (LSD, $p<0.05$). Dependentiae, Elusimcrobia, FCPU426 and WPS-2 significantly more abundant in pp (LSD, $p<0.05$). At a finer taxonomic level, we also found significant differences among three different shading in some bacterial genera, the abundance of the 50 most-abundant genera among three different shading was shown in Figure S6.

Correlation and KEGG functional analysis in bacterial communities

Based on the changes in abundance of different genera in different soil samples, the relationships between the abundance values of the various bacterial genera were determined. The relationship network of these genera indicated a complex functional collaboration within the microbiota (Fig. S7). To analyze the functional diversities of bacteria, the KEGG functional enrichment analysis of bacterial microbiota was compared among the rhizosphere soils under three different shade sheds. The abundance of the top 50 functional groups was analyzed and the heat map was drawn (Fig. 5). Number of OTUs of common functional soil bacteria in three sample were shown in a Venn diagram (Fig. 6). This indicated a functional divergence of bacterial microbiota in response to the rhizosphere of ginkgo roots.

Discussion

In this study, we investigated the changes of American ginseng rhizosphere soil bacterial communities under three different shade sheds. Different shade sheds will cause the change of temperature, light, humidity and other environmental factors, and the changes in environmental conditions can affect the diversity and composition of soil microorganisms. Nicholas et al. reported that the bacterial diversity changed in the different environmental conditions of grapes *(Bokulich et al. 2014)*. In our study, the temperature in the high arch shed is lower than the other two types of shed (Table S1). The bacterial diversity is the most abundant in high arch shed, while that of traditional shed is the lowest. The diversity of soil microorganisms is related to the maintenance of soil health and quality *(Anderson 2003; Garbeva et al. 2004)*. A decrease in soil microbial diversity was responsible for the development of soil-borne plant diseases. The mounting risks of some phytopathogens under changing growing conditions have already been considered *(Burie et al. 2011; Pugliese et al. 2011)*. In our study, we found that the disease rate of American ginseng was the lowest under the high arch shed. Therefore, it is very important to prevent the American ginseng from getting sick by building the shade shed.

American ginseng is a kind of perennial plant. Its root exudates accumulate in the rhizosphere and provide a substrate for several kinds of biological communities. Root exudates are thought to be a driving force in the selection of specific microbial populations in the rhizosphere *(Bais et al. 2006; Garbeva et al. 2004; Mazzola & Manici 2012)*. For example, the proportion of Proteobacteria and Bacteroidetes were increased, because their relative abundance will increase in the high nitrogen area *(Fierer et al. 2012)*. Proteobacteria are dominant in three soil environments, most of them are Gram-negative, responsible for legume symbiotic nitrogen fixation *(Raymond et al. 2004)*. Among them, *Bradyrhizobium* belongs to Proteobacteria, which is the highest relative abundance in the rhizosphere soil microorganism of American ginseng in our samples. *Bradyrhizobium* is a Azotobacterthat induces nodule formation in the host root of legumes *(Long 1996)*. However, the absence of root nodule in American ginseng may be due to the lack of nod receptor or the defect of subsequent kinase cascade in American ginseng, which is essential for nodule formation in legumes *(Gage 2004; Smit et al. 2007)*. *Sphingomonas* was the second largest accumulated in the rhizosphere of American ginseng under three type shades. The genus of *Sphingomonas* have the common ability to degrade a broad range of aromatic compounds *(Fredrickson et al. 1995)*. Therefore, the accumulation of *Sphingomonas* in the rhizosphere indirectly implies the secretion of different aromatic secondary metabolites in American ginseng root, thus attracting the accumulation of *Sphingomonas* consuming aromatic substances. The genus *Nitrospira* is composed of a group of species widely distributed in many natural environments, which are believed to play an important role in the nitrogen cycle in water and soil *(Bartosch et al. 2002)*. The content of both *Sphingomonas* and *Nitrospira* in the soil under the high arch shed is higher than that of the other two shed types. The relative abundance of *Gemmatimonas* was also high in our study. It is reported that the abundance of *Gemmatimonas* participates in the acquisition of various plant processing resources , and it is negatively correlated with plant growth *(H. et al. 2015)*.

The relative abundance of some microbial communities decreased. This may be due to the fact that there are a series of antimicrobial metabolites in the root exudates *(Bais et al. 2006; Berg & Smalla 2009; Mazzola & Manici 2012)*. In addition, changes in soil chemical properties may lead to changes in microbial community composition *(Lauber et al. 2008)*. Our analyses show significant differences in pH, total salt, organic matter, hydrolytic nitrogen, available phosphorus among the three different shade sheds (Table S1-S2)， which effect on microbial community needs further verification. Changes in the composition of bacterial communities may lead to changes in metabolism, biodegradation and disease inhibition *(Bell et al. 2013; Garbeva et al. 2004)*. The results show that the shade shed may have a positive impact on soil productivity. The balance of soil microbial community can be adjusted by setting up appropriate shade shed.

Half of the 50 most abundance functional groups of microbial community for the three types of shed are transporters. The other half is mainly related to metabolism, such as amino acid metabolism, fatty acid metabolism and secondary metabolism, and so on. Among the 26 transporters, 22 belong to ATP-binding cassette (ABC) transporters. ABC transporters are essential for the survival of bacteria. It is an important factor for bacteria to catalyze the uptake of nutrients and the efflow of toxic or antibacterial drugs *(Davidson & Chen 2004)*. ABC transporter is a integral membrane protein that combines substrate trans lipid bilayers transport with ATP hydrolysis *(Hollenstein et al. 2007)*. The accumulation of bacterial rich in ABC transporters in the rhizosphere is consistent with the role of the rhizosphere, which mediates the exchange of inorganic and organic matter between the root and the soil *(Ryan et al. 2001)*.The structure and composition of microbial community are closely related to the metabolic function of microbial community. It can be seen from Venn diagram (Fig. 6) that there are 5625 functional groups in total for the three types of shed, including 15 special functional groups for flat shed, 23 special functional groups for traditional shed and 58 special functional groups for high arch shed. The specific functional groups of high arch shade sheds were significantly higher than the other two shade shelters.

Conclusions

In summary, the diversities of American ginseng soil microbial community structure and function were changed under three different shade sheds. The shade shed may have a positive impact on soil productivity. It is an effective measure to prevent American ginseng from getting sick. It also regulates the balance of soil microbial communities. Shading can also enrich the metabolic functions of soil microorganisms. Our work will be of great significance for promote the optimization and upgrading of American ginseng shade shed type and ensure the healthy development of American ginseng planting industry.

Acknowledgement

This study was supported by Agricultural Science and Technology Innovation Project of Shandong Academy of Agricultural Sciences, China (NO. CXGC2018D03); the National Natural Science Foundation of China (No. 82003632); the Provincial Major Scientific and Technological Innovation Project of Shandong, China (NO. 2019JZZY020612); the Province Agricultural Major Application Technology Innovation Project of Shandong, China (NO. SD2019ZZ016); the Provincial Key Research and Development Program of Shandong, China (NO. 2019LYXZ025, 2019GSF109087); the Taishan Scholars's Program of Shandong for Jinyue Sun.

References

Anderson T-H. 2003. Microbial eco-physiological indicators to asses soil quality. *Agriculture, Ecosystems & Environment* **98**:285-293 DOI 10.1016/S0167-8809(03)00088-4.

Bais HP, Weir TL, Perry LG, Gilroy S, and Vivanco JM. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* **57**:233-266 DOI 10.1146/annurev.arplant.57.032905.105159.

Bartosch S, Hartwig C, Spieck E, and Bock E. 2002. Immunological detection of Nitrospira-like bacteria in various soils. *Microbial Ecology* **43**:26-33 DOI 10.1007/s00248-001-0037-5.

Bell TH, Yergeau E, Maynard C, Juck D, Whyte LG, and Greer CW. 2013. Predictable bacterial composition and hydrocarbon degradation in Arctic soils following diesel and nutrient disturbance. *ISME Journal* **7**:1200-1210 DOI 10.1038/ismej.2013.1.

Berendsen RL, Pieterse CM, and Bakker PA. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**:478-486 DOI 10.1016/j.tplants.2012.04.001.

Berg G, Grube M, Schloter M, and Smalla K. 2014. Unraveling the plant microbiome: looking back and future perspectives. *Frontiers in Microbiology* **5**:148 DOI 10.3389/fmicb.2014.00148.

Berg G, and Smalla K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology* **68**:1-13 DOI 10.1111/j.1574-6941.2009.00654.x.

Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, and Caporaso JG. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods* **10**:57-59 DOI 10.1038/nmeth.2276.

Bokulich NA, Thorngate JH, Richardson PM, and Mills DA. 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America* **111**:E139-148 DOI 10.1073/pnas.1317377110.

Broeckling CD, Broz AK, Bergelson J, Manter DK, and Vivanco JM. 2008. Root exudates regulate soil fungal community composition and diversity. *Applied and Environmental Microbiology* **74**:738-744 DOI 10.1128/aem.02188-07.

Burie JB, Langlais M, and Calonnec A. 2011. Switching from a mechanistic model to a continuous model to study at different scales the effect of vine growth on the dynamic of a powdery mildew epidemic. *Annals of Botany* **107**:885-895 DOI 10.1093/aob/mcq233.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, and Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**:335-336 DOI 10.1038/nmeth.f.303.

Chisholm ST, Coaker G, Day B, and Staskawicz BJ. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* **124**:803-814 DOI 10.1016/j.cell.2006.02.008.

Cruse-Sanders JM, and Hamrick JL. 2004. Genetic diversity in harvested and protected populations of wild American ginseng, Panax quinquefolius L. (Araliaceae). *American Journal of Botany* **91**:540-548 DOI 10.3732/ajb.91.4.540.

Davidson AL, and Chen J. 2004. ATP-binding cassette transporters in bacteria. *Annual Review of Biochemistry* **73**:241-268 DOI 10.1146/annurev.biochem.73.011303.073626.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, and Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* **72**:5069-5072 DOI 10.1128/aem.03006-05.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**:2460-2461 DOI 10.1093/bioinformatics/btq461.

Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**:996-998 DOI 10.1038/nmeth.2604.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, and Knight R. 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME Journal* **6**:1007-1017 DOI 10.1038/ismej.2011.159.

Fredrickson JK, Balkwill DL, Drake GR, Romine MF, Ringelberg DB, and White DC. 1995. Aromatic-degrading Sphingomonas isolates from the deep subsurface. *Applied and Environmental Microbiology* **61**:1917-1922 DOI 10.1128/aem.61.5.1917-1922.1995.

Gage DJ. 2004. Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. *Microbiology and Molecular Biology Reviews* **68**:280-300 DOI 10.1128/mmbr.68.2.280-300.2004.

Garbeva P, van Veen JA, and van Elsas JD. 2004. Microbial diversity in soil: selection microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of Phytopathology* **42**:243-270 DOI 10.1146/annurev.phyto.42.012604.135455.

H. I, Franke-Whittle, Manici LM, and Heribert Insam BS. 2015. Rhizosphere bacteria and fungi associated with plant growth in soils of three replanted apple orchards. *Plant and Soil* **395**:317-333 DOI 10.1007/s11104-015-2562-x

Hollenstein K, Dawson RJ, and Locher KP. 2007. Structure and mechanism of ABC transporter proteins. *Current Opinion in Structural Biology* **17**:412-418 DOI 10.1016/j.sbi.2007.07.003.

Hongpeng LI, Yingjiao LI, Zhao Y, and Xiangguo LI. 2018. Effects of Different Shading Treatments on Agronomic Characters of Cultivated Ginseng (Panax ginseng) in Farmland. *Agricultural Biotechnology* **v.7**:146-149+153 DOI 10.19759/j.cnki.2164-4993.2018.02.034.

Izzo AA. 2009. Phase II clinical trial reveals the excellent safety profile of Panax quinquefolius (American ginseng). *Focus on Alternative and Complementary Therapies* **14**:24-25 DOI 10.1111/j.2042-7166.2009.tb01862.x.

Jiao XL, Zhang XS, Lu XH, Qin R, Bi YM, and Gao WW. 2019. Effects of maize rotation on the physicochemical properties and microbial communities of American ginseng cultivated soil. *Scientific Reports* **9**:8615 DOI 10.1038/s41598-019-44530-7.

Lauber CL, Strickland MS, Bradford MA, and Fierer N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* **40**:2407-2415 DOI 10.1016/j.soilbio.2008.05.021

Li B, Wang CZ, He TC, Yuan CS, and Du W. 2010. Antioxidants potentiate American ginseng-induced killing of colorectal cancer cells. *Cancer Letter* **289**:62-70 DOI 10.1016/j.canlet.2009.08.002.

Long SR. 1996. Rhizobium symbiosis: nod factors in perspective. *Plant Cell* **8**:1885-1898 DOI 10.1105/tpc.8.10.1885.

Magoč T, and Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**:2957-2963 DOI 10.1093/bioinformatics/btr507.

Mazzola M, and Manici LM. 2012. Apple replant disease: role of microbial ecology in cause and control. *Annual Review of Phytopathology* **50**:45-65 DOI 10.1146/annurev-phyto-081211-173005.

Nadeau I, and Olivier A. 2003. The biology and forest cultivation of American ginseng (Panax quinquefolius L.) in Canada. *Canadian Journal of Plant Science* **83**:877-891

Poddar K, Kolge S, Bezman L, Mullin GE, and Cheskin LJ. 2011. Nutraceutical supplements for weight loss: a systematic review. *Nutrition in Clinical Practice* **26**:539-552 DOI 10.1177/0884533611419859.

Proctor JTA, and Palmer JW. 2017. Optimal light for greenhouse culture of American ginseng seedlings. *Journal of Ginseng Research* **41**:370-372 DOI 10.1016/j.jgr.2016.04.002.

Pugliese M, Gullino ML, and Garibaldi A. 2011. Effect of climate change on infection of grapevine by downy and powdery mildew under controlled environment. *Communications in Agricultural & Applied Biological Sciences* **76**:579-582

Punja ZK. 2011. American ginseng: research developments, opportunities, and challenges. *Journal of Ginseng Research* **35**:368-374 DOI 10.5142/jgr.2011.35.3.368.

Qin Z, Jia C, Liao D, Chen X, and Li X. 2018. Comparison of Serum Metabolite Changes of Radiated Mice Administered with Panax quinquefolium from Different Cultivation Regions Using UPLC-Q/TOF-MS Based Metabolomic Approach. *Molecules* **23** DOI 10.3390/molecules23051014.

Raymond J, Siefert JL, Staples CR, and Blankenship RE. 2004. The natural history of nitrogen fixation. *Molecular Biology and Evolution* **21**:541-554 DOI 10.1093/molbev/msh047.

Ryan P, Delhaize E, and Jones D. 2001. Function and mechanism of organic anion exudation from plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**:527-560 DOI 10.1146/annurev.arplant.52.1.527.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, and Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**:7537-7541 DOI 10.1128/aem.01541-09.

Schmidt R, Ulanova D, Wick LY, Bode HB, and Garbeva P. 2019. Microbe-driven chemical ecology: past, present and future. *ISME Journal* **13**:2656-2663 DOI 10.1038/s41396-019-0469-x.

Smit P, Limpens E, Geurts R, Fedorova E, Dolgikh E, Gough C, and Bisseling T. 2007. Medicago LYK3, an entry receptor in rhizobial nodulation factor signaling. *Plant Physiology* **145**:183-191 DOI 10.1104/pp.107.100495.

Tsao R, and Liu Z. 2007. Exploration and characterization of bioactive phytochemicals in native Canadian plants for human health. *Canadian journal of plant science Revue canadienne de phytotechnie* **87**:1045-1053 DOI 10.4141/cjps07149.

Turner TR, James EK, and Poole PS. 2013. The plant microbiome. *Genome Biology* **14**:209 DOI 10.1186/gb-2013-14-6-209.

Wang CZ, Mehendale SR, and Yuan CS. 2007. Commonly used antioxidant botanicals: active constituents and their potential role in cardiovascular illness. *American Journal of Chinese Medicine* **35**:543-558 DOI 10.1142/s0192415x07005053.

Wu X, Zhang H, Chen J, Shang S, Wei Q, Yan J, and Tu X. 2016. Comparison of the fecal microbiota of dholes high-throughput Illumina sequencing of the V3-V4 region of the 16S rRNA gene. *Applied Microbiology and Biotechnology* **100**:3577-3586 DOI 10.1007/s00253-015-7257-y.