https://doi.org/10.17221/320/2020-PSE

Crop rotation alleviates replant failure in *Panax notoginseng* (Burkill) F.H. Chen by changing the composition but not the structure of the microbial community

Binbin Tang[1,2,3], Yaojun Dong[1,2,3], Kai Wu[1,2,3], Mimi He[1,2,3], Jianfeng Liu[1,2,3], Fang Yin[1,2,3], Wudi Zhang[1,2,3], Ming Gong[1]**

[1]*Yunnan Normal University, Kunming, P.R. China*
[2]*Yunnan Research Center of Biogas Technology and Engineering, Yunnan Normal University, Kunming, P.R. China*
[3]*Jilin Dongsheng Institute of Biomass Energy Engineering, Tonghua, P.R. China*
**Corresponding authors: kaiwu8868@hotmail.com; gongming63@163.com*
Binbin Tang and Yaojun Dong contributed equally to this work.

Citation: Tang B.B., Dong Y.J., Wu K., He M.M., Liu J.F., Yin F., Zhang W.D., Gong M. (2020): Crop rotation alleviates replant failure in *Panax notoginseng* (Burkill) F.H. Chen by changing the composition but not the structure of the microbial community. Plant Soil Environ., 66: 493–499.

Abstract: Consecutive monocropping with sanqi (*Panax notoginseng* (Burkill) F.H. Chen) can increase the abundances of pathogens in soil, resulting in soil sickness. Crop rotation is one way to alleviate this problem. In the present study, there were no differences in microbial structure or bacterial alpha diversity among one-year monocropping soil, one-year rotation soil, and ten-year rotation soil. However, monocropping practices decreased fungal alpha diversity. The relative abundance of copiotrophic bacteria decreased after sanqi monocropping, while that of oligotrophic bacteria increased. Ten-year rotation significantly increased the abundance of potential beneficial bacterial genera. Moreover, the potential beneficial fungal genera were also enriched by rotation for ten years. Furthermore, the relative abundance of *Cylindrocarpon* spp. decreased dramatically after a ten-year rotation. The results of pot experiments showed that disease incidences after ten-year rotation were significantly decreased among the three treatments. Hence, we suggested that pausing sanqi cultivation for a long time can increase the abundance of potentially beneficial soil bacteria and fungi that are helpful for overcoming soil sickness in sanqi cultivation.

Keywords: medicinal herb; microflora; rotation; phytopathogen; microorganism; pathogenic fungi

Panax notoginseng (Burkill) F.H. Chen (sanqi) is an expensive Chinese medicinal herb that provides efficient blood stasis elimination (Kim 2012), cultivated in a restricted area of Yunnan for more than 200 years. Soil sickness is a common phenomenon for many crops such as maize (Gentry et al. 2013), peanut (Li et al. 2018), and cucumber (Jin et al. 2019b). An imbalance in the microflora can cause the soil sickness in cash crops, such as banana (Fu et al. 2017), *Panax quinquefolius* L. (Jiang et al. 2019), and also sanqi (Tan et al. 2017). Plant autotoxins have also been found to disrupt the microbial com-

Supported by the National Natural Science Foundation of China, Grant No. 51366015; by the Yunnan Ten Thousand Talents Plan Industrial Technology Project (2019), Collaborative Innovation Center for Renewable Energy R&D in Southwestern China, Project No. 05300205020516009; by the Program of the Provincial Education Department of Yunnan Province, Project No. 2015Y107; by the China Postdoctoral Science Foundation, Grant No. 2015M582765XB, and by the Yunnan Science and Technology Programs, Projects No. 2016FB076 and 2016FD019; by the Doctoral Candidate Academic Award of Yunnan Province, Project No. 01701205020503068. Founders were involved no role in the experimental design, data collection, and the decision to prepare a manuscript.

https://doi.org/10.17221/320/2020-PSE

munity, resulting in cucumber growth inhibition. Soil sickness in sanqi, which occurs as a consequence of soil-borne phytopathogens such as *Fusarium oxysporum*, *Cylindrocarpon* spp., and *Phoma* spp. (Li et al. 2020); imbalanced of soil physicochemical properties; and allelochemical accumulation (Yang et al. 2015), seriously affects sustainable production in the sanqi medicine industry. Niche vacancies in an imbalanced microflora increased the abundance of specific pathogens, which increased the disease incidence and decreased yields (Berendsen et al. 2012).

Manipulating the microflora seems to be a sustainable way of controlling soil-borne disease (Chaparro et al. 2012). The application of beneficial microorganisms (Durairaj et al. 2018) or bio-organic fertiliser (Fu et al. 2017) could successfully control the disease incidences in crops by regulating the microflora (Ye et al. 2020). Crop rotation reduces the soil-borne disease by regulating the soil microflora (Larkin 2008). However, it takes nearly 15 to 20 years for sanqi to successfully be replanted (Fan et al. 2016). This threatens the sustainable development of the sanqi industry due to the lack of appropriate fields in the restricted area. Furthermore, the effects of rotation on the microflora and the replant failure of sanqi have rarely been studied. Hence, we hypothesised that rotation could reduce soil sickness by regulating the microbial community. The microbial community composition and structure were analysed. The seedling survival incidence was also determined in pot experiments.

MATERIAL AND METHODS

Soil sample collection. Bulk soil samples were collected from a one-year sanqi monocropping soil (MCS), a one-year vegetable rotation soil (OCS), and a ten-year vegetable rotation soil (TCS) in Yanshan county with triplicates in each treatment. The bulk soil samples were collected from the upper layer soil (2–10 cm) in late April 2015. The collected soil samples were passed through a 2 mm mesh to remove plant debris and stones. The soil type was a ferralic anthrosol, according to the World Reference Base for Soil Resources (FAO 2006). The soil samples were used to test the incidence of sanqi replant failure in pots and for further soil DNA extraction.

High-throughput MiSeq sequencing. The bulk soil DNA of 0.7 g soil from each sample was extracted with the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, USA) according to the manufacturer's instructions. The isolated soil DNA was then stored at –80 °C for further use.

The primers and PCR amplification procedures were performed according to the previous report (Zhao et al. 2018). Briefly, the V4 region of bacteria (Xiong et al. 2017) and the internal transcribed spacer (ITS) region of fungi were used. The bacterial and fungal templates were amplified according to methods in a previous report (Xiong et al. 2017). Finally, the paired-end sequencing amplicons of bacteria and fungi were sequenced on an Illumina MiSeq sequencing platform at Personal Biotechnology, Shanghai. The data were submitted to the NCBI SRA database with the accession numbers PRJNA638218 for bacteria and PRJNA638219 for fungi.

Sequencing data processing and analysis. The sequenced amplicons were then trimmed and analysed, as described in a previous report (Zhao et al. 2018). Data sequencing was performed with the QIIME software package (version 1.9.1). Chimeras and singletons were also removed. After quality control, the operational taxonomic units were obtained with a 97% sequence similarity against the Greengenes database (McDonald et al. 2012) and the UNITE database (Kõljalg et al. 2013), respectively. The RDP naïve Bayesian rRNA classifier with confidence thresholds of 80% and 50% for bacteria and fungi, respectively, was used for taxonomic classification.

Replant failure incidences in pot experiments. The bulk soil samples collected above were mixed and sieved with 2 mm mesh sieves to remove pebbles as described above. Three seedlings were transplanted into each plastic bottle with 500 g soil. Each replicate included 5 individuals. The replant failure incidences (disease incidences) were calculated as the proportion of diseased seedlings to the total seedlings transferred after four months.

Statistical analysis. The alpha diversity indexes of bacteria and fungi in each sample were calculated with Mothur (Zhao et al. 2018). The principal coordinate analysis (PCoA) plots were generated with the vegan package in R, based on the Bray-Curtis index. The Permanova test was also performed with the Adonis function using the vegan package in R. Statistical analysis of each sample was performed with the SPSS 17 (SPSS Inc., Chicago, USA). Significant differences among the treatments were identified with Duncan's multiple range test.

https://doi.org/10.17221/320/2020-PSE

Table 1. Bacterial and fungal alpha diversity indexes of different samples

	Treatment	Goods coverage	OTUs	Equitability	Shannon index
Bacteria	OCS	0.887 ± 0.001[a]	5 931.00 ± 35.38[a]	0.888 ± 0.001[a]	11.13 ± 0.01[a]
	MCS	0.882 ± 0.010[a]	6 075.33 ± 400.71[a]	0.887 ± 0.004[a]	11.15 ± 0.13[a]
	TCS	0.889 ± 0.002[a]	5 881.33 ± 31.34[a]	0.887 ± 0.001[a]	11.11 ± 0.01[a]
Fungi	OCS	0.977 ± 0.002[a]	1 376.33 ± 32.25[a]	0.624 ± 0.017[a]	6.50 ± 0.16[a]
	MCS	0.978 ± 0.001[a]	1 275.33 ± 45.57[b]	0.586 ± 0.009[b]	6.05 ± 0.12[b]
	TCS	0.978 ± 0.001[a]	1 338.33 ± 51.48[ab]	0.627 ± 0.007[a]	6.51 ± 0.07[a]

OCS – one-year rotation soil; MCS – one-year sanqi ginseng monocropping soil; TCS – ten-year rotation soil; OTUs – operational taxonomic units. The means and standard errors from three replicates are shown. Letters within column indicated the significant difference with $P < 0.05$

RESULTS AND DISCUSSION

Alpha diversity analysis of bacterial and fungal communities. Crop rotation can change the microflora and decrease the soil-borne disease (Larkin and Lynch 2018, Jin et al. 2019a). The results showed that there were no significant differences in the Good's coverage, the number of OTUs (operational taxonomic units), equitability, and Shannon indexes of the bacterial communities among the treatments (Table 1). However, the number of OTUs, fungal equitability, and Shannon indexes of the MCS obviously decreased in contrast to those of the OCS and TCS, suggesting an imbalance in the fungal community and functional losses that resulted in replant failure (Li et al. 2020). A previous report suggested that maize rotation for three years significantly increased bacterial diversity, while no significant differences in fungal diversity were observed (Zhao et al. 2017). Our results showed the opposite effects of crop rotation and sanqi monocropping on the bacterial and fungal diversity as a previous report (Zhao et al. 2017). This could have been caused by the types of rotation plants, as different plants secrete different components of root exudates and attract a specific microbial community (Badri and Vivanco 2009).

Community structure and composition in different treatments. The PCoA plots showed that there were no significant differences in bacterial or fungal community structure among the three treatments (Figure 1). Although the rotation had little effect on the structural variations in the microbial community, the relative abundances in the microbial community composition from phylum to the genus changed after rotation. The dominant phylum in each sample was *Proteobacteria* (Figure 2A), which was in accordance with a previous report (Zhao et al. 2017). After ten years of rotation, the relative abundances of the *Acidobacteria* and *Gemmatimonadetes* significantly decreased in contrast to those in OCS and MCS, respectively. The relative abundance of *Proteobacteria*,

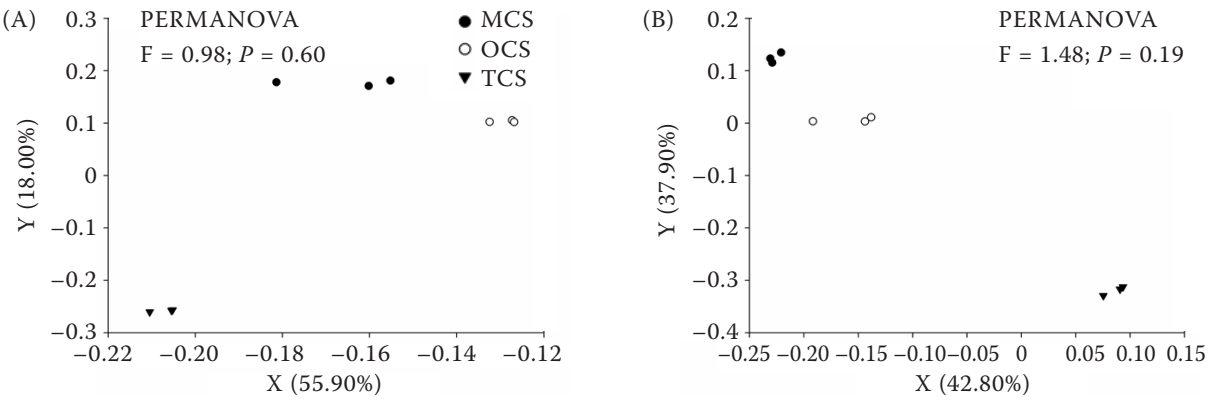

Figure 1. Principal coordinates analysis (PCoA) plots of (A) bacterial and (B) fungal communities from different treatments. OCS – one-year rotation soil; MCS – one-year sanqi ginseng monocropping soil; TCS – ten-year rotation soil

https://doi.org/10.17221/320/2020-PSE

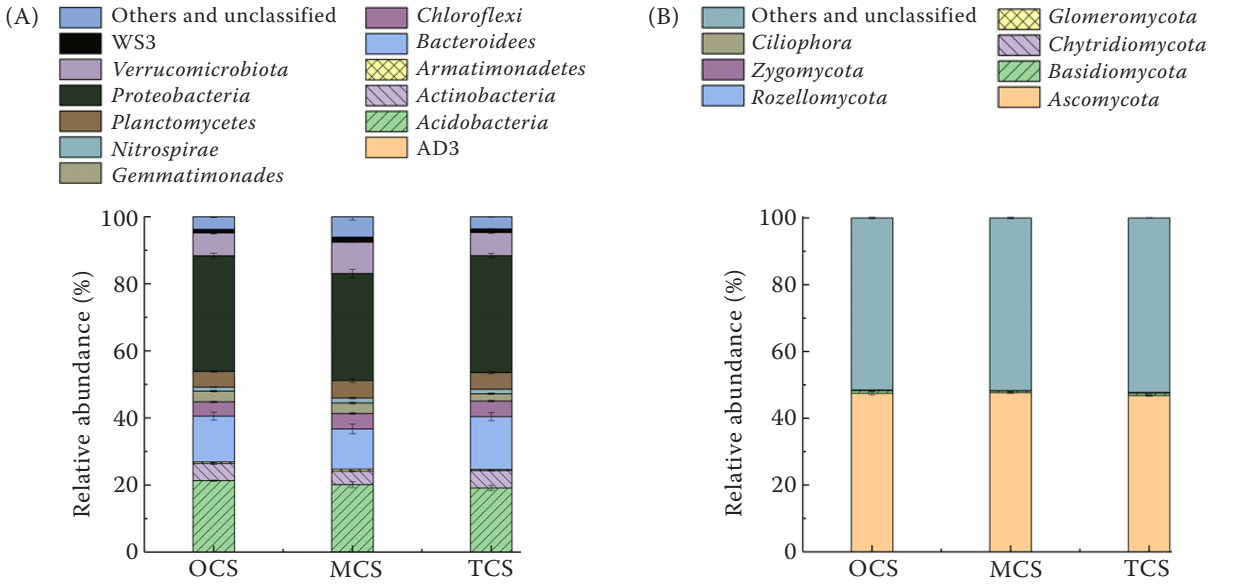

Figure 2. The composition of main (A) bacterial and (B) fungal from different samples at the phylum level. OCS – one-year rotation soil; MCS – one-year sanqi ginseng monocropping soil; TCS – ten-year rotation soil

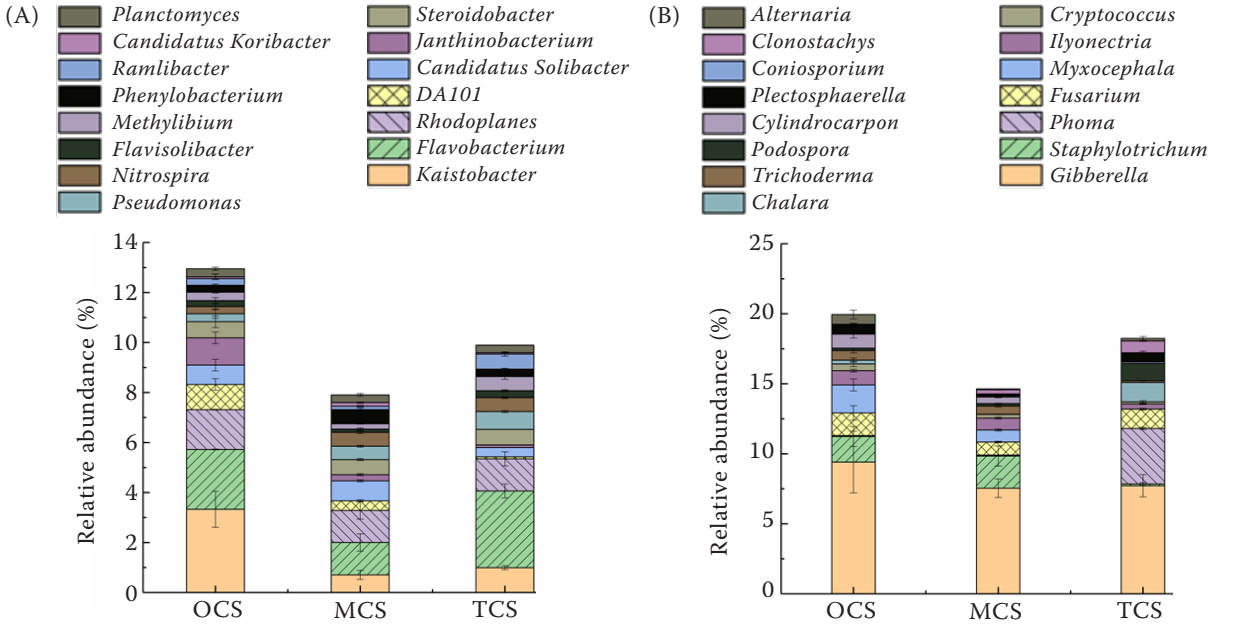

Figure 3. The composition of main (A) bacterial and (B) fungal from different samples at the genus level (Top 15). OCS – one-year rotation soil; MCS – one-year sanqi ginseng monocropping soil; TCS – ten-year rotation soil

Betaproteobacteria, and *Actinobacteria*, as copiotrophic bacteria, decreased after sanqi monocropping. However, the abundance of oligotrophic bacteria, such as *Verrucomicrobia*, increased after the monocropping practices, suggesting that soil resources were limited after the consecutive monocropping (Pianka 1970). For fungi, the dominant phylum was Ascomycota (Figure 2B), which was in line with a previous report (Miao et al. 2016). However, Zhao et al. (2017) found that the phylum *Sordariomycetes* was dominant in bulk soil, mainly due to root exudates after a maize-sanqi rotation. The relative abundance of *Ciliophora* in MCS significantly decreased in contrast to that in OCS and TCS.

At the genus level, the relative abundance of *Kaistobacter* spp., *Flavobacterium* spp., and *Ramlibacter* spp. in MCS significantly decreased (Figure 3A). The abundance of potential beneficial

https://doi.org/10.17221/320/2020-PSE

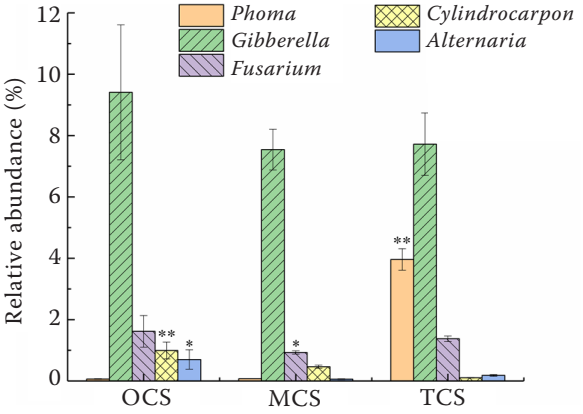

Figure 4. The relative abundances of five potential pathogenic fungal genera from different samples. *P < 0.05; **P < 0.01. OCS – one-year rotation soil; MCS – one-year sanqi ginseng monocropping soil; TCS – ten-year rotation soil

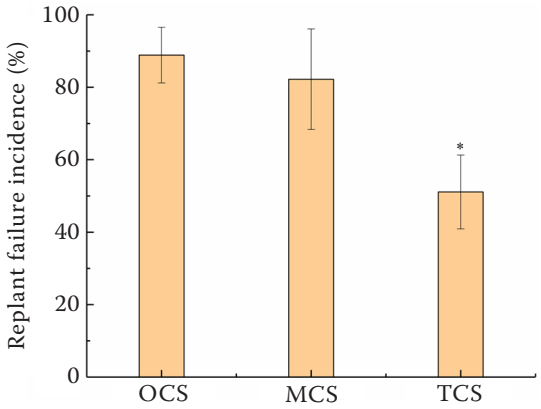

Figure 6. Disease incidences of different treatments in pot experiment. *P < 0.05; **P < 0.01. OCS – one-year rotation soil; MCS – one-year sanqi ginseng monocropping soil; TCS – ten-year rotation soil

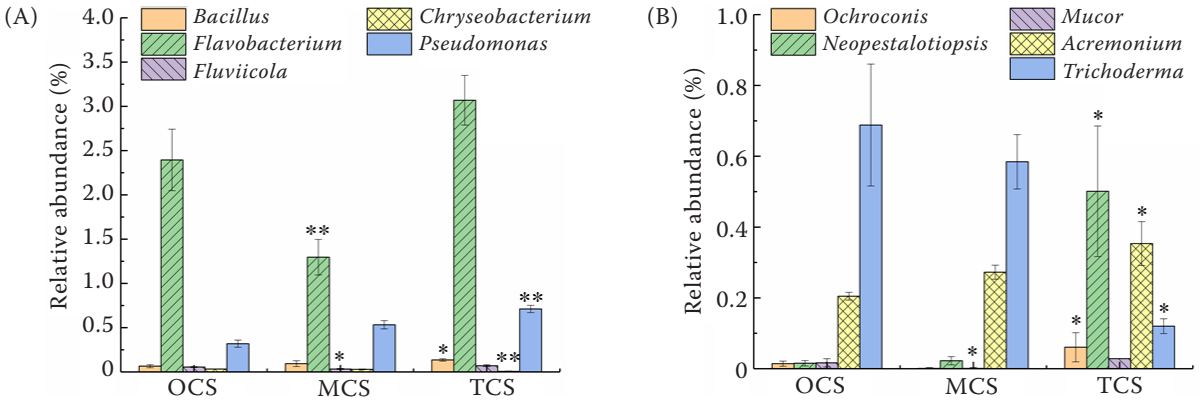

Figure 5. The relative abundances of five potential beneficial (A) bacterial and (B) fungal genera from different samples. *P < 0.05; **P < 0.01. OCS – one-year rotation soil; MCS – one-year sanqi ginseng monocropping soil; TCS – ten-year rotation soil

bacterial genera that are capable of controlling of soil-borne diseases, such as *Bacillus* sp. (Song et al. 2014), *Flavobacterium* sp. (Sang and Kim 2012), and *Pseudomonas* sp. (Durairaj et al. 2018), significantly increased after rotation for ten years (Figure 5A). For fungal genera, the dominant genus of each treatment was *Gibberella* spp. (Figure 3B). The relative abundances of potential pathogenic genera such as *Ilyonectria* spp., *Cylindrocarpon* spp., and *Alternaria* spp. in OCS were 2.79-, 9.39-, and 3.89-times higher than those in TCS (Figures 3B and 4), suggesting that rotation could decrease the abundance of pathogen agents and thereby reduce soil-borne disease. This may have been caused by the elimination of allelochemicals such as ginsenoside, which could promote the abundance of pathogenic genera (Li et al. 2020) after rotation. However, the results showed that the abundance of potential pathogenic genus like *Phoma* spp. increased 60.95-times higher after ten-year rotation than that in OCS, while the relative abundance of *Coniosporium* spp. significantly decreased with the increasing rotation duration (Figure 3B). For potentially beneficial fungi, the results showed that the relative abundances of *Acremonium* spp., *Ochroconis* spp., *Neopestalotiopsis* spp., and *Mucor* spp. (Li et al. 2020) significantly increased as the rotation duration increased, while those of *Trichoderma* spp. showed a negative trend as the rotation duration increased (Figure 5B).

Pot experiments. It was found that the disease incidences in OCS was the highest, as high as 88.9 ± 7.7% (Figure 6). However, there were no significant differences between OCS and MCS. The results also showed that the disease incidences in TCS

https://doi.org/10.17221/320/2020-PSE

was 1.74- and 1.61- times lower than those in OCS and MCS, respectively. This result suggested that it takes time for rotation to reduce replant failure incidences, which is in accordance with the previous report that at least 3 years of rotation are needed to overcome the replant failure in American ginseng (Jiao et al. 2019).

Acknowledgment. We thank the assistant professor Jun Zhao and Ph.D. Baoying Wang from Nanjing Normal University for helping us to analyse the high throughput sequencing data.

REFERENCES

Badri D.V., Vivanco J.M. (2009): Regulation and function of root exudates. Plant, Cell and Environment, 32: 666–681.

Berendsen R.L., Pieterse C.M.J., Bakker P.A.H.M. (2012): The rhizosphere microbiome and plant health. Trends in Plant Science, 17: 478–486.

Chaparro J.M., Sheflin A.M., Manter D.K., Vivanco J.M. (2012): Manipulating the soil microbiome to increase soil health and plant fertility. Biology and Fertility of Soils, 48: 489–499.

Durairaj K., Velmurugan P., Park J.-H., Chang W.-S., Park Y.-J., Senthilkumar P., Choi K.-M., Lee J.-H., Oh B.-T. (2018): An investigation of biocontrol activity *Pseudomonas* and *Bacillus* strains against *Panax ginseng* root rot fungal phytopathogens. Biological Control, 125: 138–146.

Fan Z.Y., Miao C.P., Qiao X.G., Zheng Y.K., Chen H.H., Chen Y.W., Xu L.H., Zhao L.X., Guan H.L. (2016): Diversity, distribution, and antagonistic activities of rhizobacteria of *Panax notoginseng*. Journal of Ginseng Research, 40: 97–104.

FAO (2006): World Reference Base for Soil Resources 2006. A Framework for International Classification, Correlation and Communication. World Soil Resources Reports 103. Rome, Food and Agriculture Organisation.

Fu L., Penton C.R., Ruan Y.Z., Shen Z.Z., Xue C., Li R., Shen Q.R. (2017): Inducing the rhizosphere microbiome by biofertilizer application to suppress banana *Fusarium wilt* disease. Soil Biology and Biochemistry, 104: 39–48.

Gentry L.F., Ruffo M.L., Below F.E. (2013): Identifying factors controlling the continuous corn yield penalty. Agronomy Journal, 105: 295–303.

Jiang J.L., Yu M., Hou R.P., Li L., Ren X.M., Jiao C.J., Yang L.J., Xu H. (2019): Changes in the soil microbial community are associated with the occurrence of *Panax quinquefolius* L. root rot diseases. Plant and Soil, 438: 143–156.

Jiao X.L., Zhang X.S., Lu X.H., Qin R., Bi Y.M., Gao W.W. (2019): Effects of maize rotation on the physicochemical properties and microbial communities of American ginseng cultivated soil. Scientific Reports, 9: 8615.

Jin X., Wang J., Li D.L., Wu F.Z., Zhou X.G. (2019a): Rotations with Indian Mustard and Wild Rocket suppressed cucumber *Fusarium wilt* disease and changed rhizosphere bacterial communities. Microorganisms, 7: 57.

Jin X., Zhang J.H., Shi Y.J., Wu F.Z., Zhou X.G. (2019b): Green manures of Indian mustard and wild rocket enhance cucumber resistance to *Fusarium wilt* through modulating rhizosphere bacterial community composition. Plant and Soil, 441: 283–300.

Kim J.-H. (2012): Cardiovascular diseases and *Panax ginseng*: a review on molecular mechanisms and medical applications. Journal of Ginseng Research, 36: 16–26.

Kõljalg U., Nilsson R.H., Abarenkov K., Tedersoo L., Taylor A.F.S., Bahram M., Bates S.T., Bruns T.D., Bengtsson-Palme J., Callaghan T.M., Douglas B., Drenkhan T., Eberhardt U., Dueñas M., Grebenc T., Griffith G.W., Hartmann M., Kirk P.M., Kohout P., Larsson E., Lindahl B.D., Lücking R., Martín M.P., Matheny M.B., Nguyen N.H., Niskanen T., Oja J., Peay K.G., Peintner U., Peterson M., Põldmaa K., Saag L., Schüβler A., Scott J.A., Senée C., Smith M.E., Suija A., Taylor D.L., Telleria M.T., Weiss M., Larsson K.-H. (2013): Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology, 22: 5271–5277.

Larkin R.P. (2008): Relative effects of biological amendments and crop rotations on soil microbial communities and soilborne diseases of potato. Soil Biology and Biochemistry, 40: 1341–1351.

Larkin R.P., Lynch R.P. (2018): Use and effects of different *Brassica* and other rotation crops on soilborne diseases and yield of potato. Horticulturae, 4: 37.

Li X.G., De Boer W., Zhang Y.N., Ding C.F., Zhang T.L., Wang X.X. (2018): Suppression of soil-borne *Fusarium* pathogens of peanut by intercropping with the medicinal herb *Atractylodes lancea*. Soil Biology and Biochemistry, 116: 120–130.

Li Y.L., Dai S.Y., Wang B.Y., Jiang Y.T., Ma Y.Y., Pan L.L., Wu K., Huang X.Q., Zhang J.B., Cai Z.C., Zhao J. (2020): Autotoxic ginsenoside disrupts soil fungal microbiomes by stimulating potentially pathogenic microbes. Applied and Environmental Microbiology. doi: 10.1128/AEM.00130-20

McDonald D., Price M.N., Goodrich J., Nawrocki E.P., DeSantis T.Z., Probst A., Andersen G.L., Knight R., Hugenholtz P. (2012): An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME Journal, 6: 610–618.

Miao C.P., Mi Q.L., Qiao X.G., Zheng Y.K., Chen Y.W., Xu L.H., Guan H.L., Zhao L.X. (2016): Rhizospheric fungi of *Panax notoginseng*: diversity and antagonism to host phytopathogens. Journal of Ginseng Research, 40: 127–134.

Pianka E.R. (1970): On *r*- and *K*-selection. American Naturalist, 104: 592–597.

Sang M.K., Kim K.D. (2012): The volatile-producing *Flavobacterium johnsoniae* strain GSE09 shows biocontrol activity against *Phytophthora capsici* in pepper. Journal of Applied Microbiology, 113: 383–398.

https://doi.org/10.17221/320/2020-PSE

Song M.J., Yun H.Y., Kim Y.H. (2014): Antagonistic *Bacillus* species as a biological control of ginseng root rot caused by *Fusarium* cf. *incarnatum*. Journal of Ginseng Research, 38: 136–145.

Tan Y., Cui Y.S., Li H.Y., Kuang A.X., Li X.R., Wei Y.L., Ji X.L. (2017): Rhizospheric soil and root endogenous fungal diversity and composition in response to continuous *Panax notoginseng* cropping practices. Microbiological Research, 194: 10–19.

Xiong W., Guo S., Jousset A., Zhao Q.Y., Wu H.S., Li R., Kowalchuk G.A., Shen Q.R. (2017): Bio-fertilizer application induces soil suppressiveness against *Fusarium wilt* disease by reshaping the soil microbiome. Soil Biology and Biochemistry, 114: 238–247.

Yang M., Zhang X., Xu Y., Mei X., Jiang B., Liao J., Yin Z., Zheng J., Zhao Z., Fan L. (2015): Autotoxic ginsenosides in the rhizosphere contribute to the replant failure of *Panax notoginseng*. PLoS One, 10: e0118555.

Ye X.F., Li Z.K., Luo X., Wang W.H., Li Y.K., Li R., Zhang B., Qiao Y., Zhou J., Fan J.Q., Wang H., Huang Y., Cao H., Cui Z.L., Zhang R.F. (2020): A predatory myxobacterium controls cucumber *Fusarium wilt* by regulating the soil microbial community. Microbiome, 8: 49.

Zhao J., Li Y.L., Wang B.Y., Huang X.Q., Yang L., Lan T., Zhang J.B., Cai Z.C. (2017): Comparative soil microbial communities and activities in adjacent Sanqi ginseng monoculture and maize-Sanqi ginseng systems. Applied Soil Ecology, 120: 89–96.

Zhao Q., Xiong W., Xing Y., Sun Y., Lin X., Dong Y. (2018): Long-term coffee monoculture alters soil chemical properties and microbial communities. Scientific Reports, 8: 6116.

Received: June 28, 2020
Accepted: August 25, 2020
Published online: September 14, 2020