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# INFESTATION OF *NECTRIA HAEMATOCOCCA* IN SOIL, A CLUE FOR THE REPLANT DISEASE OF *PANAX NOTOGINSENG* REVEALED BY 454 PYROSEQUENCING

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**Abstract**

*Panax notoginseng* (Burk.) F. H. Chen (Sanchi or Sanqi in Chinese) is a well-known and valuable traditional Chinese herbal medicine, which is currently planted over 20 000 $hm^2$ with an output value of more than 10 billion RMB per year. However, the replant disease characterized by severe root rot has been becoming a serious problem for the *P. notoginseng* industry, and the etiology of replant disease in *P. notoginseng* remains unclear. The microbial communities in the rhizosphere and bulk soils of *P. notoginseng* that had been planted 1, 2 and 3 years previously were investigated using deep amplicon pyrosequencing and analyzed by principal component analysis (PCA). The microbial communities in the rhizosphere and bulk soils represented 94 253, 18 871 and 15 116 reads that were obtained from archaeal, bacterial and fungal PCR amplicons, respectively. A principal component analysis showed that rhizosphere and bulk soils shared similar archaeal and bacterial communities. While fungal communities were greatly different between bulk and rhizosphere soils, and the fungi in the rhizosphere soils had a high diversity level. *Nectria haematococca* (asexual name Fusarium solani), the cause of root rot, was found to accumulate as a consequence of the continuous cultivation of *P. notoginseng*. The accumulation and long-time survival of *N. haematococca* provides a new clue to investigate the mechanism underling replant disease in *P. notoginseng*.

**Key words:** *Panax notoginseng*, *Nectria haematococca*, Replant disease, Principal component analysis (PCA), 454 pyrosequencing.

## Introduction

Radix et Rhizoma Notoginseng (Sanchi or Sanqi) are the dry roots and rhizomes of *Panax notoginseng* (Burk.) F.H. Chen that belongs to the *Panax* genus (Wang *et al*., 2006). Modern studies have found that this herb has multiple active constituents, such as triterpene saponins, dencichine, flavonoids, aliphatic acetylene hydrocarbons, phytosterols, volatile oils, fatty acids, non-protein amino acids, polysaccharides, polyacetylenes, and trace elements (Wang *et al*., 2006; Niu *et al.*, 2014). Among them, the major bioactive components are recognized as triterpene saponins, and at least 56 saponins were isolated and characterized from *P. notoginseng* (Wang *et al*., 2006). Extensive pharmacological findings showed that these sponins possess a wide range of pharmacological activities, such as protective measures for prevention of cerebral ischaemia, beneficial to the cardiovascular system, as well as haemostatic, antioxidant, antitumor, hypolipidaemic, anti-inflammatory, hepatoprotective, renoprotective, antiarrhythmic and estrogen-like activities (Ng, 2006; Chen *et al*., 2014; Zhou *et al*., 2014).

*P. notoginseng* which plays an important role in traditional Chinese herbal medicine is used as a key component for more than 400 Chinese patent medicines, including Yunnan baiyao powder, Danshen dripping pill, and Xuesaitong, and is listed as a dietary supplement by the US Dietary Supplement Health and Education Act (Xia *et al*., 2014). Wenshan, a state of Yunnan Province in China, has been planting for more than 400 years as the Geo-authentic product area of *P. notoginseng* cultivation (Zheng *et al*., 2013; Wang *et al*., 2013). The Plantations are usually distributed in mountain areas with altitudes between 1200 and 2000 m of the Wenshan area, at approximately N23.5° and E104° (Guo *et al*., 2010; Xia *et al*., 2014). In recent years, *P. notoginseng* has been becoming one of the fastest developing species of traditional Chinese medicines with growing demand. It is planted over 20,000 $hm^2$ with an output value of more than 10 billion RMB per year (Cui *et al*., 2014). More than 85% of *P. notoginseng* is from Wenshan area (Qian *et al*., 2008; Yuan *et al*., 2013).

Replant disease usually occurs in a soil where some specific crops are continuously grown. The symptoms induced by replant disease usually display severe plant growth inhibition or necrosis, thus leading to reduced yield and shortened production life (Yang *et al*., 2012). *P. notoginseng* shows the typical characteristics of replant disease (Li *et al*., 2013). The local farmers did not consecutively replanted *P. notoginseng* in the same fields because it would lead to severe root rot with a mortality of approximately 100%. In general, fields that had been used for *P. notoginseng* cultivation can only be reused after 10 years of soil recovery (Zhang *et al*., 2013). Thus, the plantations of *P. notoginseng* need to be exploited in virgin soils after a 2-3 year crop cycle. The cost of developing plantations and the limited numbers of suitable fields are serious problems for the *P. notoginseng* industry (Zhang *et al*., 2013). Obviously, there is an urgent need to understand the underlying mechanism of

replant disease in *P. notoginseng*. Unfortunately, the etiology remains unclear.

Previous studies have speculated soil microorganisms in replant disease (Yang *et al*., 2012). For example, replant diseases in prunus (Benizri *et al*., 2005), peach and apple (Mazzola *et al*., 2012) have been associated with soils microorganisms. Thus, we hypothesized that continuous cropping-driven changes in soil microbes might, at least in part, explain the replant problem of *P. notoginseng*. In this study, the microbial communities in rhizosphere and bulk soils of *P. notoginseng* that had been planted 1-3 years previously were investigated using deep amplicon pyrosequencing. The aim of the present study was to find some microbial clues to explain possible mechanism resulting in replant disease of *P. notoginseng*.

## Materials and Methods

**Soil samples and treatments:** The field experiment was developed at the farm of Miaoxiang Sanqi Ltd. (Wenshan County, Yunnan Province, China) and located at 23° 31' 36"N and 104° 19' 27"E. *P. notoginseng* had been planted under Good Agriculture Practices (GAPs) for 1-3 years previously on the farm of Miaoxiang Ltd. Soil samplings from the three year's plots were collected in October 2012. Fresh plants were carefully rooted out of the soil with a forked spade. Their roots and tubers were slightly shaken to remove the loosely-attached soil. The rhizospheric soil firmly attached to the roots and rhizomes was collected. The rhizospheric soil from five random plants in each plot of five plots were mixed to generate composite samples and then sieved through 2 mm mesh to remove plant roots, leaf remains and insects. The samples were then stored at -20°C for further analyses. The rhizosphere soil of *P. notoginseng* planted 1, 2 and 3 years previously were denoted as R1, R2 and R3, respectively. The bulk soil samples from approximately 20 cm deep cores (at least 0.5 m outside of the plant rows), were collected and each plot of five plots were combined, sieved and stored as described above. The bulk soils of *P. notoginseng* planted 1, 2 and 3 years previously were denoted as B1, B2 and B3, respectively.

**DNA extraction, PCR amplification, amplicon quantitation, and pyrosequencing:** Metagenomic DNA from each soil sample was extracted using the E.Z.N.A.$^{TM}$ Soil DNA Kit (Omega Bio-Tek, Inc., Georgia, USA) according to the manufacturer's instructions. Universal primers ARC 906F (5′-GAAACTTAAAKGAATTG-3′) and ARC 1492R (5′-GGCTACCTTGTTACGACTT-3′), BAC 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and BAC 533R (5′-TTACCGCGGCTGCTGGCAC-3′), and FUN NS1F (5′-CAGTAGTCATATGCTTGTCTC-3′) and FUN NS8R (5′-GCAGGTTCACCTACGGA-3′), incorporating the FLX Titanium adapters targeting archaeal *16S rRNA*, the region of V1-V3 bacterial *16S rRNA* and fungal *18S rRNA* gene, respectively, were selected for the amplification, and then the PCR products were subjected to subsequent pyrosequencing. Platinum® PCR SuperMix (Invitrogen, Carlsbad, CA, USA) was used for PCR. Each PCR consisted of 0.5 μL Platinum Taq (5 U/μL), 5 μL 10-fold PCR buffer, 0.5 μL dNTP(10 mM each), 1 μL Bar-PCR primer (50 μM), 10 ng of genomic DNA and dd $H_2O$ to a final volume of 50 μL. The amplification conditions were as follows: 94°C for 30 s; five cycles of 94°C for 20 s, 45°C for 20 s, 65°C for 60 s; 20 cycles of 94°C for 20 s, 60°C for 20 s, 72°C for 60 s and 72°C for 5 min. All PCR products were visualized on agarose gels (2% in TBE buffer) containing ethidium bromide. For each sample, three independent PCRs were performed. The triplicate products were pooled and purified using AxyPrep PCR Clean-up Kit (Axygen Biosciences, CA, USA). The DNA concentration of each PCR product was determined using a Quant-iT PicoGreen double-stranded DNA assay (Invitrogen, Germany) and was quality controlled on an Agilent 2100 bioanalyzer (Agilent, USA). Amplicon pyrosequencing was performed from the A-end using a 454/Roche A sequencing primer kit on a Roche Genome Sequencer GS-FLX Titanium platform at Enocode Genomics Bio-Technology Co., Ltd., Suzhou, Jiangsu, China.

**Pyrosequencing data processing and bioinformatic analysis:** Data preprocessing was mainly analyzed by using MOTHUR software (Schloss *et al*., 2009; Schloss *et al*., 2011). After the barcode and primers were pruned, further analysis was carried out on less than 200 bp or containing ambiguous bases. The chimeric sequences were also excluded using chimera.uchime command with default parameters (Edgar *et al*., 2011). When the similarity of the sequence is greater than 97%, it would be clustered into one operational taxonomic unit (OTU) by MOTHUR. The taxonomical assignment of each OTU was performed using the classify. seqs command (Naïve Bayesian Classifier) against the SILVA 16S rRNA gene database (release 111) at an 80% confidence level (Wang *et al*., 2007; Huse *et al*., 2008; Quast *et al*., 2013). By using MOTHUR, we obtained community richness and diversity indices (Chao1 estimator, ACE and Shannon indices, respectively) and rarefaction curves (Kemp *et al*., 2004).

To compare bacterial community structures based on the OTU composition across all samples, a principal component analysis (PCA) of the sites was performed using the SPSS software package (SPSS 19.0, Chicago, IL, USA).

**Nucleotide sequence accession numbers:** The 454 pyrosequencing data generated for this study were deposited in the Sequence Read Archive (SRA) database (accession number SRR1610276).

## Results and Discussion

After removing low quality and chimeric sequences, a total of 94 253, 18 871 and 15 116 high quality sequences with average read lengths of approximately 509 497 and 530 bp, were, obtained from run of the archaeal, bacterial and fungal PCR amplicons, respectively. Each library contains 1 117-17 309 reads with different phylogenetic OTUs ranging from 43 to 1 942 (Table 1). Rarefaction curves at a 3% cutoff are shown in Fig. 1. In addition, the Chao1 estimation, and ACE and Shannon indices are shown in Table 1.

**Table 1. Pyrosequencing of rhizosphere and bulk soil samples of *P. notoginseng* planted 1, 2 and 3 years ago.**

| | Sample | No. of reads | OTUs | Richness estimators | | Diversity indices | |
|---|---|---|---|---|---|---|---|
| | | | | ACE | Chao1 | Shannon | Simpson |
| Archaea | B1 | 22787 | 294 | 864.98 | 577.50 | 0.45 | 0.89 |
| | R1 | 9559 | 1942 | 4901.43 | 3401.81 | 5.26 | 0.04 |
| | B2 | 28688 | 590 | 1318.94 | 1023.65 | 1.19 | 0.69 |
| | R2 | 6433 | 969 | 2162.69 | 1680.01 | 4.21 | 0.12 |
| | B3 | 20473 | 739 | 2293.82 | 1557.42 | 1.55 | 0.61 |
| | R3 | 6313 | 1429 | 3282.54 | 2518.04 | 4.91 | 0.06 |
| Bacteria | B1 | 2484 | 675 | 2317.47 | 1485.27 | 5.33 | 0.01 |
| | R1 | 3284 | 1441 | 8399.13 | 3993.50 | 6.44 | 0.01 |
| | B2 | 5082 | 1196 | 4076.87 | 2711.13 | 5.80 | 0.01 |
| | R2 | 3829 | 1247 | 5097.36 | 3142.51 | 6.22 | 0.00 |
| | B3 | 1457 | 661 | 3020.84 | 1711.78 | 5.89 | 0.01 |
| | R3 | 3865 | 1643 | 9061.86 | 5258.08 | 6.48 | 0.01 |
| Fungi | B1 | 1672 | 43 | 68.58 | 70.14 | 0.58 | 0.83 |
| | R1 | 3846 | 283 | 620.08 | 534.78 | 2.74 | 0.29 |
| | B2 | 1478 | 54 | 225.63 | 153.17 | 1.01 | 0.59 |
| | R2 | 2566 | 248 | 543.97 | 443.03 | 3.14 | 0.20 |
| | B3 | 1569 | 78 | 228.77 | 154.15 | 0.95 | 0.73 |
| | R3 | 3985 | 370 | 758.50 | 641.78 | 4.13 | 0.04 |

Note: B1, 2, 3 and R1, 2, 3 indicated the samples of bulk and rhizosphere soils, respectively.

**Soil microbial community structure:** The overall microbial communities at the class level were summarized in Figure. 2. The relative abundance (%) of archaea, bacteria and fungi in each soil sample were shown in Tables 2, 3 and 4, respectively. The dominated archaea were classified as Crenarchaeota and Thermoprotei, accounting for approximately 31.59-98.71% of the 16S *rRNA* gene sequences per sample (Fig. 2A). Additionally, in the rhizosphere soil, the relative abundance of Euryarchaeota increased (Table 2, Fig. 2A). PCA analyses showed that the archaeal communities of B1, B2, B3, R2 and R3 were grouped, while R1 was separated from the group (Fig. 3A), suggesting that the archaeal community of R1 differed from those of B1, B2, B3, R2 and R3.

These bacterial OTUs were classified into 25 phyla and 49 classes (Table 4, Fig. 2B). At the class level, the bacteria belonging to Acidobacteria (13.16-31% of the total reads of each sample), Alphaproteobacteria (9.8-23.7%), Betaproteobacteria (3.99-11.49%) and Gammaproteobacteria (4.92-23.63%) were the dominants. PCA analyses showed that the bacterial communities of all samples were grouped (Fig. 3B), indicating that they did not differ from each other. Except unclassified fungi, fungal OTUs were classified into eight phyla and 33 classes (Table 3, Fig. 2C). PCA analysis showed that the fungal communities of B1, B2 and B3 were grouped even on the third principal component axis (Fig. 3C), indicating that bulk soils have similar fungal assemblages with dominant fungi of Dacrymycetes (75.51-91.21% of the each samples reads). However, the fungal communities of R1, R2 and R3 were separate from each other, indicating that fungal communities of rhizosphere soils had a high level of diversity, which was further corroborated by the greater Shannon index of rhizosphere soils than that of bulk soils ($p< 0.05$, Table 1). The group of B1, B2 and B3 was separated from R1, R2 and R3, suggesting that fungal communities shifted greatly between bulk and rhizosphere soils (Fig. 3C). The dominant classes in rhizosphere soils were Agaricomycetes (13.81-57.09%), Blastocladiomycetes, (0.27-54.03%), Glomeromycetes (4.75-17.19%) and unclassified Chytridiomycota (5.77-21.18%) (Fig. 2C). Among them, fungi belonging to Glomeromycetes were further identified to have the species belonging *Acaulospora*, *Gigaspora* and *Glomus* genera which were known to form arbuscular mycorrhiza (Kohout *et al*., 2014; Borriello *et al*., 2014).

**Accumulation of *N. haematococca* in soil:** To investigate the relationship between soil microorganisms and the replant disease of *P. notoginseng*, we further analyzed the plant pathogens. Interestingly, *N. haematococca* was detected in the rhizosphere and bulk soils of *P. notoginseng* planted for 3 years, representing 2.79% and 1.40% of each samples reads, respectively; however, this fungus was not detected in the other years' plantings. This suggested that this fungus colonized as a consequence of the consecutive cultivation of *P. notoginseng*. The ascomycetous fungus *N. haematococca*, (asexual name *Fusarium solani*) has the ability to cause disease on > 100 genera of plants (Coleman *et al*., 2009), such as foot rot disease of peas (Etebu & *Osborn*, 2010), physic nut (*Jatropha curcas*) (Wu *et al*., 2011) and pepper (*Piper nigrum* L.) (Ikeda, 2010), fruit and stem rots of Capsicum (Tyson *et al*., 2001) and sudden death syndrome of soybean (Abney *et al*., 1993). By *in vitro* pathogenicity tests, Miao *et al.,* (2006) revealed that *N. haematococca* could cause root rot disease of *P. notoginseng* (Miao *et al*., 2006). Morever, *N. haematococca* has strong vitality in the soil and can survive unchecked in infected soil (Bahar & Shahab., 2012). Taken together, these results suggest that the durable accumulation of *N. haematococca* in replant soil might play a key role in the replant disease of *P. notoginseng*. Further separation, infection and colonization will be needed to assess the possible role of *N. haematococca* in this replant disease.

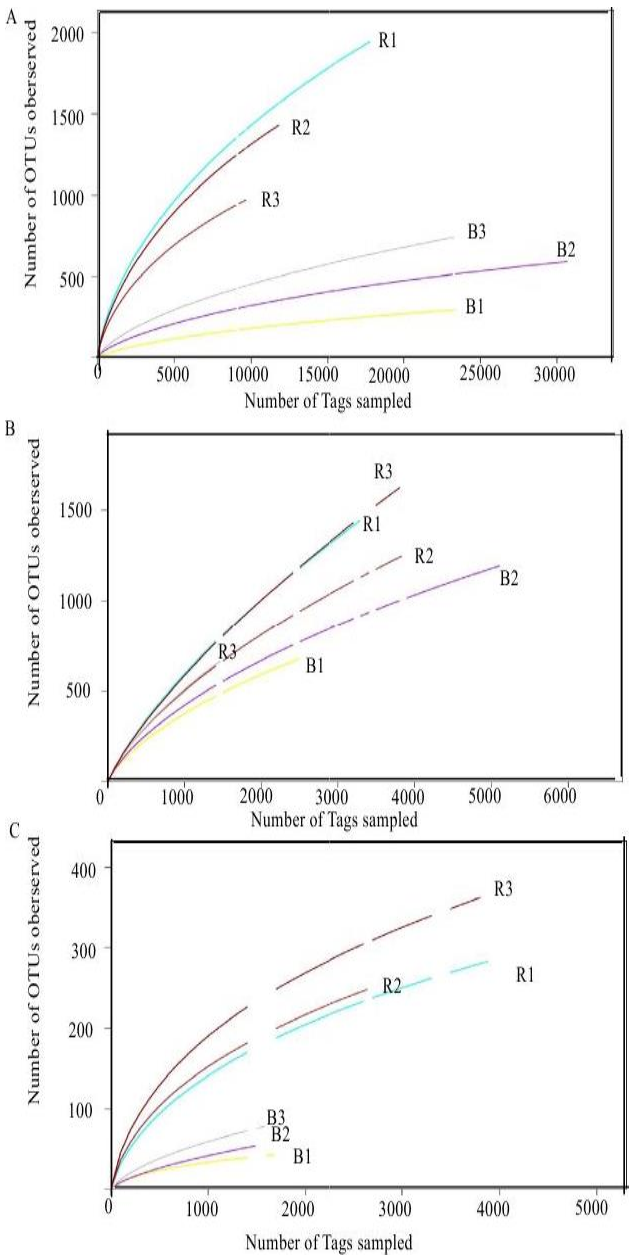


Fig. 1. Rarefaction curves indicating the observed number of operational taxonomic units (OTUs) at a genetic distance of 3% of archaea (a), bacteria (b) and fungi (c) in rhizosphere and bulk soils of *P. notoginseng* planted 1, 2 and 3 years ago. B1, 2, 3 and R1, 2, 3 indicated the samples of bulk and rhizosphere soils, respectively, of *P. notoginseng* planted 1, 2 and 3 years ago, respectively.

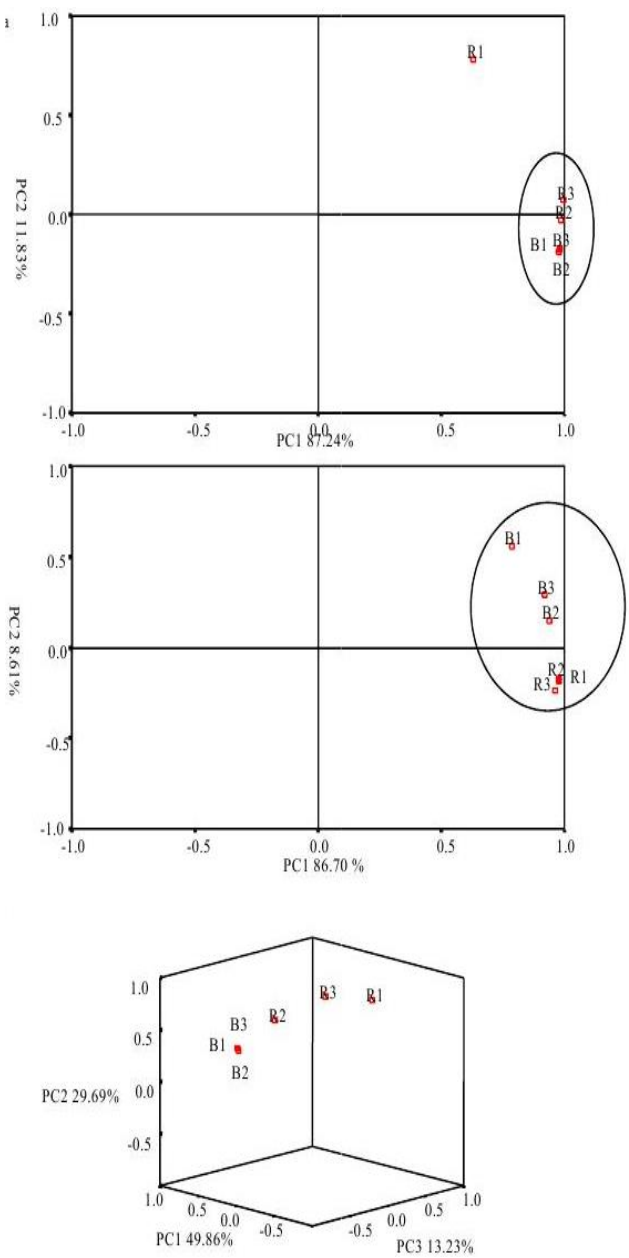


Fig. 2. Relative abundance of archaea (a), bacteria (b) and fungi (c) obtained from a pyrosequencing analysis in rhizosphere and bulk soils of *P. notoginseng* planted 1, 2 and 3 years ago. Other are composed of the classes showing a percentage of reads < 1.0% of the total reads in each sample. B1-3 and R1-3 indicated the samples of bulk and rhizosphere soils, respectively, of *P. notoginseng* planted 1, 2 and 3 years ago, respectively.

## Conclusion

The microbial communities in rhizosphere and bulk soils that had been planted *P. notoginseng* for 1, 2 or 3 years were analyzed by 454 pyrosequencing method and compared using PCA. There were no differences in both archaeal and bacterial communities between rhizosphere and bulk soils. However, the fungal communities differed greatly between bulk and rhizosphere soils, and fungi in rhizosphere soils had a high diversity levels. We identified *N. haematococca* whose accumulation was the cause of root rot disease, which provided a new clue for elucidating the mechanism of replant disease of *P. notoginseng*.

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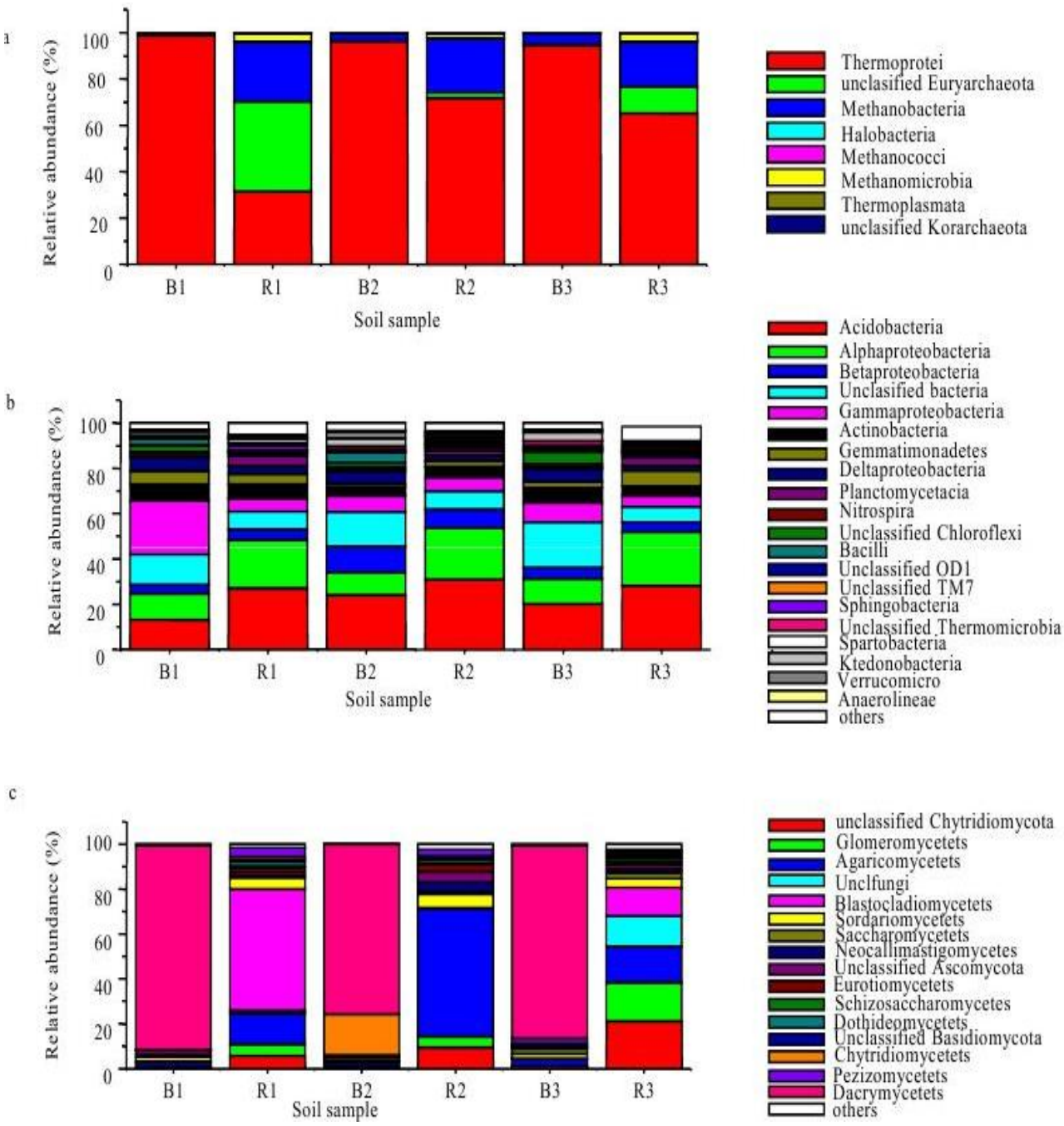


Fig. 3. Principal component analysis of archaeal (a), bacterial (b) and fungal (c) communities in rhizosphere and bulk soils of *P. notoginseng* planted 1, 2 and 3 years ago, based on the relative abundances of the sample reads at the class level.

**Table 3. Relative abundance (%) of archaea in each soil sample.**

| Taxonomic groups | | B1 | R1 | B2 | R2 | B3 | R3 |
|---|---|---|---|---|---|---|---|
| **Phylum** | **Class** | | | | | | |
| Crenarchaeota | Thermoprotei | 98.71% | 31.59% | 96.29% | 71.77% | 94.76% | 65.25% |
| Euryarchaeota | Halobacteria | 0.00% | 0.03% | 0.00% | 0.06% | 0.00% | 0.14% |
| | Methanobacteria | 0.93% | 25.64% | 3.10% | 23.53% | 4.48% | 19.29% |
| | Methanococci | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% |
| | Methanomicrobia | 0.15% | 3.44% | 0.30% | 2.24% | 0.28% | 3.67% |
| | Thermoplasmata | 0.00% | 0.41% | 0.10% | 0.09% | 0.14% | 0.16% |
| | Unclasified Euryarchaeota | 0.20% | 38.88% | 0.20% | 2.27% | 0.34% | 11.47% |
| Korarchaeota | Unclasified Korarchaeota | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% |

Note: B1, 2, 3 and R1, 2, 3 indicated the samples of bulk and rhizosphere soils, respectively

**Table 2. Relative abundance (%) of fungi in each soil sample.**

| Taxonomic groups / Phylum | Class | B1 | R1 | B2 | R2 | B3 | R3 |
|---|---|---|---|---|---|---|---|
| Ascomycota | Dothideomycetes | 0.00% | 2.21% | 0.20% | 1.64% | 0.83% | 1.25% |
| | Eurotiomycetes | 0.42% | 2.08% | 0.07% | 3.31% | 0.51% | 1.51% |
| | Laboulbeniomycetes | 0.00% | 0.10% | 0.00% | 0.00% | 0.06% | 0.00% |
| | Lecanoromycetes | 0.00% | 0.05% | 0.00% | 0.04% | 0.00% | 0.05% |
| | Leotiomycetes | 0.00% | 0.29% | 0.00% | 0.04% | 0.00% | 0.28% |
| | Orbiliomycetes | 0.00% | 0.00% | 0.00% | 0.12% | 0.00% | 0.03% |
| | Pezizomycetes | 0.06% | 4.11% | 0.20% | 2.84% | 2.29% | 0.78% |
| | Pneumocystidomycetes | 0.00% | 0.08% | 0.00% | 0.00% | 0.00% | 0.03% |
| | Saccharomycetes | 0.84% | 0.10% | 1.56% | 1.33% | 2.29% | 2.23% |
| | Schizosaccharomycetes | 0.00% | 0.60% | 0.07% | 0.55% | 0.00% | 1.48% |
| | Sordariomycetes | 2.15% | 5.04% | 0.54% | 6.16% | 2.10% | 4.24% |
| | Taphrinomycetes | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% |
| | Unclassified Ascomycota | 1.91% | 1.59% | 0.20% | 4.60% | 0.57% | 1.68% |
| Basidiomycota | Agaricomycetes | 2.03% | 13.81% | 1.49% | 57.09% | 2.80% | 15.98% |
| | Agaricostilbomycetes | 0.00% | 0.13% | 0.00% | 0.23% | 0.00% | 0.38% |
| | Atractiellomycetes | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | Dacrymycetes | 91.21% | 0.00% | 75.51% | 0.04% | 85.79% | 0.00% |
| | Exobasidiomycetes | 0.00% | 0.05% | 0.00% | 0.31% | 0.00% | 0.43% |
| | Tremellomycetes | 0.30% | 0.42% | 0.00% | 0.19% | 0.19% | 0.35% |
| | Tritirachiomycetes | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | Unclassified Basidiomycota | 0.00% | 0.73% | 0.14% | 0.66% | 0.00% | 1.05% |
| | Ustilaginomycetes | 0.00% | 0.00% | 0.00% | 0.12% | 0.00% | 0.03% |
| | Wallemiomycetes | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| Blastocladiomycota | Blastocladiomycetes | 0.18% | 54.03% | 0.20% | 0.27% | 0.06% | 12.55% |
| Chytridiomycota | Chytridiomycetes | 0.00% | 1.33% | 18.13% | 1.05% | 0.00% | 0.98% |
| | Unclassified Chytridiomycota | 0.42% | 5.77% | 0.54% | 9.51% | 1.15% | 21.18% |
| Entomophthoromycota | Basidiobolomycetes | 0.06% | 0.16% | 0.00% | 0.00% | 0.00% | 0.05% |
| | Entomophthoromycetes | 0.00% | 0.00% | 0.00% | 0.55% | 0.32% | 0.20% |
| Glomeromycota | Glomeromycetes | 0.36% | 5.02% | 0.27% | 4.75% | 0.25% | 17.19% |
| | Unclassified Glomeromycota | 0.00% | 0.18% | 0.07% | 0.55% | 0.13% | 0.43% |
| Monoblepharidomycota | Monoblepharidomycetes | 0.00% | 0.03% | 0.00% | 0.16% | 0.00% | 0.18% |
| Neocallimastigomycota | Neocallimastigomycetes | 0.00% | 0.73% | 0.07% | 3.90% | 0.32% | 1.76% |
| Unclassified fungi | Unclassified fungi | 0.06% | 1.38% | 0.74% | 0.00% | 0.32% | 13.70% |

Note: B1, 2, 3 and R1, 2, 3 indicated the samples of bulk and rhizosphere soils, respectively

**Table 4. Relative abundance (%) of bacteria in each soil sample.**

| Taxonomic groups | | B1 | R1 | B2 | R2 | B3 | R3 |
|---|---|---|---|---|---|---|---|
| **Phylum** | **Class** | | | | | | |
| Acidobacteria | Acidobacteria | 13.16% | 26.95% | 24.10% | 31.00% | 20.18% | 28.18% |
| | Solibacteres | 0.32% | 0.52% | 0.20% | 0.44% | 0.07% | 0.44% |
| | TM1 | 0.04% | 0.09% | 0.51% | 0.00% | 0.27% | 0.03% |
| | Unclassified Acidobacteria | 0.04% | 0.88% | 0.20% | 0.21% | 0.34% | 0.98% |
| Actinobacteria | Actinobacteria | 7.41% | 6.24% | 3.84% | 4.78% | 6.52% | 4.27% |
| | Unclassified Actinobacteria | 0.04% | 0.06% | 0.02% | 0.00% | 0.07% | 0.03% |
| Bacteroidetes | Bacteroidia | 0.08% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | Flavobacteria | 0.12% | 0.18% | 0.43% | 0.03% | 0.00% | 0.47% |
| | Sphingobacteria | 0.40% | 2.10% | 0.87% | 0.81% | 1.17% | 1.47% |
| | Unclassified Bacteroidetes | 0.04% | 0.09% | 0.12% | 0.31% | 0.07% | 0.28% |
| Chloroflexi | Anaerolineae | 0.56% | 1.00% | 0.55% | 0.47% | 0.41% | 1.16% |
| | Caldilineae | 0.00% | 0.09% | 0.00% | 0.00% | 0.07% | 0.05% |
| | Chloroflexi | 0.08% | 0.67% | 0.02% | 0.21% | 0.34% | 0.93% |
| | Ktedonobacteria | 1.49% | 0.18% | 3.42% | 0.63% | 3.77% | 0.08% |
| | Unclassified Chloroflexi | 3.10% | 0.85% | 1.91% | 1.25% | 5.42% | 0.96% |
| Deinococcus-Thermus | Deinococci | 0.20% | 0.00% | 0.00% | 0.00% | 0.07% | 0.00% |
| Firmicutes | Bacilli | 3.10% | 0.09% | 4.43% | 0.13% | 0.75% | 0.10% |
| | Clostridia | 0.93% | 0.06% | 0.14% | 0.18% | 0.14% | 0.18% |
| | Erysipelotrichi | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% |
| Gemmatimonadetes | Gemmatimonadetes | 5.80% | 4.60% | 1.44% | 2.64% | 2.68% | 6.78% |
| | Unclassified Gemmatimonadetes | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.05% |
| Nitrospira | Nitrospira | 1.53% | 1.22% | 1.14% | 1.31% | 1.44% | 1.42% |
| | Unclassified Nitrospira | 0.04% | 0.43% | 0.55% | 0.16% | 0.34% | 0.13% |
| Planctomycetes | Planctomycetacia | 0.89% | 4.11% | 0.91% | 1.80% | 0.89% | 3.60% |
| | Unclassified Planctomycetes | 0.00% | 0.30% | 0.02% | 0.24% | 0.07% | 0.70% |
| Proteobacteria | Alphaproteobacteria | 11.43% | 21.44% | 9.80% | 22.67% | 11.05% | 23.70% |
| | Betaproteobacteria | 3.99% | 4.57% | 11.49% | 8.31% | 5.01% | 4.32% |
| | Deltaproteobacteria | 5.84% | 3.62% | 5.39% | 2.45% | 5.56% | 2.15% |
| | Gammaproteobacteria | 23.63% | 5.72% | 7.22% | 6.11% | 8.72% | 4.92% |
| | Unclassified Proteobacteria | 0.04% | 0.27% | 0.10% | 0.24% | 0.62% | 0.34% |
| Spirochaetes | Spirochaetes | 0.00% | 0.00% | 0.02% | 0.08% | 0.07% | 0.03% |
| TG1 | Elusimicrobia | 0.00% | 0.03% | 0.04% | 0.03% | 0.07% | 0.00% |
| Thermomicrobia | Thermomicrobia | 0.00% | 0.03% | 0.00% | 0.03% | 0.00% | 0.05% |
| | Unclassified Thermomicrobia | 0.32% | 1.22% | 1.32% | 0.78% | 1.99% | 1.29% |
| Verrucomicrobia | Opitutae | 0.12% | 0.18% | 0.47% | 0.24% | 0.07% | 0.18% |
| | Spartobacteria | 0.28% | 1.64% | 0.10% | 0.76% | 0.21% | 0.54% |
| | Verrucomicrobiae | 0.48% | 0.67% | 2.83% | 0.60% | 0.55% | 1.03% |
| BRC1 | Unclassified BRC1 | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.05% |
| Chlorobi | Unclassified Chlorobi | 0.04% | 0.09% | 0.00% | 0.00% | 0.00% | 0.05% |
| Fibrobacteres | Unclassified Fibrobacteres | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.05% |
| OD1 | Unclassified OD1 | 0.08% | 0.30% | 0.51% | 1.04% | 0.27% | 0.36% |
| OP10 | Unclassified OP10 | 0.56% | 0.88% | 0.18% | 0.63% | 0.27% | 0.60% |
| OP11 | Unclassified OP11 | 0.04% | 0.00% | 0.04% | 0.05% | 0.00% | 0.05% |
| OP3 | Unclassified OP3 | 0.00% | 0.06% | 0.02% | 0.00% | 0.00% | 0.00% |
| OP5 | Unclassified OP5 | 0.04% | 0.03% | 0.02% | 0.00% | 0.00% | 0.00% |
| TM6 | Unclassified TM6 | 0.00% | 0.00% | 0.08% | 0.44% | 0.00% | 0.39% |
| TM7 | Unclassified TM7 | 0.28% | 0.33% | 0.22% | 1.04% | 0.41% | 0.52% |
| WS3 | Unclassified WS3 | 0.00% | 0.06% | 0.02% | 0.03% | 0.07% | 0.08% |
| Unclasified bacteria | Unclasified bacteria | 13.45% | 8.04% | 15.31% | 7.86% | 19.97% | 6.83% |

Note: B1, 2, 3 and R1, 2, 3 indicated the samples of bulk and rhizosphere soils, respectively

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