Effects of Different Leaf Litters on the Physicochemical Properties and Microbial Community Structure of Ginseng Soil (Postprint)

Authors: Sun Hai, Wang Qiuxia, Zhang Chunge, Li Le, Liu Zhengbo, The input appears to be incomplete. Please provide the full academic paper content containing the ···tags, LaTeX formulas, and text that requires translation. The current input only contains a name "刘宁" (Liu Ning), which does not constitute translatable academic content under the specified format., Shao Cai, Zhang Yayu, Yan Jun, Yuexiong Li

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Abstract

Tree species selection is critical to the success of forest understory ginseng cultivation. This study investigated the effects of leaf litter on soil nutrients and microbial community structure in ginseng soils, aiming to provide a scientific basis and theoretical guidance for selecting suitable forest lands for understory ginseng cultivation and for improving soils in farmland ginseng cultivation. Through a pot experiment, 5.0 g of leaf litter from Acer mono.Maxim.var.mono (A), Pinus densiflora Sieb.et Zucc. (B), Juglans mandshurica Maxim. (C), Tilia amurensis Rupr. (D), and Quercus mongolica Fisch.ex Ledeb. (E) were added to soil, and changes in soil physicochemical properties and microbial community structure were examined after planting Panax ginseng C.A.meyer. The results indicated that the addition of different leaf litters altered ginseng soil properties, with soil pH being significantly higher than the control value of 5.91 ($P < 0.05$). Soil total nitrogen, available nitrogen and phosphorus, and microbial carbon and nitrogen increased significantly in all leaf litter treatments ($P < 0.05$), whereas soil bulk density, available potassium, and C/N ratio decreased in the leaf litter addition treatments. Genome sequencing of 18 soil samples yielded 6064 and 1900 OTUs through 16S and ITS1 sequencing, respectively. The bacterial sequences encompassed 42 phyla, 117 classes, 170 orders, 213 families, and 225 genera, while fungal sequences encompassed 24 phyla, 98 classes, 196 orders, 330 families, and 435 genera. The status of bacteria and fungi in ginseng soils changed under different leaf litter treatments. Bacterial Proteobacteria were the key microorganisms for leaf decomposition, with their diversity being significantly higher than the control after leaf addition ($P < 0.05$). Bacterial Bacteroidetes and fungal Basidiomycota may be key microorganisms distinguishing broadleaf and coniferous forest tree species, with their abundance being significantly lower in coniferous forests than in broadleaf forests ($P < 0.05$), while fungal Ascomycota were the key microorganisms for coniferous litter decomposition. Furthermore, specific bacteria and fungi associated with particular leaf litters were identified at different taxonomic levels. Canonical correlation analysis (CDA) revealed that changes in the composition and diversity of bacterial Bacteroidetes, Chloroflexi, Actinobacteria and fungal Basidiomycota, Zygomycota, Chytridiomycota, and Ascomycota were all correlated with soil factors including SMBN, TN, AP, SOC, AK, C/N, and pH. In summary, the addition of different leaf litters not only increased soil microbial biomass carbon and nitrogen and improved soil physicochemical properties, but also altered microbial community structure composition. Differences in soil physicochemical properties under different leaf litter treatments led to variations in ginseng soil microbial composition. These results provide theoretical guidance for site selection in forest understory ginseng cultivation and for microbial improvement of farmland ginseng cultivation soils.

Full Text

Effects of Different Leaf Litters on the Physicochemical Properties and Microbial Community Structure of Ginseng-Growing Soil

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Abstract: Tree species selection is critical for the success of understory ginseng cultivation. Leaf litter plays a significant role in determining soil physicochemical properties and shaping soil microbial communities in forest ecosystems, yet its effects on understory wild ginseng and cultivated ginseng soils remain unknown. To address this knowledge gap, leaf litters from five tree species—*Juglans mandshurica* (C), *Acer mono* (A), *Tilia amurensis* (D), *Pinus densiflora* (B), and *Quercus mongolica* (E)—were added to ginseng-growing soil in a pot experiment. MiSeq high-throughput sequencing was used to analyze and compare bacterial and fungal diversity and community composition across different leaf litter treatments. Sequencing of the V4 region of 16S rDNA and ITS1 rDNA from eighteen soil samples yielded 6,064 bacterial OTUs and 1,900 fungal OTUs. Bacterial sequences covered 42 phyla, 117 classes, 170 orders, 213 families, and 225 genera, while fungal sequences covered 24 phyla, 98 classes, 196 orders, 330 families, and 435 genera.

Results indicated that all leaf litter treatments significantly affected soil physicochemical properties. Soil total nitrogen, available NPK, and microbial biomass carbon and nitrogen increased significantly ($P < 0.05$) across all treatments, while soil bulk density and C/N ratio decreased compared to the control (no litter addition). Significant changes in bacterial and fungal community composition were observed, with the relative abundance of *Proteobacteria* being higher in all treatments than in the control. The bacterial phylum *Bacteroidetes* and fungal phylum *Basidiomycota* showed lower abundance in coniferous litter treatments compared to broadleaf litter treatments ($P < 0.05$), while the fungal phylum *Ascomycota* may represent key microbes for coniferous litter decomposition. LEfSe (Linear Discriminant Analysis Effect Size) analysis revealed specific bacterial and fungal taxa overexpressed at kingdom, phylum, class, order, family, genus, and species levels based on relative abundance. These included the bacterial genus *Sphingomonas* and fungal species *Podospora glutinans* and *Exophiala equina* in treatment D, and specific fungal taxa in treatment B. Canonical discriminant analysis (CDA) confirmed that shifts in microbial community composition and diversity were closely related to changes in soil microbial biomass nitrogen, total nitrogen, available phosphorus, soil organic carbon, available potassium, C/N ratio, and pH across all treatments.

Our findings suggest that leaf litter addition significantly affects soil bacterial community development, leading to higher soil nutrients and microbial biomass as well as altered bacterial community composition. The resultant soil bacterial communities influence litter decomposition and wild understory ginseng management. These results provide theoretical guidance for site selection in understory ginseng cultivation and soil microbial improvement in field-cultivated ginseng systems.

Keywords: *Panax ginseng*; leaf litter; soil nutrients; soil microbial carbon; soil microbial nitrogen; 16S; ITS sequences; community structure

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1. Collection of Potting Soil and Leaves

Soil and leaf litter were collected from the Wujie understory ginseng cultivation base in Lengchang Village, Fusong County, Jilin Province, a major production area for forest-cultivated ginseng (127°75 78 E, 42°81 12 N). The potting soil was obtained from abandoned farmland adjacent to the base in 2015. Concurrently, fallen leaves of *Acer mono*, *Tilia amurensis*, and *Quercus mongolica* were collected from the understory ginseng base. Collected leaves were oven-dried at 60°C to constant weight and passed through a 2 mm sieve. The initial physicochemical properties of the potting soil were: pH 5.89, organic carbon 20.89 g/kg, total nitrogen 1.61 g/kg, available phosphorus 25.83 mg/kg, and available potassium 220.54 mg/kg. The elemental composition of different leaf litters is shown in .

2. Experimental Design and Soil Sample Preparation

The pot experiment was arranged using a completely randomized block design at the Institute of Special Animal and Plant Science, Chinese Academy of Agricultural Sciences in May 2015. Based on Zhang Xinping’ s survey method for leaf litter quantity per unit area at the Wujie understory ginseng base, the final determined leaf addition rate was 5.0 g per 3.5 kg soil. Six treatments were established: A) *Acer mono* (5.0 g), B) *Pinus densiflora* (5.0 g), C) *Juglans mandshurica* (5.0 g), D) *Tilia amurensis* (5.0 g), E) *Quercus mongolica* (5.0 g), and F) control (no leaf addition). *Acer mono*, *Tilia amurensis*, and *Quercus mongolica* are broadleaf species, while *Pinus densiflora* is a coniferous species. Leaf litters were sieved through a 0.01 mm mesh and thoroughly mixed with soil as the test substrate.

Uniformly sized 2-year-old ginseng seedlings were planted in black nutrient pots (120 mm diameter × 180 mm height), with each pot serving as one replicate. All treatments were watered weekly to maintain 70–80% water holding capacity. Pot temperature was maintained at 17–28°C. After one growing season in October 2015, rhizosphere soil from each plant was collected. Soil samples from three plants per pot were mixed to form one replicate. Samples were divided into two portions: one air-dried for physicochemical analysis and another stored at -80°C for microbial analysis.

3. Measurement Indicators and Methods

Soil pH was measured at a 1:2.5 soil-water ratio using a Mettler Toledo pH meter. Soil organic carbon and total nitrogen were determined using a Vario EL III elemental analyzer. Available phosphorus was measured by sodium bicarbonate extraction followed by molybdenum-antimony anti-colorimetry. Available potassium was extracted with ammonium acetate and measured by flame photometry. Alkaline hydrolyzable nitrogen was determined by the petri dish diffusion method. Soil microbial biomass carbon and nitrogen were measured using chloroform fumigation-potassium sulfate extraction, with organic carbon content in extracts measured by a Vario TOC analyzer and total nitrogen by continuous flow analyzer (AA3). Results were multiplied by correction factors KEC (0.45) and KEN (0.54) to obtain microbial biomass carbon and nitrogen contents.

For DNA extraction, 0.1 g soil was processed using the Power Soil™ DNA Isolation Kit (MoBio, USA) following manufacturer instructions. Bacterial and fungal ribosomal gene amplification and sequencing were performed by Novogene Bioinformatics Technology Co., Ltd. Bacterial 16S rDNA V4 region was amplified using primers 515F (5 -GTGCCAGCMGCCGCGGTAA-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3) following Caporaso et al. [29]. Fungal ITS1 region was amplified using primers ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG). PCR conditions were: 98°C for 10 s, 50°C for 30 s, 72°C for 60 s (30 cycles), and final extension at 72°C for 5 min. Sequencing was performed on an Illumina MiSeq platform.

4. Data Statistics and Analysis

Raw sequences were processed using QIIME software. After trimming barcode and primer sequences, paired-end reads were merged using FLASH software. Quality filtering was performed to obtain high-quality sequences, which were then compared against databases to detect chimeric sequences, resulting in effective data (Clean Tags). UPARSE pipeline (V7.0.1001) was used for clustering at 97% similarity to obtain OTUs. RDP classifier (V2.2) was used for species annotation against databases. SAS 9.0 was used for variance analysis, Excel 2013 for data organization, and CANOCO 5.0 for correlation analysis.

1. Soil Properties

After one growing season, soil physicochemical properties under different leaf treatments are shown in . Soil pH values in all leaf addition treatments were significantly higher than the control ($P < 0.05$). Soil bulk density in treatments A, B, C, D, and E decreased to 0.90, 0.96, 0.95, 0.94, and 0.95 g/cm^3, respectively, significantly lower than the control (0.96 g/cm^3). Soil organic carbon and total nitrogen ranged from 20.20-28.90 g/kg and 2.20–4.11 g/kg, respectively. The C/N ratio in all treatments (7.04-8.35) was significantly lower than the control (8.36, $P < 0.05$). Microbial biomass carbon and nitrogen, as well as available nitrogen and phosphorus, increased significantly. Treatment D showed the highest microbial biomass carbon content, while treatment E showed the highest microbial biomass nitrogen content. All treatments were significantly higher than the control ($P < 0.05$).

2. Basic Sequencing Data Analysis

MiSeq sequencing yielded 16S rDNA reads ranging from 47,500 per sample. After quality filtering, high-quality reads were obtained. A total of 6,064 bacterial OTUs were identified across all samples, with individual sample OTU numbers ranging from 1,370. Over 90% of sequences were retained for further analysis. ITS1 sequencing yielded reads ranging from 47,500 per sample, with 1,900 fungal OTUs identified. At the phylum level, unclassified sequences accounted for 10% of bacterial reads. The dominant bacterial phyla were *Proteobacteria* (41%), *Acidobacteria* (18%), *Actinobacteria* (14%), *Gemmatimonadetes* (8%), *Chloroflexi* (5%), *Crenarchaeota* (3%), *Nitrospirae* (3%), *Firmicutes* (2%), *Verrucomicrobia* (2%), and *Bacteroidetes* (2%) [Figure 1a: see original paper].

At the class level, the most abundant bacterial classes were *Alphaproteobacteria* (18.07%), *Acidobacteria-6* (18.07%), *Betaproteobacteria* (9.11%), *Deltaproteobacteria* (7.69%), *Actinobacteria* (6.72%), and *Gammaproteobacteria* (5.90%). At the phylum level for fungi, unclassified sequences accounted for 10% of reads, with *Ascomycota* (47%), *Basidiomycota* (24%), *Chytridiomycota* (5%), and *Zygomycota* (1%) being dominant [Figure 1b: see original paper]. At the class level, the most abundant fungal classes were *Sordariomycetes* (30.81%), *Agaricomycetes* (23.78%), *Eurotiomycetes* (11.83%), *Dothideomycetes* (3.99%), and *Chytridiomycetes* (3.08%).

3. Effects of Different Leaf Treatments on Soil Microbial Community Structure

Cluster analysis based on weighted UniFrac UPGMA revealed that bacterial communities clustered into four groups: treatments A and D, treatments B and C, treatment E, and the control [Figure 2: see original paper]. Fungal communities also formed four distinct groups. At the phylum level, the top five dominant bacterial phyla were consistent across all leaf treatments: *Proteobacteria* (35.38-43.69%), *Acidobacteria* (17.10-20.34%), *Actinobacteria* (12.77-14.76%), *Gemmatimonadetes* (7.85-9.36%), and *Chloroflexi* (4.37-5.02%). However, the relative abundances of *Firmicutes*, *Crenarchaeota*, *Verrucomicrobia*, *Nitrospirae*, and *Bacteroidetes* varied among treatments.

At the phylum level, all leaf treatments showed *Ascomycota* as the dominant fungal group, while the control showed *Basidiomycota* as the second most abundant phylum. Variance analysis using SAS 9.0 revealed that *Bacteroidetes* abundance was significantly higher in broadleaf treatments than in coniferous treatments ($P < 0.05$) and significantly higher in all leaf treatments compared to the control. The abundance of *Ascomycota* differed significantly between coniferous treatment B and the control ($P < 0.01$), while *Basidiomycota* abundance was significantly lower in treatment B compared to treatments A, C, and D ($P < 0.01$) [Figure 4: see original paper].

4. Correlations Between Soil Properties and Microbial Diversity

LEfSe analysis identified statistically significant biomarkers at different taxonomic levels. The indicator bacterial population for *Acer mono* leaf litter soil was *Rhizobiales* at the order level, with *Colletotrichum anthrisci* as the indicator fungus. For *Pinus densiflora* leaf litter soil, indicator fungi included two genera (*Chalara* and *Xenopolyscytalum*) and two species (*Exophiala equina* and *Podospora glutinans*), with *Rhodospirillales* as the indicator bacteria. *Quercus mongolica* leaf litter soil showed four indicator fungal genera: *Trichoderma*, *Pilidiella*, *Minimedusa*, and *Talaromyces*. For *Tilia amurensis* leaf litter soil, the indicator bacterium was *Sphingomonas* and the indicator fungus was *Harzia acremonioides*.

To reveal how soil physicochemical factors influence microbial community structure, redundancy analysis (RDA) was performed using CANOCO 5.0. The analysis included soil factors (pH, SOC, TN, C/N, AN, AP, AK, SMBC, SMBN) and microbial phyla (*Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Crenarchaeota*, *Chloroflexi*, *Verrucomicrobia*, *Nitrospirae*, *Bacteroidetes*, *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Zygomycota*).

The first axis explained 83.24% of variation (eigenvalue = 0.7928) and the second axis explained 10.07% (eigenvalue = 0.0959), with cumulative explanatory power reaching 93.31% [Figure 6: see original paper]. *Bacteroidetes*, *Chloroflexi*, and *Actinobacteria* were closely correlated with SMBN, TN, AP, SOC, AK, C/N, and pH. *Basidiomycota*, *Zygomycota*, and *Chytridiomycota* showed similar correlations, while *Ascomycota* responded differently to leaf litter types, possibly related to soil physicochemical properties.

1. Effects of Different Leaf Treatments on Soil Physicochemical Properties

Leaf addition reduced soil bulk density, a key indicator for ginseng site selection that directly affects root morphology. Previous studies indicate ginseng grows best in soils with bulk density <1 g/cm³ and slightly acidic pH with high nutrient supply capacity. In this study, leaf treatments reduced bulk density and altered pH, potentially enhancing ginseng' s resistance to environmental stress. Continuous ginseng cropping leads to nutrient depletion and disrupted microbial communities, but leaf litter addition increased soil organic carbon, total nitrogen, and available nitrogen and phosphorus, particularly in *Juglans mandshurica*, *Tilia amurensis*, and *Quercus mongolica* treatments. This confirms that litter decomposition enhances soil nutrients through microbial carbon flow, supporting plant growth.

2. Effects of Different Leaf Treatments on Soil Microbial Community Structure

Leaf litter addition altered microbial community composition and functional microbial status. Both bacterial and fungal communities were significantly influenced by forest type (broadleaf vs. coniferous). Broadleaf litters introduced more diverse carbon sources and higher carbon content than coniferous litters, leading to greater microbial diversity. Coniferous soils contained more recalcitrant lignin and humic acids, favoring K-strategist microbes that decompose complex organic carbon. While dominant microbial groups remained similar across treatments, their relative abundances changed significantly. *Proteobacteria* dominated all leaf treatments, consistent with its role as a primary functional group in litter decomposition. *Bacteroidetes*, key for broadleaf litter decomposition, showed higher abundance in broadleaf than coniferous treatments. *Ascomycota* status also changed, likely due to varying carbon structure complexity and environmental preferences among fungi.

Specific taxa were associated with particular leaf types. *Sphingomonas*, a plant probiotic that secretes hydrogen peroxide to enhance stress resistance, was specific to *Tilia amurensis* treatment. *Colletotrichum anthrisci*, a pathogen causing ginseng anthracnose, was specific to *Acer mono* treatment. *Trichoderma*, *Pilidiella*, *Minimedusa*, and *Talaromyces* were specific to *Quercus mongolica* treatment, potentially participating in litter decomposition.

4. Conclusion

Different leaf litter additions significantly affected soil physicochemical properties and microbial community structure. While dominant microbial groups remained largely unchanged, functional microbial status shifted considerably. Leaf addition increased microbial biomass carbon and nitrogen, enhanced available nitrogen and phosphorus, and altered microbial community composition. *Proteobacteria* and *Bacteroidetes* were key microbes distinguishing broadleaf from coniferous treatments, while *Ascomycota* functioned in coniferous litter decomposition. Specific bacterial and fungal taxa were identified at different classification levels for each leaf type. Changes in functional microbial status and diversity were closely correlated with soil factors including SMBN, TN, AP, SOC, AK, C/N, and pH. These findings provide theoretical and practical guidance for site selection in understory ginseng cultivation and microbial improvement of field-cultivated ginseng soils.

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