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Effects of chemical properties of mixed leaf litter, phyllosphere microbial communities, and leaf litter decomposition

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Abstract

Purpose Litter decomposition is crucial for maintaining soil fertility, regulating energy flow and nutrient balance in forest ecosystems. However, current research often focuses on the role of soil microorganisms in litter decomposition while neglecting the role of phyllosphere microorganisms. Therefore, this study focuses on the impact of phyllosphere microorganisms on litter decomposition.

Methods In mid-October 2022, litter collectors were deployed, and the collected leaf litter from different tree species was subsequently placed into litterbags. In mid-April 2023, plastic frames were placed under the forest canopy, and the decomposition bags were suspended in the air with protective covers installed on top. Sampling was conducted in mid-July 2023. The leaf litter in the decomposition bags was mixed and weighed for the determination of chemical properties, microbial indicators and other parameters.

Results There are significant differences in the chemical properties, weight loss rate (WLR) and decomposition rate (DR) of different leaf litter. At the same time, it was found that the chemical properties are significantly correlated with the phyllosphere microorganisms, the WLR and the DR. Moreover, the microbial community composition and diversity of the mixed leaf litter are also significantly different from those of a single tree species.

Conclusion The various indicators of the mixed leaf litter show a significant intermediate effect, and the mixed leaf litter can reshape the microbial community structure at the leaf interface. At the same time, chemical properties and microbial communities can affect the decomposition of leaf litter. These research results have important theoretical and practical value for promoting the nutrient cycling in forest areas and maintaining the stability of artificial forest ecosystems.

Keywords Leaf litter; Leaf litter decomposition; Phyllosphere microorganisms; Chemical properties; *Populus* × *canadensis*; *Pinus sylvestris* var. *mongolica*

1 Introduction

Leaf litter decomposition is a crucial biogeochemical process in terrestrial ecosystems, which promotes soil fertility formation and regulates the cycling of carbon, nitrogen, and phosphorus nutrients (Gripp et al. 2018; Jiang et al. 2025; Liu et al. 2020; Mao et al. 2024). Globally, 70% of the carbon emissions from soil each year come from leaf litter decomposition, with an annual carbon release of up to 68 Pg $C \cdot a^{-1}$, being the core driving force of carbon flux and nutrient cycling in forest ecosystems (Fu et al. 2025; Jeong et al. 2015; Mao et al. 2017; Peh et al. 2012; Rosenfield et al. 2020). This process encompasses multiple reactions such as physical, chemical, and biological, directly participating in the regulation of greenhouse gases, the formation of soil humus, and nutrient mineralization, determining forest productivity and the carbon balance of the ecosystem (Ge et al. 2017; Mao et al. 2017). The decomposability of leaf litter is a key factor regulating nutrient availability. Recalcitrant leaf litter retains nutrients in the detritus pool for long periods, whereas easily decomposable leaf litter enables rapid nutrient cycling and transformation into plant-available forms (Sayad et al. 2015). Leaf litter decomposition is also a key factor in determining the composition of forest vegetation communities and the productivity of forest stands (Wan et al. 2021). In studies of nutrient cycling in temperate forests, leaf litter decomposition is a process that has received extensive attention, and revealing its nutrient dynamics is a key link in long-term ecological research plans for understanding the structure and function of forest ecosystems (Jeong et al. 2015).

However, most previous studies have focused on the decomposition characteristics and nutrient dynamics of the leaf litter from a single tree species. In fact, whether in natural ecosystems or in artificially managed ecosystems, the leaf litter usually decomposes in a mixed form involving multiple species (Lin et al. 2026; Mao & Zeng 2012; Zhao et al. 2013). Compared to the decomposition of a single type of leaf litter, the decomposition of mixed leaf litter exhibits two typical effects: additive effect and non-additive effect, and the dominant decomposition mechanisms differ significantly. The additive effect refers to the fact that the decomposition rate of the mixed leaf litter is basically consistent with the average rate of decomposition of each component species, indicating that no significant interaction occurs among different leaf litter components (Xie et al. 2020). The non-additive effect is triggered by the changes in the physicochemical properties of the mixed leaf litter, and can be further divided into synergistic effect and antagonistic effect, which manifest as the decomposition rate of the mixed leaf litter being significantly higher or lower than the average rate of decomposition of single species (Xie et al. 2020). A large number of existing studies have confirmed that the non-additive effect is a common phenomenon in the mixed decomposition system of leaf litter (Ristok et al. 2017; Song et al. 2019; Xie et al. 2020; Zhang et al. 2019). Under natural conditions, the coexistence of different types of leaf litter will not only change the nutrient endowment of the leaf litter itself, but also cause significant changes in the surrounding soil microenvironment. Existing studies have shown that mixed decomposition can reconstruct the physical spatial structure of the leaf litter, enhance the chemical diversity of the decomposition substrates, create more suitable microhabitats for decomposers, provide more heterogeneous nutrient supply, effectively enhance the activity of decomposers, and accelerate the decomposition rate of leaf litter, ultimately changing the composition of decomposer populations and soil biodiversity (Bruder et al. 2014; Zhang et al. 2019).

Most current studies regard the contact of leaf litter with the soil as the starting point of the decomposition process, and focus mainly on the decomposition effect of soil microorganisms, while

neglecting the crucial initial role of the microbial communities at the leaf interface. In forest ecosystems, a large amount of leaf litter will remain in a dead-standing form or be intercepted by the understory vegetation, remaining in a non-soil-contact state for a long time. In subtropical forests, the annual decomposition rate of dead-standing leaf litter can reach over 40% (Liu et al. 2024). The decomposition process of leaf litter is jointly regulated by multiple factors such as climate, terrain, soil nutrients, chemical properties of leaf litter, and microbial communities (del Campo et al. 2021; Wang et al. 2010; Wu et al. 2022; Scheid et al. 2009; Sohng et al. 2014; Vaidelys et al. 2020; Zhao et al. 2025). The microbial communities colonized on the leaves before they fell are the initial triggers and sources of the decomposition of leaf litter, with significant species specificity, but their initial regulatory role has long been overlooked.

Among them, the decomposition rate of leaf litter is closely related to their initial physical properties, mainly including C, N, P content, C/N, C/P, N/P ratio, cellulose, lignin, and lignin/N ratio (Langenbruch et al. 2013; Mayor & Henkel 2006; Wu et al. 2022). Lignin is a typical recalcitrant carbon source, with a large and complex molecular structure and strong resistance to decomposition. Microbial degradation of this substance requires more metabolic energy, thus the corresponding decomposition rate of leaf litter is lower (Bastías et al. 2018; Shi et al. 2025; Yue et al. 2024). Compared to lignin, cellulose and hemicellulose are more easily decomposed and utilized by microorganisms (Wu et al. 2023). Previous studies indicate that leaf litter with higher lignin/N and C/N ratio typically decomposes more slowly, and exhibits significantly lower overall decomposition efficiency than leaf litter containing fewer recalcitrant components (Ferreira et al. 2022; Schoffer et al. 2021).

Pinus sylvestris var. *mongolica* possesses strong characteristics of cold tolerance, drought tolerance, and tolerance to infertility, and exhibits prominent ecological functions in sand fixation, soil conservation, and wind erosion prevention (Ren et al. 2024). It is the core afforestation species for soil and water conservation and desertification control in sandy and desert areas in northern China (Ren et al. 2022; Zheng et al. 2020). However, the slow decomposition rate of leaf litter from Mongolian Scots pine hinders nutrient cycling in the forest, leading to slow tree growth, widespread decline, and death. This severely restricts the stability and sustainable management of Mongolian Scots pine plantation ecosystems and has attracted widespread attention from academia and forestry management departments at all levels (Chen et al. 2006; Mao et al. 2016).

Populus × *canadensis* is a fast-growing timber species widely cultivated in the temperate monsoon climate regions of northern China (Hou et al. 2024; Zeng et al. 2010). With its short rotation period, high yield, and strong adaptability, it has been extensively planted (Li et al. 2026; Pérez et al. 2021; Xu et al. 2023; Yang et al. 2026). It serves as a key afforestation species in mid-latitude plains worldwide and is an important component of China's forestry resources (Yin et al. 2024). Compared to the delayed decomposition characteristics of Mongolian Scots pine leaf litter, the nutrient return potential of Poplar leaf litter is prominent. Mixing the two for decomposition can effectively improve the low decomposition efficiency of single Mongolian Scots pine leaf litter, playing an important role in restoring the degraded ecological functions of sandy artificial forests. Therefore, this study takes the mixed leaf litter of Poplar and Mongolian Scots pine as the research object, systematically explores the decomposition dynamics and nutrient release characteristics of mixed leaf litter, reveals the mixed decomposition effect and its inherent laws of needle-broadleaf leaf litter, and aims to improve the nutrient cycling mechanism of sandy artificial forests, alleviate the soil fertility decline and stand decline issues of Mongolian Scots pine artificial forests, and provide theoretical basis and technical support for the construction of mixed artificial forests in northern sandy areas, ecological restoration of forest land, and refined forest

management.

2 Materials and methods

2.1 Overview of the study area

The study site is located at the Liaohe Plain Forest Ecological Station of the National Forestry and Grassland Administration (43°21'143"N-42°53'623"N, 123°53'623"E-123°53'623"E), in Tieling City, Liaoning Province, China. It sits on the southeastern edge of the Horqin Sandy Land and is at the intersection of Liaoning, Jilin, and Inner Mongolia provinces. The terrain belongs to the Liaohe River alluvial plain, with an elevation ranging from 91.10 to 173.40 meters. It belongs to the temperate semi-humid and semi-arid continental climate, with an average annual precipitation of 400-550 mm, mostly concentrated in July and August each year. The annual evaporation is 1843 mm, with an extreme maximum temperature of 35.6℃ and an extreme minimum temperature of -31.5℃. The daily average temperature is 6.3℃. The soil type is aeolian sandy soil, with low contents of organic matter and other nutrients. The original vegetation consisted of shrubs and herbaceous plants, while current vegetation is primarily composed of windbreak and sand-fixing forests dominated by Poplar and Mongolian Scots pine (Fig. 1).

2.2 Collection of leaf litter

In mid-October 2022, multiple 1m×1m leaf litter collectors were set up in the study area to collect fresh leaf litter samples from Poplar and Mongolian Scots pine. The leaf litter samples were mixed evenly, with one part stored in a -80℃ refrigerator and the other air-dried at room temperature for subsequent experiments.

2.3 Experiment setup

Each bag contained 8 grams of air-dried and constant-weight leaf litter, with the mass ratio of poplar: Mongolian Scots pine leaf litter (Unit: g) being 8:0, 4:4, and 0:8, denoted as Py, PyPs, and Ps, respectively. Put them into the nylon mesh bag of size 20 cm × 20 cm (the pore size of the decomposition bag is 1 mm×1 mm). In mid-April 2023, plastic frames were placed beneath the forest canopy. Litterbags were suspended 0.5 m above the ground using nylon threads, with protective covers installed 0.5 m above the litterbags. This treatment was termed “leaf litter isolated from soil”. The leaf litter on the protective cover and the decomposition bag was removed regularly. Different types of litterbags were placed randomly.

2.4 Sample collection

On July 12, 2023, destructive sampling was conducted. Surface attachments on the litterbags were carefully removed. Three replicates were collected for each leaf litter mass-ratio treatment, placed in ice boxes, and immediately transported back to the laboratory. Subsequently, mix the leaf litter in each decomposition bag, weigh it, and divide it into two parts. One part of the leaf litter is dried to constant weight at 65°C and weighed, then crushed for chemical property determination; the other part of the sample is stored in a -80°C refrigerator for DNA extraction and microbial-related index determination.

2.5 Indicator measurement

Total carbon (TC) content in leaf litter was determined using an elemental analyzer (Elementar Vario EL III Germany);

Total nitrogen (TN) in leaf litter was determined using an elemental analyzer (Elementar Vario EL III Germay);

The total phosphorus (TP) content in leaf litter was determined using the $HClO_4$-H_2SO_4 digestion-molybdenum antimony colorimetric method;

Lignin (Lig) in leaf litter was determined using the klason method;

Cellulose (Cel) in leaf litter was determined using the Van Soest detergent-fiber method;

The hemicellulose (Hemi) content in leaf litter was determined using gas chromatography;

The WLR and DR of leaf litter were determined using the weighing method and calculated based on formulas (1) (Yang et al. 2021; Guimarães de Alencar et al. 2025) and (2) (Swan and Palmer 2006; Gao et al. 2015):

$$D_w = \frac{M_t - M_0}{M_0} \times 100 \quad (1)$$

$$M_t = M_0 \times e^{-kt} \quad (2)$$

where D_w is the WLR of leaf litter, %; M_0 is the initial dry mass of the sample in the decomposition bag, g; M_t is the dry mass of the sample in the litterbags after t months of decomposition, g; k is the DR constant, $month^{-1}$.

2.6 Calculation of mixed decomposition effect of leaf litter

The expected DR of mixed leaf litter (k_0) is calculated using formula (3) (Xie et al. 2024):

$$k_0 = a \times k_1 + b \times k_2 \quad (3)$$

where k_0 is the expected DR of mixed leaf litter, with units of $month^{-1}$; k_1 and k_2 are the DRs of poplar and Mongolian Scots pine leaf litter decomposing alone, respectively, with units of $month^{-1}$; a and b represent the proportions of poplar and Mongolian Scots pine leaf litter in the mixed litter.

When k =k_0, the mixed leaf litter decomposition exhibits an additive effect; when k >k_0, the mixed leaf litter decomposition exhibits a synergistic effect; when k <k_0, the mixed leaf litter decomposition exhibits an antagonistic effect.

2.7 DNA extraction, high-throughput sequencing and PCR reaction system

Total DNA from leaf litter was extracted using the MoBio PowerSoil DNA Isolation Kit (MP Biomedicals, Santa Ana, CA, USA) from the American company OMEGA. Approximately 0.5 g of fresh sample was weighed for each sample and extracted according to the kit's extraction steps. The purity and integrity of the extracted DNA were assessed using 1% agarose gel electrophoresis, and the concentration and purity of the extracted DNA were determined using a NanoDrop ND-1000 nucleic acid quantifier (Thermo Fisher Scientific, Waltham, MA, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified using primer 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'); the Internal Transcribed Spacer (ITS) region of fungi was amplified using primer ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'). The raw data from high-throughput sequencing were uploaded to the NCBI database. The library was constructed using the TruSeq Nano DNA LT Library Prep Kit from Illumina, and final fragment selection and purification were performed through 2% agarose gel electrophoresis. A 1 μL library was taken and subjected to 2100 quality control using the Agilent High Sensitivity DNA Kit on an Agilent Bioanalyzer. For qualified libraries, 2×300 bp dual-end sequencing was performed on a MiSeq machine using the MiSeq Reagent Kit V3 (600 cycles).

The Polymerase Chain Reaction (PCR) reaction mixture consisted of 2 μL template DNA (40-50 ng), 1 μL each of forward and reverse primers (10 μM), 5 μL of 5 × reaction buffer, 5 μL of 5 × Q5 High-Fidelity buffer, 0.25 μL Q5 High-Fidelity DNA polymerase (5 U/μL), 2 μL dNTP mix (2.5 mM), and 8.75 μL ddH_2O. The PCR amplification program was set as follows: initial denaturation at 98 °C for

2 min, followed by 25 cycles of 98 °C for 15 s (denaturation), 55 °C for 30 s (annealing), and 72 °C for 30 s (extension), with a final extension at 72 °C for 5 min. The PCR amplification products were detected by 2% agarose gel electrophoresis, and the target fragments were gel-extracted using the gel extraction kit from AXYGEN. The products were then sent to Shanghai Paisenuo Company for sequencing. The raw high-throughput sequencing data were uploaded to the NCBI database.

2.8 Data analysis

Statistics were conducted using Excel Office 2019, and data analysis was performed using Data Processing System v7.50. Subsequently, significance tests were conducted, and the analysis graphs were generated by genescloud software.

3 Results

3.1 Differences in chemical properties, WLR, and DR of different leaf litter types

Significant differences exist between any two groups among Py, Ps, and PyPs in terms of various chemical properties. In terms of TC, significant differences exist between any two groups among Py, Ps, and PyPs. Ps has the highest TC content, at 506.11 g/kg, while Py has the lowest, at 409.64 g/kg. In terms of TN, significant differences exist among the three types of leaf litter. Py has the highest TN content, at 24.86 g/kg, while Ps has the lowest, at 15.98 g/kg. Similarly, TP follows the same pattern as TN, with significant differences among the three types of leaf litter. Py has the highest TP content, at 1.67 g/kg, while Ps has the lowest, at 1.39 g/kg. In terms of total carbon/total nitrogen (C/N) ratio, significant differences exist among the three types of leaf litter. Ps has the highest C/N ratio, at 31.68, while Py has the lowest, at 16.48. In terms of total nitrogen/total phosphorus (N/P) ratio, significant differences also exist among the three types of leaf litter. Py has the highest N/P ratio, at 14.90, while Ps has the lowest, at 11.53. The total carbon/total phosphorus (C/P) and C/N ratios among the three types of leaf litter show consistent significant results, with Ps having the highest C/P ratio, at 365.24, and Py having the lowest, at 245.64. Significant differences exist among the three types of leaf litter in terms of Lig, Cel, Hemi, and lignin/total nitrogen (Lig/N) ratio, with Ps having the highest values, at 216.74 g/kg, 224.33 g/kg, 103.96 g/kg, and 13.56, respectively. Py has the lowest values in all four chemical properties mentioned above, at 207.96 g/kg, 173.82 g/kg, 72.29 g/kg, and 8.37, respectively (Table 1).

In terms of WLR, significant differences were observed between any two groups among Py, Ps, and PyPs, with Py being the highest at 29.37% and Ps being the lowest at 18.56%. Similarly, in terms of DR, significant differences were also observed between any two groups among Py, Ps, and PyPs, with Py being the fastest at 0.118 and Ps being the slowest at 0.067 (Table 2). Further, the expected DR of mixed leaf litter was calculated based on a 1:1 mixing ratio, k_0=0.0917; the mean measured DR of mixed leaf litter was 0.0850. The one-sample t-test indicated that there was no significant difference between the measured DR of mixed leaf litter and the theoretical expected DR (t=-0.735, df=1, P=0.597), suggesting that the decomposition of poplar-Mongolian Scots pine mixed leaf litter exhibited an additive effect, with no significant synergistic or antagonistic non-additive interactions detected.

3.2 The correlation between the chemical properties, WLR, DR of different leaf litter types and the phyllosphere microorganisms

In the fungal community, most dominant fungal genera are significantly correlated with the chemical properties of the leaf litter. Genera such as *Paraconiothyrium*, *Diplodia*, and *Heterotruncatella* have extremely significant positive correlations with TC, C/N ratio, C/P ratio, Lig, Cel, Hemi, and Lig/N ratio ($P < 0.01$), and show extremely significant negative correlations with TN and TP ($P < 0.01$); while

Pseudocoleophoma, *Articulospora*, *Coniothyrium*, and *Neosetophoma* exhibit the opposite pattern, showing extremely significant negative correlations with TC, C/N ratio, C/P ratio, Lig, Cel, Hemi, and Lig/N ratio ($P < 0.01$), and extremely significant positive correlations with TN and TP ($P < 0.01$). Some fungi such as *Acremonium* and *Trichothecium* have relatively weak correlations with the chemical properties of the leaf litter overall, with only a few significant correlations ($P < 0.05$) (Fig. 2a).

The dominant phyllosphere bacterial genera also have extensive significant correlations with the chemical properties of the leaf litter. Most bacterial genera such as *Sphingomonas*, *Mucilaginibacter*, *Kosakonia*, *Luteibacter*, *Pantoea*, *Burkholderia-Caballeronia-Paraburkholderia*, and *Amantichitinum* have significant ($P < 0.05$) or extremely significant ($P < 0.01$) positive correlations with TC, C/N ratio, C/P ratio, Lig, Cel, Hemi, and Lig/N ratio, and show negative correlations with TN and TP ($P < 0.05$); *Actinoplanes*, *Nocardioides*, and *Aureimonas* have extremely significant negative correlations with most of the leaf chemical properties ($P < 0.01$). A few groups such as Kineosporia, Cellulomonas, and Amnibacterium showed significant correlations with only a few specific chemical properties ($P < 0.05$) (Fig. 2b).

As shown in Fig. 3, the correlations between the phyllosphere fungi, bacteria and WLR, DR are relatively consistent. In terms of the fungi, WLR and DR show extremely significant positive correlations with *Pseudocoleophoma*, *Articulospora*, *Coniothyrium*, and *Neosetophoma* ($P < 0.01$), while they show extremely significant negative correlations with *Paraphaeosphaeria*, *Diplodia*, and *Heterotruncatella* ($P < 0.01$). In addition, WLR also shows an extremely significant negative correlation with *Fusarium* ($P < 0.01$) (Fig. 3a).

In the bacterial community, WLR and DR show extremely significant positive correlations with *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Actinoplanes*, *Nocardioides*, *Aureimonas*, and *Cellulomonas* ($P < 0.01$). DR also shows an extremely significant positive correlation with *Pedobacter* ($P < 0.01$). WLR, DR show extremely significant negative correlations with *Mucilaginibacter*, *Kosakonia*, *Luteibacter*, *Burkholderia-Caballeronia-Paraburkholderia*, and *Amantichitinum* ($P < 0.01$). WLR also shows an extremely significant negative correlation with *Pantoea* ($P < 0.01$) (Fig. 3b).

WLR and DR show strong correlations with all the leaf chemical properties. Specifically, they show extremely significant positive correlations with TN, TP, and N/P ratio ($P < 0.01$), and extremely significant negative correlations with all the other chemical properties ($P < 0.01$) (Fig. 4).

3.3 Composition of phyllosphere microbial communities on different leaf litter types

A total of 1,245 Amplicon Sequence Variants (ASVs)/Operational Taxonomic Units (OTUs) were detected in the fungal communities. Py, Ps, and PyPs contained 599, 304, and 608 ASVs/OTUs, respectively. Py had the highest number of unique ASVs/OTUs at 441, while Ps had the fewest at 189. The three leaf litter types shared 51 ASVs/OTUs. Py and PyPs shared the most (151), indicating high similarity in their fungal community composition; whereas Py and Ps shared the least (58), suggesting significant differences in community structure (Fig. 5a).

At the bacterial level, a total of 14,027 ASVs/OTUs were detected. Py, Ps, and PyPs contained 5,477, 4,348, and 6,730 ASVs/OTUs, respectively. PyPs had the highest number of unique ASVs/OTUs at 4,563, while Ps had the lowest at 3,459. The three leaf litter types shared 321 ASVs/OTUs. Py and PyPs shared the most (1,639), indicating highly similar bacterial community compositions; whereas Py and Ps shared the least (361), indicating considerable differences in community structure (Fig. 5b).

At the phylum level, 4 phyllosphere fungi and 20 phyllosphere bacteria were detected. In the fungal community, *Ascomycota* was the dominant group in the three sets of leaf litter, with an average abundance of 0.9947. It had the highest proportion in Ps, at 0.9982, and the lowest in PyPs, at 0.9882. *Basidiomycota*

was a subdominant group, with an average abundance of 0.00007. It had the highest proportion in Ps, at 0.00017, while Py and PyPs had equal proportions, at 0.00003 (Fig. 6a). In the bacterial community, *Proteobacteria* was the dominant phylum in the three sets of leaf litter, with an average abundance of 0.6617. It reached its peak in Ps, at 0.8579, while Py had the lowest proportion, at 0.5423. *Actinobacteriota* was a subdominant phylum in Py and PyPs, with relative abundances of 0.3490 and 0.2714, respectively. *Bacteroidota* was a subdominant phylum in Ps litter, with a relative abundance of 0.0641 (Fig. 6b).

At the genus level, a total of 20 phyllosphere fungi and 20 bacteria were detected. *Pseudocoleophoma* was the dominant genus of Py fungi, with a relative abundance of 0.2078; *Cyphellophora* was the subdominant genus, with a relative abundance of 0.0940. *Paraconiothyrium* was the dominant genus of Ps litter fungi, with a relative abundance of 0.2784; *Fusarium* was the subdominant genus, with a relative abundance of 0.2183. The dominant and subdominant fungal genera of PyPs were *Paraphaeosphaeria* and *Fusarium*, accounting for 0.1236 and 0.1064, respectively (Fig. 6c). *Methylobacterium-Methylorubrum* was the dominant genus of Py, with a value of 0.1267; *Massilia* was the subdominant genus, with a relative abundance of 0.1111. The dominant and subdominant genera of Ps were *Sphingomonas* and *Kosakonia*, with values of 0.2163 and 0.0842, respectively. The dominant and subdominant genera of PyPs were *Methylobacterium-Methylorubrum* and *Sphingomonas*, with relative abundances of 0.1284 and 0.0810, respectively (Fig. 6d).

As can be seen from Fig. 7a, when the threshold of Linear Discriminant Analysis (LDA) is set at 2, a comparison was made of the average relative abundance from class to species across three fungal communities, revealing 43 significant differences ($p<0.05$) among the three groups. These included one class, five orders, eight families, 13 genera, and 16 species (Fig. 7a).

In the bacterial community, the average relative abundance from phylum to species was compared, and 62 significant differences ($p<0.05$) were found. These differences were distributed across 2 phyla, 3 classes, 4 orders, 12 families, 21 genera, and 20 species (Fig. 7b).

3.4 Alpha and beta diversity analysis of microbial communities associated with different litter types

In fungal communities, Py showed the highest values in Goods_coverage and Shannon index at 0.999986 and 5.03607, respectively, while exhibiting the lowest Simpson index at 0.812457. PyPs had the highest values in Chao1, Simpson, Pielou_e, and Observed_species indices (312.84, 0.927932, 0.620578, and 306, respectively), with a Goods_coverage of 0.999728, ranking last among the three samples. Ps recorded the lowest values across Chao1, Pielou_e, Shannon, and Observed_species indices (126.46, 0.460587, 3.22411, and 126, respectively) (Fig. 8a).

At the bacterial level, Py exhibited both the highest and lowest values in the Simpson index (0.993582 and 0.978053, respectively). Additionally, it showed the lowest values in Faith_pd, Allens_H, and Raos_quadratic_entropy indices (76.979, 0.81424, and 0.247428, respectively). Ps had the highest values in Goods_coverage and Raos_quadratic_entropy indices (0.99771 and 0.323232), but the lowest in Chao1, Pielou_e, Shannon, and Observed_species indices (1529.49, 0.694728, 7.32856, and 1498, respectively). PyPs ranked highest in Chao1, Pielou_e, Faith_pd, Shannon, Observed_species, and Allens_H indices (3133.35, 0.823111, 128.048, 9.54149, 3087, and 1.13387, respectively), yet had the lowest Goods_coverage at 0.99256 (Fig. 8b).

Non-metric Multidimensional Scaling (NMDS) and Principal Coordinates Analysis (PCoA) analyses were performed on microbial-community structures for different treatments based on Bray-Curtis distances, revealing the differences in beta diversity of fungal and bacterial communities among

Py, Ps, and PyPs leaf litter groups (Fig. 9, Fig. 10). The fungal NMDS analysis yielded a Stress value of 0.123; when Stress is below 0.2, the two-dimensional plot is considered interpretable. The Stress value of bacterial NMDS is 0.0026. It is generally believed that when the Stress value is less than 0.05, it is highly representative. NMDS results indicated clear separation among the Py, Ps, and PyPs groups in ordination space, with samples within each group clustered closely together and substantial distances between groups, suggesting significant differences in fungal and bacterial community compositions. Py samples were predominantly distributed on the left side of the NMDS1 axis, Ps clustered on the right side, and PyPs occupied an intermediate position, with no overlapping samples among the three groups (Fig. 9).

In the PCoA analysis, fungal PC1 (explaining 45.1% of variation) and PC2 (explaining 16.5%) together accounted for 61.6% of total variation. Bacterial PC1 (explaining 44.5%) and PC2 (explaining 10.3%) jointly explained 54.8% of variation. The PCoA analysis results were consistent with the trends observed in the above NMDS analysis (Fig. 10).

4 Discussion

4.1 Differences in chemical traits of different leaf litter types and their regulatory effects on WLR and DR

Table 1 shows that Py leaf litter had the highest TN, TP, and N/P ratio, while Ps leaf litter exhibited the highest contents of TC, C/N, C/P, Lig, Cel, Hemi, and Lig/N. The chemical properties of PyPs leaf litter fell between those of Py and Ps leaf litter, displaying typical transitional characteristics (Table 1). These findings are consistent with those of Murindangabo et al. (2026). The differences in mycorrhizal symbiosis types among tree species and their leaf litter functional traits represent a key mechanism regulating variation in leaf substrate quality and decomposition processes: Poplar is a broad-leaved deciduous species associated with arbuscular mycorrhizal fungi, exhibiting a resource-acquisitive growth strategy characterized by rapid growth rates and high specific leaf area. Its litter contains high nutrient concentrations and low proportions of recalcitrant structural components, resulting in superior substrate quality and faster decomposition rates, favoring a bacteria-dominated decomposition pathway. In contrast, Mongolian Scots pine is an evergreen coniferous species forming ectomycorrhizal associations, adopting a resource-conservative survival strategy with low nutrient-use efficiency and slow turnover. Its litter is nutrient-poor and enriched in resistant compounds such as lignin, leading to low decomposability and a slower, fungus-dominated decomposition pathway (Liu et al. 2023; Rawat et al. 2020; Zhao et al. 2013; Zhao et al. 2025). Decomposition dynamics shown in Table 2 further support the regulatory role of physicochemical traits on litter decomposition. Overall, the weight loss rates and decomposition rates followed the pattern Py > PyPs > Ps, with poplar litter showing significantly higher decomposition rates and weight loss than the other two groups, and mixed litter exceeding that of Mongolian Scots pine litter (Table 2), consistent with Liu et al. (2022a). This gradient in decomposition characteristics closely aligns with the intermediate chemical traits observed in the mixed litter.

Although the chemical properties of mixed litter showed clear transitional characteristics, this study did not detect significant non-additive effects, and the overall decomposition process exhibited additive behavior. Non-additive effects are commonly observed in many studies on conifer-broadleaf mixed litter, typically attributed to nutrient transfer among different litter types, which alters the substrate environment and stimulates microbial activity. However, this study was conducted in the sandy region of the Liaohe Plain, where soils are generally nutrient-poor. The dual limitations imposed by both substrate quality and soil nutrients weakened the nutrient complementarity among litter components,

resulting in no significant acceleration or inhibition of decomposition due to mixing.

Litter decomposition rates are jointly regulated by abiotic factors such as temperature and precipitation, as well as biotic factors including litter quality. Among these, litter quality directly influences decomposition rates and indirectly affects microbial community metabolic capacity by altering the availability of labile carbon—often playing a more important role than environmental factors (Frankenstein et al. 2025; Qu et al. 2019). High-quality litter (low lignin, high nutrient concentration) typically decomposes faster than low-quality litter, primarily because it supports higher microbial activity and promotes microbial colonization (Ferreira & Graça, 2016). Litter with low C/N ratios, high N and P contents, and low concentrations of recalcitrant compounds is more readily degraded by microbes, leading to higher decomposition rates (Di Sabatino et al. 2020). Consistent with previous findings (Ferreira et al. 2026; Liu et al. 2022b; Raposeiro et al. 2017; Wang et al. 2009; Wang et al. 2014), this study found extremely significant positive correlations between WLR, DR and TN, TP, N/P, and extremely significant negative correlations with TC, C/N, C/P, Lig, Cel, Hemi, and Lig/N (Fig. 4).

4.2 Similarities and differences in the composition of phyllosphere microbial communities associated with different leaf litter types

This study analyzed the similarities and differences in microbial communities associated with different litter types from three perspectives: alpha diversity, beta diversity, and species composition. The alpha diversity results showed that the species coverage and diversity of the Py fungal community were the highest. The Ps bacteria community has high species coverage and high phylogenetic functional diversity, consisting of clades with large differences in evolutionary history, with relatively even distribution of individual species, indicating that the representative habitat can accommodate the coexistence of highly differentiated species evolutionarily. The PyPs fungi community has the highest richness, evenness, and diversity; the bacteria community has the highest richness, evenness, diversity, and genetic diversity (Fig. 8).

The beta diversity results showed that Py was concentrated in the left region, Ps clustered in the right region, and PyPs were located in the middle position between the two. Within the three groups of samples, the samples were clustered within each group, while they were clearly separated between groups, with no overlap of samples and a relatively large distance, indicating significant differentiation in the composition of fungal and bacterial communities from different litter types (Fig. 9, Fig. 10). These results suggest that both Py and Ps treatments alone, as well as PyPs mixed treatment, significantly drove changes in the beta diversity of bacterial and fungal communities. PyPs formed a unique microbial community structure that differed from the single Py and Ps treatments.

At the phylum level, *Ascomycota* and *Basidiomycota* were the dominant and subdominant fungal groups across all three leaf litter types, consistent with findings by Hu et al. (2025) and Pan et al. (2024). *Ascomycota* and *Basidiomycota* complement each other functionally: *Ascomycota* typically dominate in early stages, excelling at degrading unstable carbon sources such as cellulose, thereby promoting rapid decomposition (Leifheit et al. 2026; Wei et al. 2026). In contrast, *Basidiomycota* produce enzymes required to break down complex polymers and facilitate differential losses of lignin and carbohydrates (Meng et al. 2024). *Proteobacteria* was the dominant bacterial phylum across all three leaf litter types, aligning with results from Hu et al. (2025) and Mao et al. (2022). *Proteobacteria* tend to decompose readily available carbon substrates and contribute significantly to carbon and nitrogen cycling (Li et al. 2023; Li et al. 2025) (Fig. 6a, b).

At the genus level, there were notable differences among the dominant fungal and bacterial genera associated with different leaf litter types. The dominant fungal genus on Py litter was *Pseudocoleophoma*,

while that on Ps litter was *Paraconiothyrium*, known for its ability to degrade lignin and participate in apoplastic substance breakdown (Liu et al. 2022a). For PyPs mixed leaf litter, the dominant fungal genus was *Paraphaeosphaeria*, differing from both Py and Ps leaf litter, reflecting the reshaping effect of mixed leaf litter from different tree species on fungal communities. The dominant bacterial genera on Py litter were *Methylobacterium-Methylorubrum*, which possess the ability to degrade cellulose and hemicellulose (Chiba et al. 2021), and can also metabolize methoxy groups in various aromatic compounds—common products of lignin depolymerization (Lee et al. 2022). The dominant bacteria in Ps leaf litter are *Sphingomonas*, which benefit from the availability of high-carbon sources during litter decomposition, and these carbon sources in turn provide the driving force for nitrogen fixation (Chiba et al. 2021). In this study, the dominant bacterial genus on mixed PyPs leaf litter matched that of Py leaf litter—*Methylobacterium-Methylorubrum*—without showing the community characteristics dominated by *Sphingomonas* observed in Ps leaf litter. This result indicates that the bacterial community in mixed leaf litter is not a simple sum of those from individual litter types. Instead, the recalcitrant lignocellulosic substrates carried by Py leaf litter exert a dominant selective pressure on microbial communities in the mixed system. Although Ps leaf litter contributes easily accessible carbon substrates, their quantity is insufficient to support *Sphingomonas* becoming dominant. Conversely, *Methylobacterium-Methylorubrum*, with broader substrate utilization capabilities, gain competitive advantages under mixed resource conditions and maintain their dominance (Fig. 6c,d).

4.3 The correlation between phyllosphere microorganisms and the chemical properties and decomposition of leaf litter

The correlation analysis in this study revealed that the nutrient components such as TC, TN, and TP in leaf litter, as well as the structural components like Lig, Cel, and Hemi, have a large number of extremely significant correlations with the phyllosphere fungi and bacteria. This indicates that the chemical properties of leaf litter are an important driving factor for the leaf-associated microbial community (Danger et al. 2012; Frost et al. 2012; Hou and Lü 2021; Zhu et al. 2025). This result is consistent with the research findings on the regulatory mechanisms of phyllosphere microbial communities. *Paraconiothorium*, *Diplodia*, and *Heterotremetalla* are significantly positively correlated with Lig, Cel, and Hemi content, and significantly negatively correlated with TN and TP nutrient content. The above microorganisms are more suitable for oligotrophic leaf litter habitats with high structural components and low nutrients. They have the potential to utilize and degrade difficult to decompose carbon substrates such as lignin and cellulose, and can obtain carbon sources through structural substances in plant cell walls. They tend to colonize in leaf litter substrates with high lignification and nutrient depletion. On the contrary, microorganisms such as *Pseudocoleophoma*, *Articulospora*, *Coniothyrium*, *Neosetophoma, Actinoplanes*, *Nocardioides* and A*ureimonas* showed a highly significant negative correlation with the content of structural components and a highly significant positive correlation with the nutrient contents of TN and TP (Fig. 2). Thus, these microorganisms have a relatively weak ability to utilize recalcitrant structural carbon sources and are more dependent on readily available nutrients such as nitrogen and phosphorus from leaf litter. They are more suitable for proliferation in the nutrient-rich leaf-litter environment.

5 Conclusion

This study systematically analyzed the effects of chemical properties and phyllosphere microbial community structure of single-species leaf litter of *Populus* × *canadensis*, *Pinus sylvestris* var. *mongolica*, as well as their mixed coniferous-broadleaf leaf litter, on leaf litter decomposition. The results showed

that the chemical characteristics of the mixed leaf litter were significantly different from those of the single tree species leaf litter, and the various indicators fell between those of the two single leaf litter, presenting a clear intermediate effect. Meanwhile, the weight loss rate and decomposition rate of the leaf litter were highly consistent with the patterns of its chemical properties. Leaf litter of *Populus* × *canadensis*, an arbuscular mycorrhizal deciduous broad-leaved tree species, exhibited the highest decomposition rate, followed by mixed leaf litter, while *Pinus sylvestris* var. *mongolica*, an ectomycorrhizal evergreen coniferous tree species, decomposed the slowest. Furthermore, the chemical properties of leaf litter were extremely significantly correlated with phyllosphere microbial community structure, weight loss rate and decomposition rate. Moreover, the phyllosphere microbial community structure could significantly regulate the litter decomposition process. We also found that, compared with the single leaf litter, there were certain differences in the microbial community composition and diversity of the mixed leaf litter of coniferous and broad-leaved trees, indicating that the mixture of coniferous and broad-leaved leaf litter can reshape the microbial community structure at the leaf interface. In summary, this study clarified the decomposition differences and microbial driving mechanisms of the single and mixed leaf litter of *Populus* × *canadensis* tremuloides and *Pinus sylvestris* var. *mongolica*, enriching the related research content on leaf litter decomposition. It can provide theoretical basis for optimizing the structure of *Pinus sylvestris* var. *mongolica* artificial forests, scientifically configuring needle-and-broad-leaved mixed forests, and sustainable management, and has important theoretical and practical value for promoting nutrient cycling in forest land and maintaining the stability of artificial forest ecosystems.

6. Declarations

Author contributions Conceptualization: Wenxu Zhu; Methodology: Jiaying Liu, Huiyu Shu; Formal analysis and investigation: Jiaqi Yang, Yang Ge; Writing-original draft preparation: Jiaqi Yang; Writing-review and editing: Wenxu Zhu; Funding acquisition: Changjun Ding; Resources: Weixi Zhang; Supervision: Siqi Wu.

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8 Tables

Table 1 Significant differences in the chemical properties of different leaf litter types

	TC g/kg	TN g/kg	TP g/kg	C/N	N/P	C/P	Lig g/kg	Cel g/kg	Hemi g/kg	Lig/N
Py	409.64±4.42c	24.86±0.21a	1.67±0.04a	16.48±0.11c	14.90±0.46a	245.64±8.39c	207.96±0.94c	173.82±2.77c	72.29±2.02c	8.37±0.08c
Ps	506.11±2.19a	15.98±0.01c	1.39±0.02c	31.68±0.12a	11.53±0.17c	365.24±6.33a	216.74±0.35a	224.33±0.83a	103.96±0.54a	13.56±0.03a
PyPs	456.51±6.14b	19.73±0.38b	1.51±0.03b	23.15±0.43b	13.09±0.10b	302.98±7.60b	212.64±1.60b	196.33±0.87b	87.48±0.75b	10.78±0.16b
F-value	112.4550	309.2220	20.5620	804.8130	34.0080	63.8320	16.2620	209.9830	152.8990	660.8150
p-value	0.0001	0.0001	0.0021	0.0001	0.0005	0.0001	0.0038	0.0001	0.0001	0.0001

Note: Data are presented as mean ± standard error. Different lowercase letters indicate significant differences in chemical properties among different leaf litter types ($p < 0.05$)

Table 2 Significant differences in WLR and DR among different leaf litter types

	WLR	DR
Py	29.37%±1.51%a	0.118±0.003a
Ps	18.56%±1.11%c	0.067±0.003c
PyPs	22.38%±1.77%b	0.085±0.005b
F-value	44.1630	52.0450
p-value	0.0007	0.0004

Note: Data are presented as mean ± standard error. Different lowercase letters indicate significant differences in WLR and DR among different leaf litter types ($p < 0.05$)

9 Figures

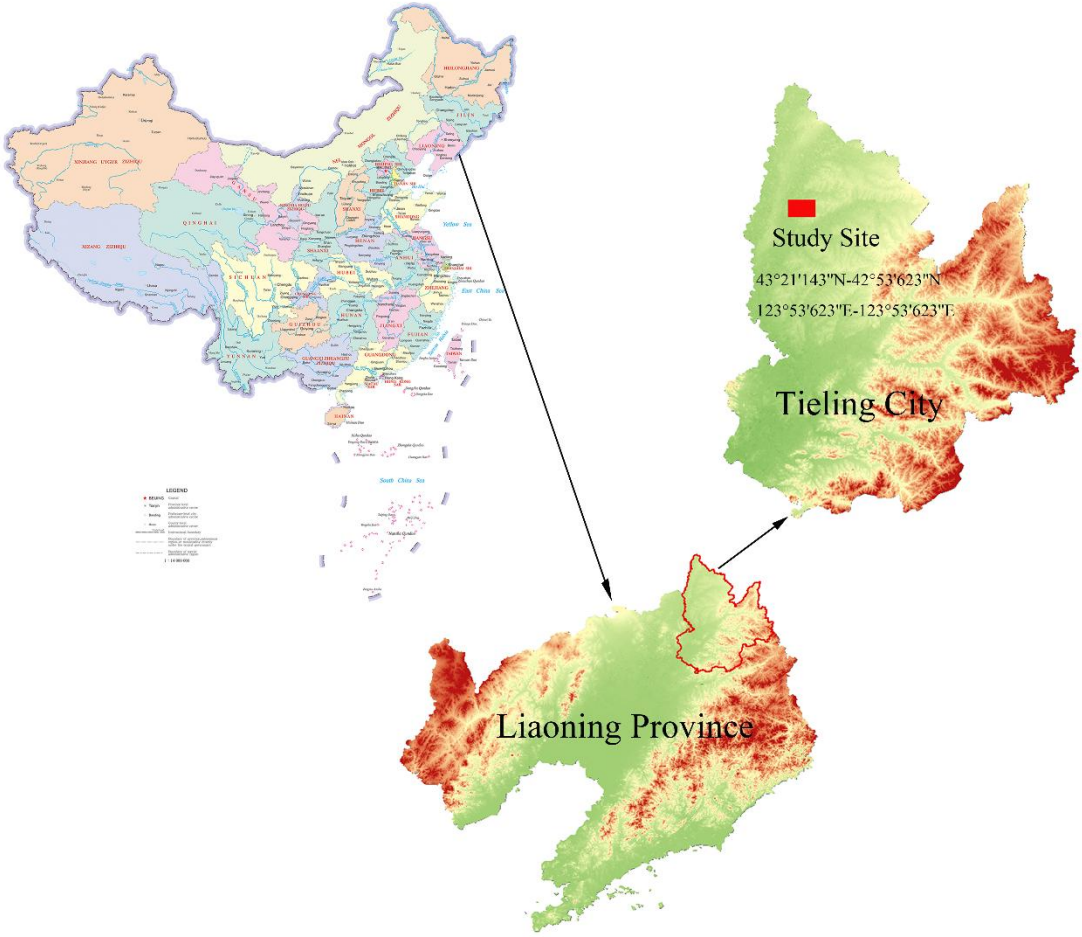


Fig. 1 Location of the study area

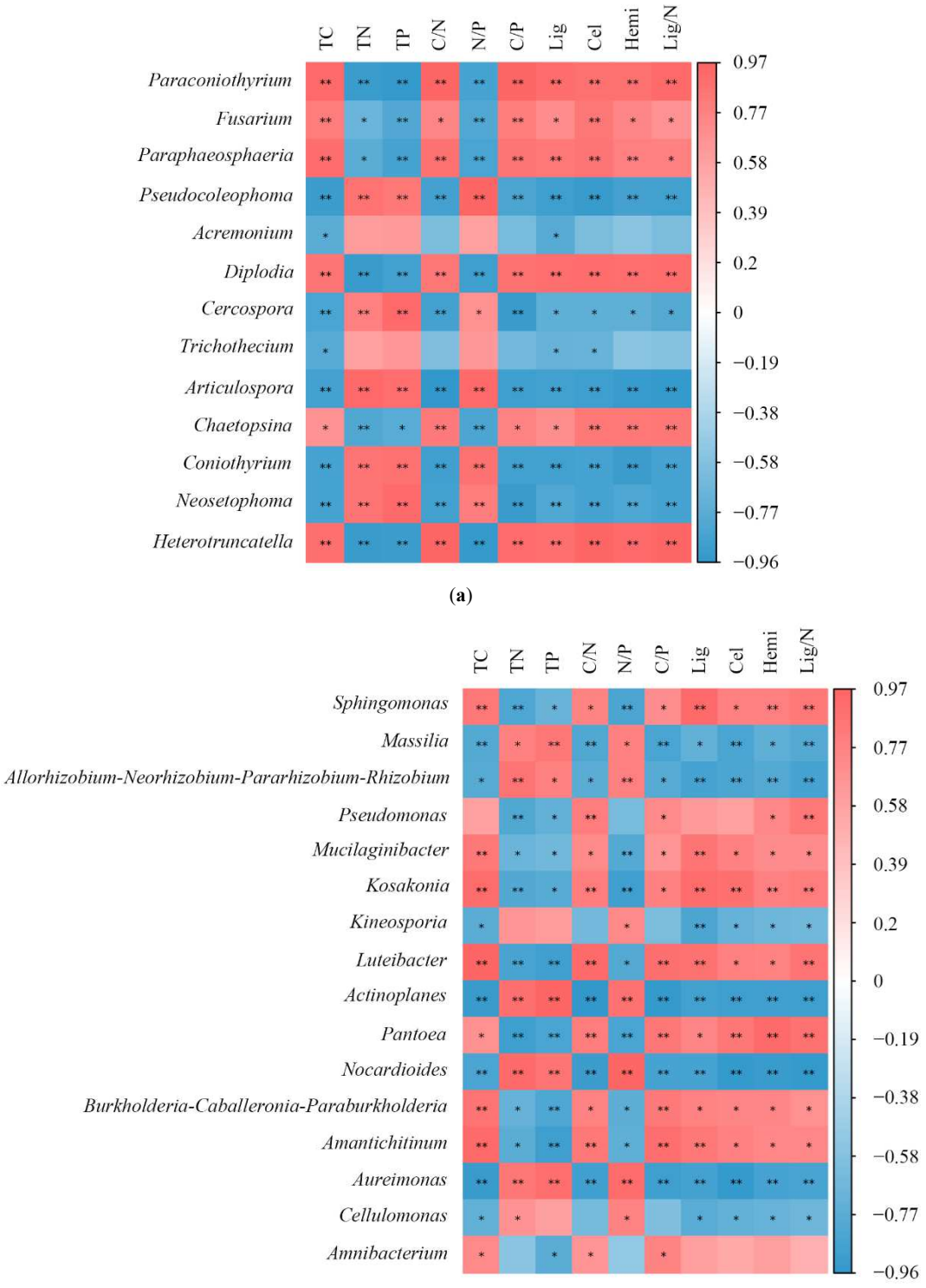
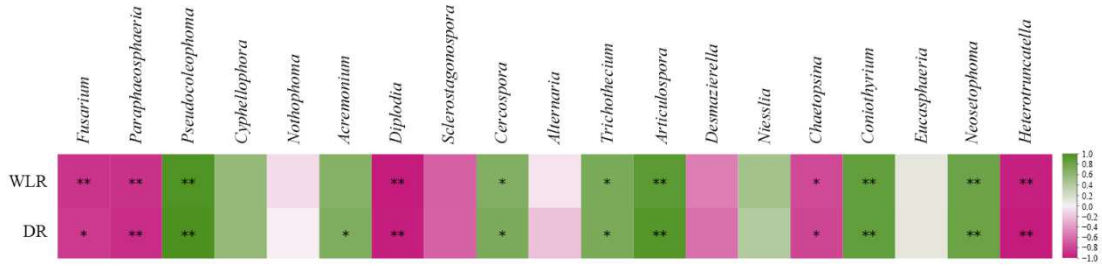
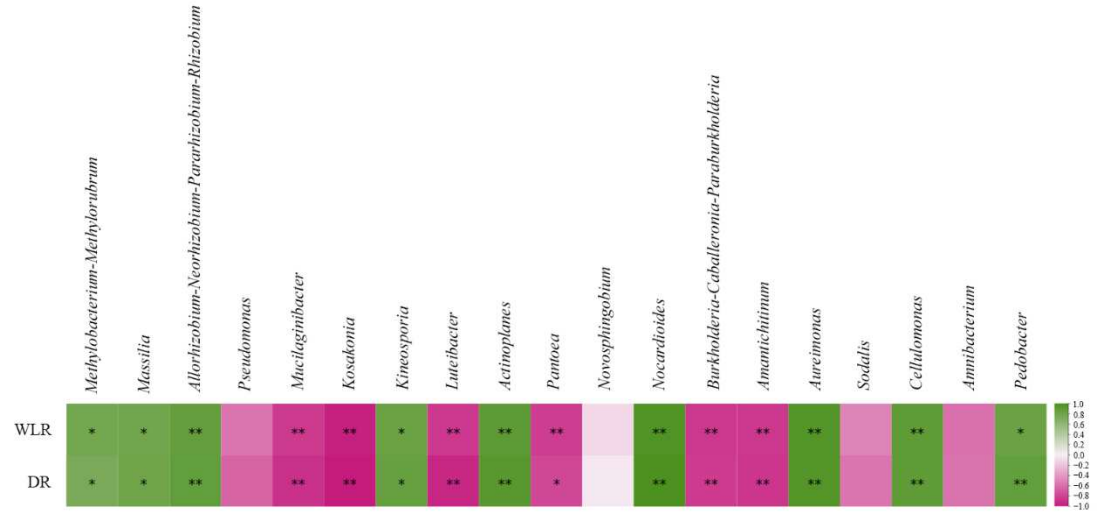


Fig. 2 Heatmap of the correlation between phyllosphere microbial community composition and chemical properties. (**a**) Fungal community. (**b**) Bacterial community. The legend shows the correlation coefficient values, with red representing positive correlations and blue by default representing negative correlations; the intensity of the color indicates the strength of the correlation; * indicates a significant difference at the 0.05 level; ** indicates a significant difference at the 0.01 level



(**a**)



(**b**)

Fig. 3 Correlation heatmap of WLR, DR, and the composition of phyllosphere microbial communities. (**a**) Fungal community. (**b**) Bacterial community. The legend shows the correlation coefficient values, with green representing positive correlations and pink by default representing negative correlations; the intensity of the color indicates the strength of the correlation; * indicates a significant difference at the 0.05 level; ** indicates a significant difference at the 0.01 level

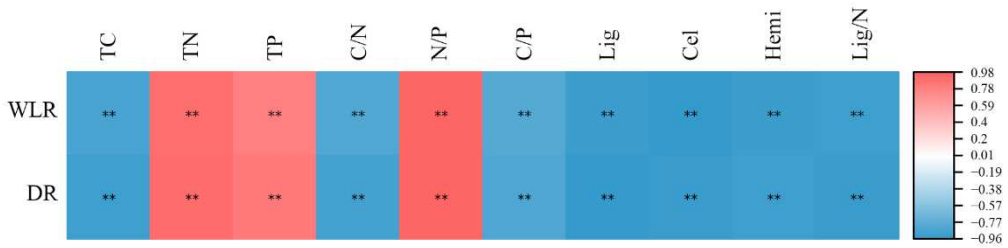
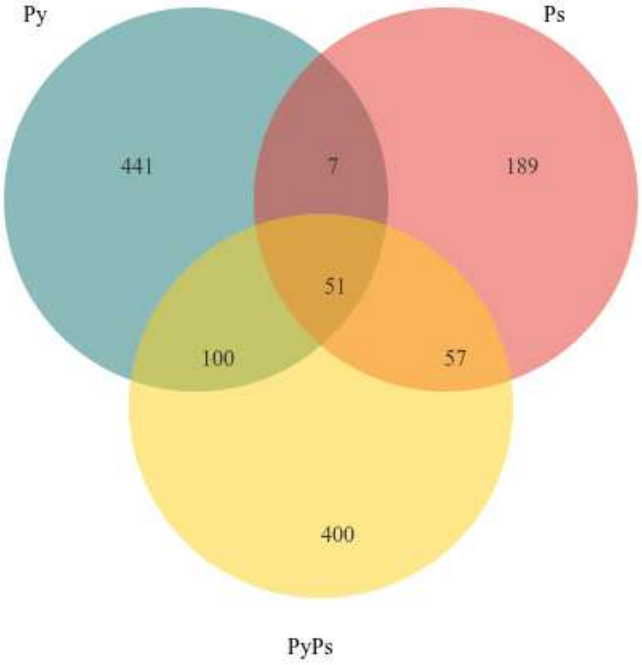
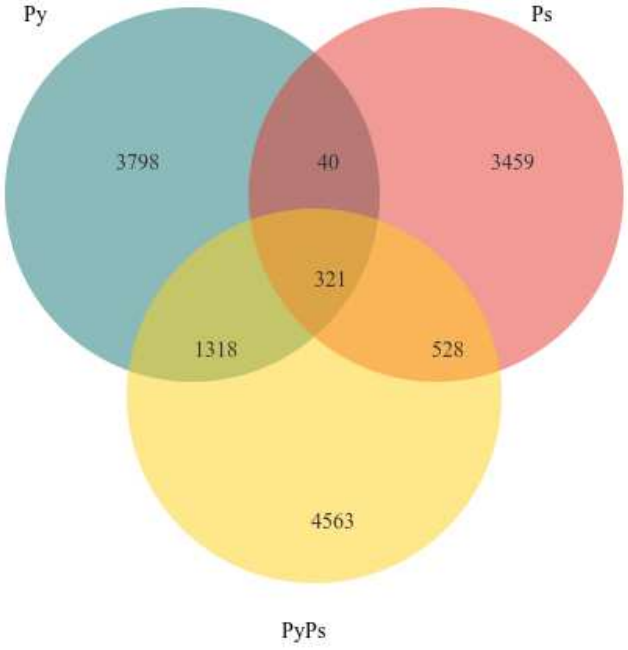


Fig. 4 Correlation heatmap of leaf litter WLR, DR, and chemical properties. The legend shows correlation coefficient values, with red indicating positive correlation and blue indicating negative correlation by default. Color intensity represents the strength of the correlation; ** denotes significance at the 0.01 level

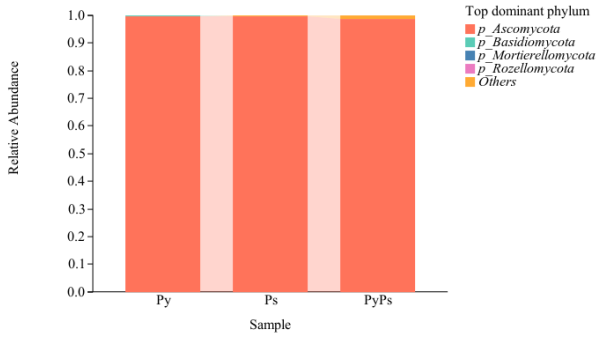


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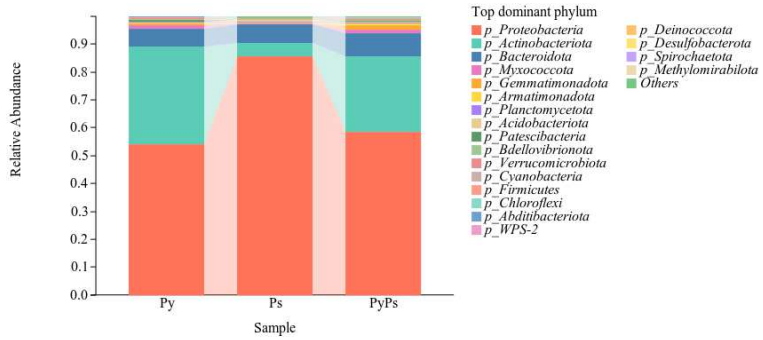


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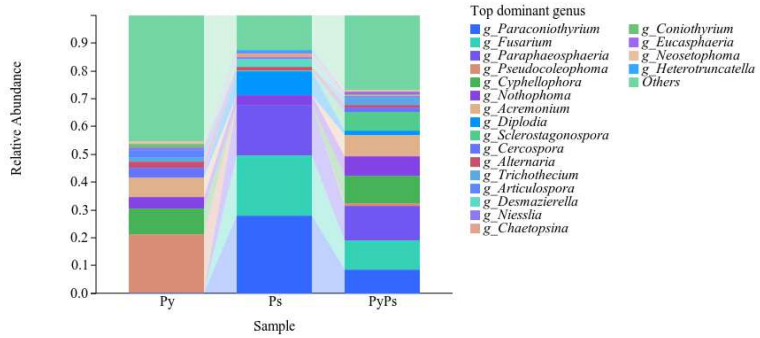
Fig. 5 Venn diagrams of the unique and shared Amplicon Sequence Variants (ASVs) in the phyllosphere microbial communities of different types of leaf litter. (**a**) Fungal communities. (**b**) Bacterial communities



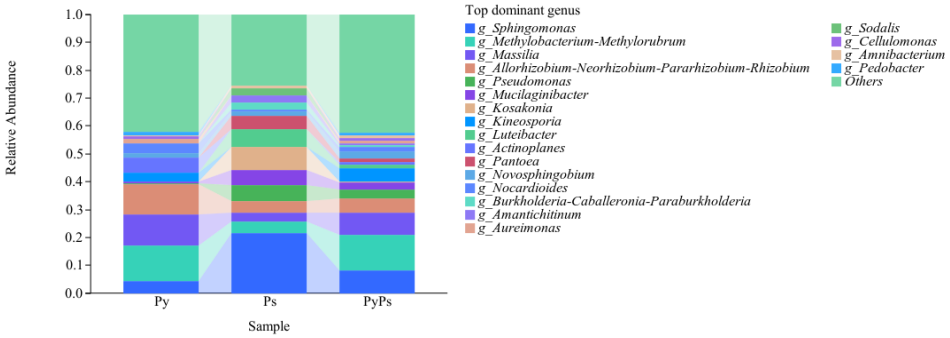
(**a**)



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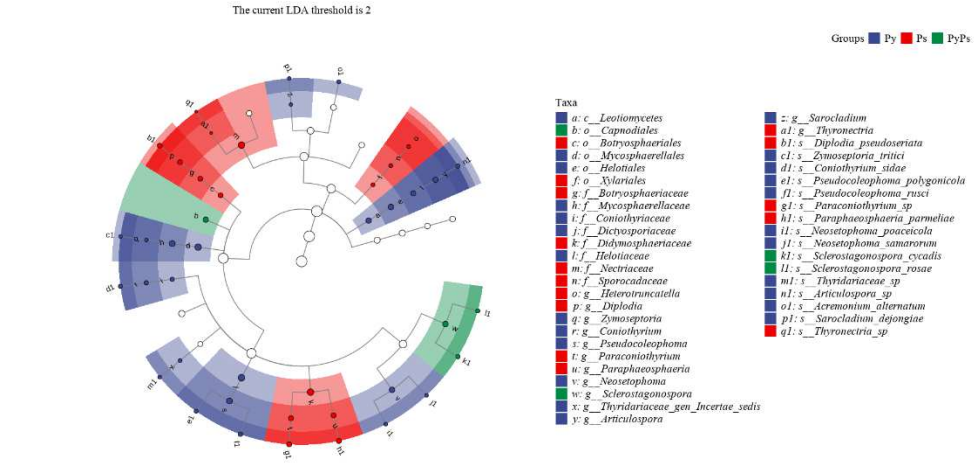


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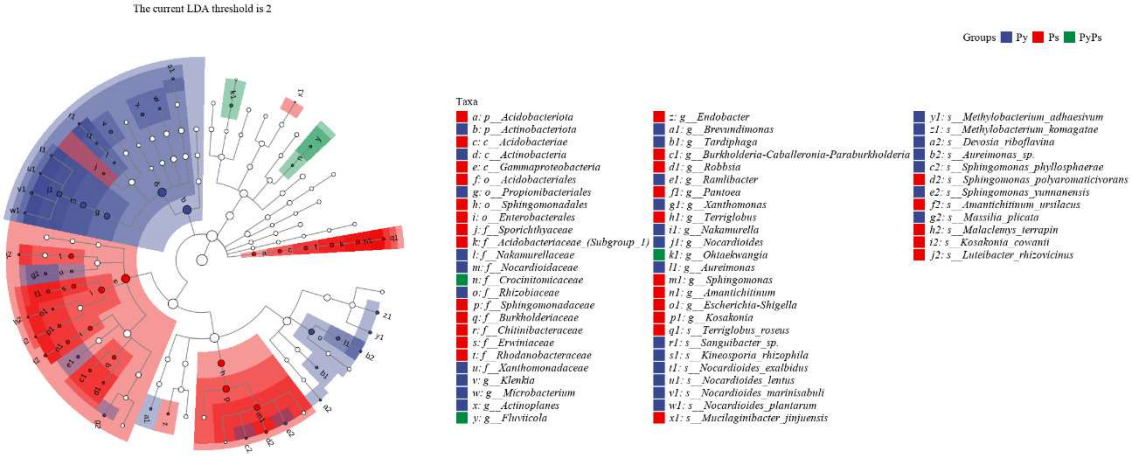


(**d**)

Fig. 6 Species composition analysis diagram. (**a**) Fungi phylum level. (**b**) Bacteria phylum level. (**c**) Fungi genus level. (**d**) Bacteria genus level

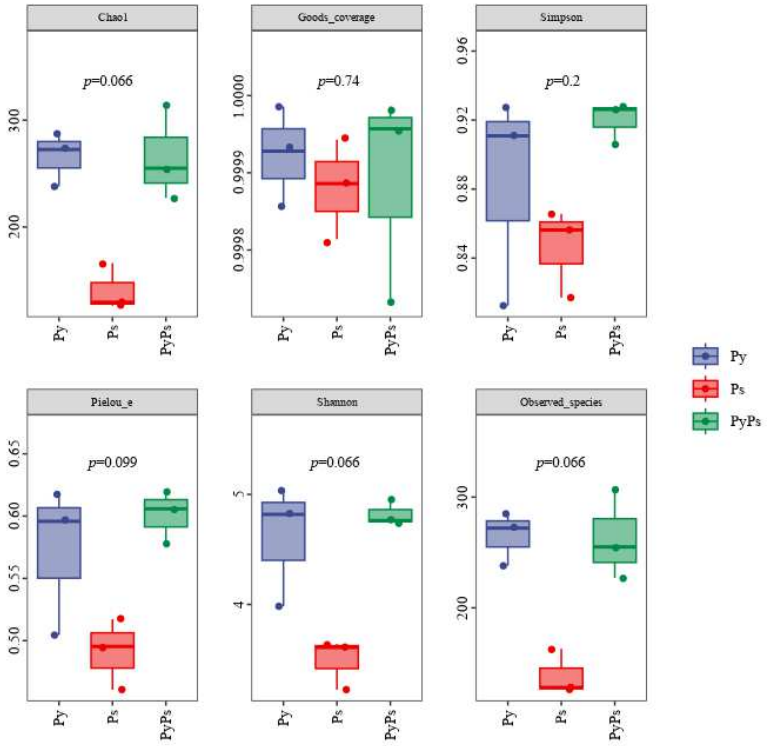


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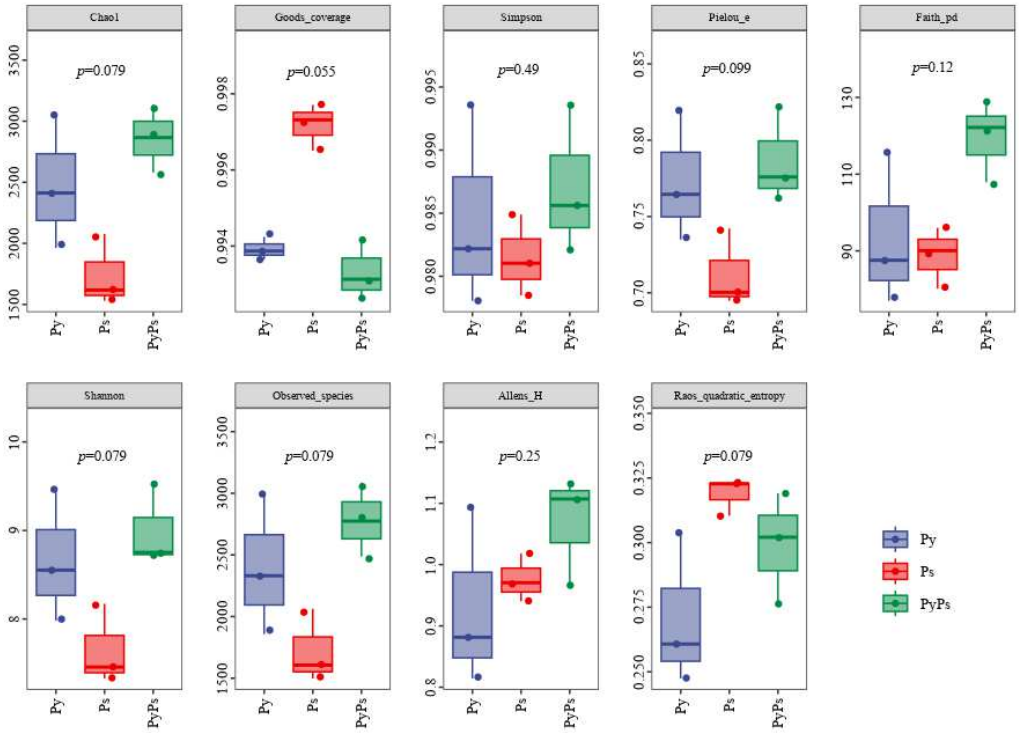


(**b**)

Fig. 7 Linear discriminant analysis Effect Size (LEfSe) analysis plot. (**a**) Fungal communities. (**b**) Bacterial communities. Node size corresponds to the average relative abundance of the taxon. Blue, red, and green nodes indicate taxa with significant intergroup differences that are more abundant in the group represented by the corresponding color, while hollow nodes represent taxa with no significant intergroup differences. Letters denote the names of taxa showing significant intergroup differences. p = phylum, c = class, o = order, f = family, g = genus, s = species

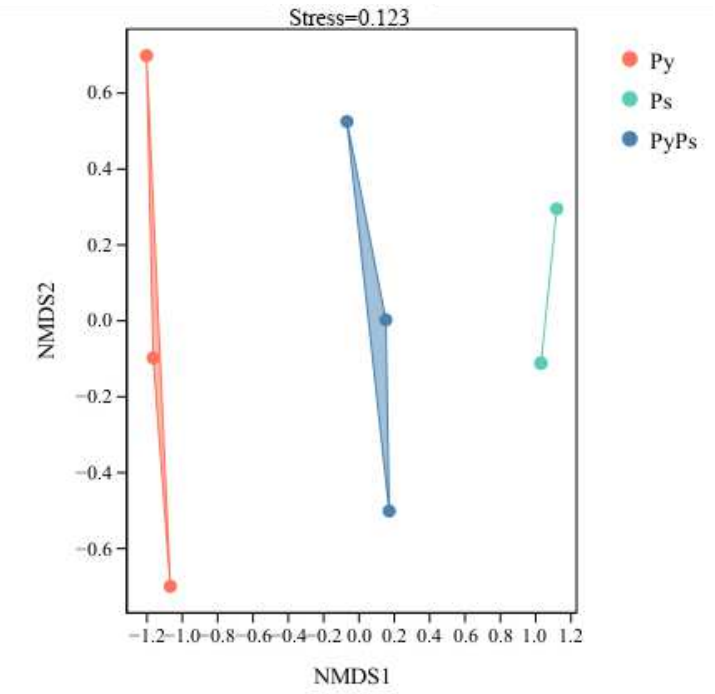


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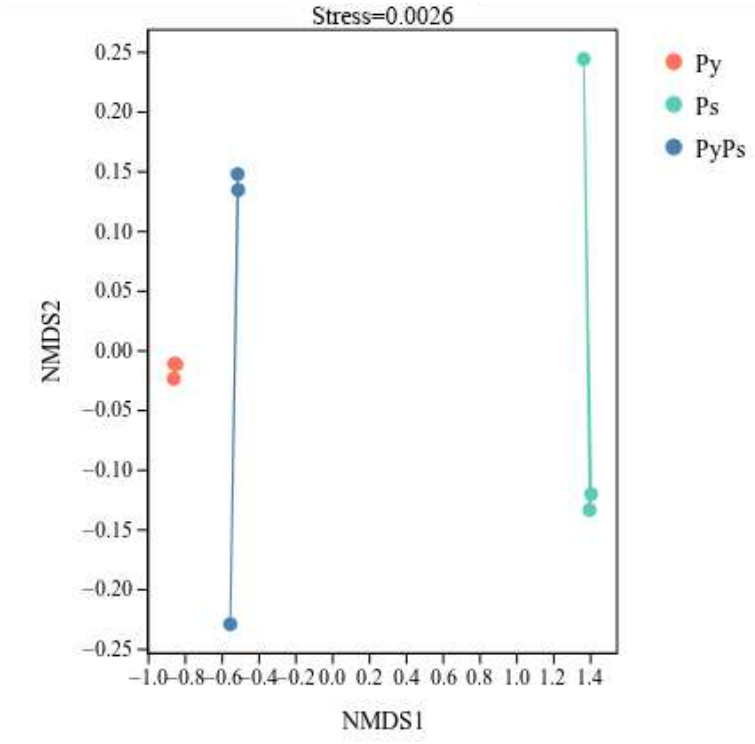


(**b**)

Fig. 8 Alpha diversity index map of phyllosphere microbial community. (**a**) Fungal communities. (**b**) Bacterial communities

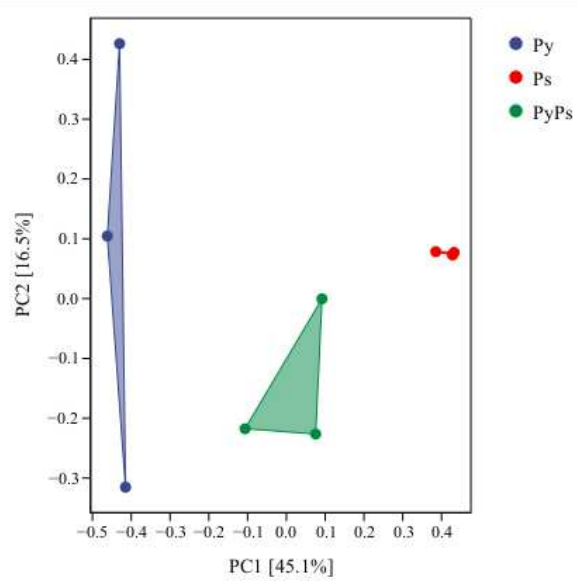


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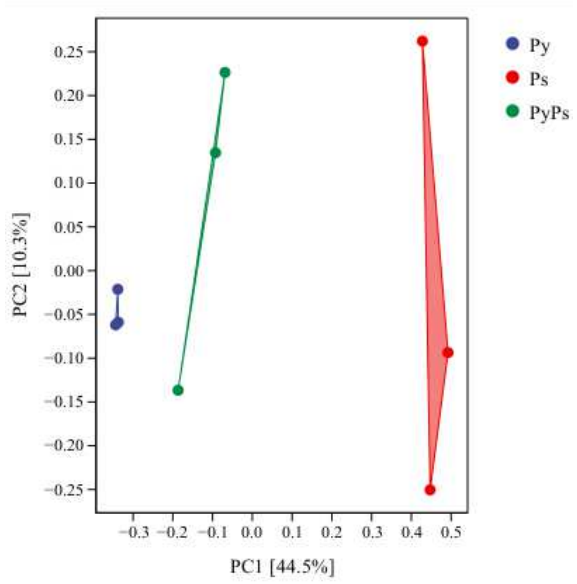


(**b**)

Fig. 9 Non-metric Multidimensional Scaling (NMDS) analysis plot. (**a**) Fungal communities. (**b**) Bacterial communities



(**a**)



(**b**)

Fig. 10 Distance matrix and Principal Coordinates Analysis (PCoA) plot. (**a**) Fungal communities. (**b**) Bacterial communities