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Functional Roles of Microbial Communities in Litter Decomposition Across Pure and Mixed Forest Types

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Abstract

Background Mixed forests are often considered superior to pure forests because they can increase biodiversity, enhance productivity, improve carbon storage, and strengthen resistance and resilience. However, the role of microorganisms in litter decomposition in mixed forests remains unclear. In this study, we examined litter decomposition in three forest stand types—pure *Robinia pseudoacacia*, pure *Platycladus orientalis*, and mixed *R. pseudoacacia–P. orientalis*—through in situ experiments combined with high-throughput sequencing.

Results Litter decomposition were fastest in mixed forests compared with pure forests. Bacterial and fungal diversity exhibited pronounced variations across forest types and decomposition stages, with Proteobacteria, Bacteroidetes, and Actinobacteria dominating bacterial communities, and Ascomycota and Basidiomycota prevailed among fungi. Early-stage cellulose and hemicellulose decomposition was driven by bacterial genera *Sphingomonas*, *Hymenobacter*, *Pseudomonas*, *Pedobacter*, and *Spirosoma,* together with fungal taxa including *Nothophoma*, *Cladosporium*, *Septoria*, and *Alfaria*. In contrast, lignin degradation after 270 days was facilitated by bacterial *Amycolatopsis* and *Streptomyces* alongsidefungal *Helicodendron, Lecanicillium, Chalara,* and *Trechispora*. Bacterial diversity indices exhibited stronger positive correlations with litter weight loss rates than fungal indices. Partial least squares path modeling further indicated that microorganisms directly regulated litter decomposition, while also indirectly modulating decay rates through enzyme-mediated alterations of organic matter composition.

Conclusions These findings demonstrate that mixed plantations enhance microbial-

mediated litter decomposition and nutrient restoration, highlighting their role as effective strategies for improving soil fertility and ecosystem functioning in temperate forests.

Keywords: Litter decomposition; Lignocellulose; Microorganisms; Mixed forest; Pure forest

1 Introduction

Litter decomposition drives ecosystem nutrient cycling through mass loss and mineralization, regulated by biotic-abiotic interactions (Bradford et al., 2016; Elias et al., 2024). As the dominant structural component (65–85% of dry mass), lignocellulose (cellulose/hemicellulose/lignin) degradation constitutes the rate-limiting step in organic matter transformation (Berg and Bjorn, 2014). Microorganisms catalyze this process by secreting lignocellulolytic enzymes, such as endoglucanases, β-glucosidases, xylanases, and lignin peroxidases, making them pivotal regulators of decomposition dynamics (Fioretto et al., 2007; Waldrop et al., 2000).

Fungal communities, particularly ascomycetes (e.g., Leotiomycetes, Dothideomycetes) and basidiomycetes—play irreplaceable roles through cellulase, laccase, and peroxidase secretion (Osono, 2007; Schneider et al., 2012; Zhang, 2019). Bacterial taxa such as β-proteobacteria, Bacteroidetes, Actinomycetes, and Acidobacteria also contribute significantly via extracellular enzyme production (Llado et al., 2017). Despite this functional diversity, the roles of specific microbial consortia across forest types remain incompletely resolved (Daunoras et al., 2024; Kim et al., 2012; Wang et al., 2020). Detrital microorganisms inhabiting litter surfaces or matrices display spatial variability modulated by species composition, geography, and climate, yet their community structure–function relationships are poorly characterized (Bai et al., 2023; Bradford et al., 2016; Peters et al., 2019).

Mixed forest systems, widely adopted as silvicultural strategies, substantially augment ecosystem services by modifying both litter biogeochemistry (organic C, N, P

stoichiometry) and soil microclimatic regimes (temperature, moisture dynamics), thereby exerting cascading effects on soil microbial communities and litter decomposition processes (An et al., 2013; Brockett et al., 2012). Although most studies report that mixed stands amplify environmental heterogeneity and substrate diversity, thereby accelerating decomposition relative to pure forests, contradictory evidence exists (Chapman and Koch, 2007; Harguindeguy et al., 2008; Liu et al., 2022; Marco et al., 2011; Porre et al., 2020). Recent findings indicate positive non-additive effects in leaf litter mixtures containing nutrient-rich species, whereas mixtures dominated by nutrient-poor species exhibit negative non-additive effects (Mukamparirwa et al., 2024). This unresolved dichotomy necessitates mechanistic investigations.

In this study, we hypothesize that (1) litter microbial community composition and diversity in mixed forest are significant different from pure forest, and (2) mixed forests litter would enhance the litter weight loss rate through increasing the activities of relevant degrading enzymes and synergistic microbial interactions. Using high-throughput sequencing, we analyze microbial succession and functional shifts over 540 days in an in-situ litter decomposition experiment conducted in pure *R. pseudoacacia*, pure *P. orientalis*, and mixed *R. pseudoacacia–P. orientalis* forests. This work aims to elucidate microbe-mediated decomposition controls critical for predicting nutrient cycling in changing forest systems.

2. Materials and Methods

2.1 Sampling sites

The research area was located in the gully region of the Loess Plateau within the

Huaiping Forest Farm, Yongshou County, Shaanxi Province, China (108°05′-108°10′E, 34°47′-34°51′N). The region has a warm temperate, semi-arid continental monsoon climate, with an average annual precipitation of 605 mm and a mean annual temperature of 10.8 °C(Zhang et al., 2024). The zonal soil is predominantly yellow–brown soil, and the area is characterized by warm temperate deciduous broadleaf forest., The dominant tree species include *R. pseudoacacia*, *P. orientalis*, *Quercus wutaishanica*, *Populus davidiana*, and *Pinus tabuliformis* (Zhang et al., 2023; Zhang et al., 2024). Formal approval for field sampling and related experiments was obtained from the forest farm before the experiment was conducted.

2.2 Experimental Design

At the end of June 2020, pure forest stands of *R. pseudoacacia* (23 years old, RL), *P. orientalis* (25 years old, PL), and mixed forest stands of *R. pseudoacacia* (23 years old) and *P. orientalis* (25 years old) (hereafter referred to as mixed forest, with a mixing ratio of 6:4; ML) were selected. Both tree species are among the most common and widely distributed tree species in Shaanxi Province and are not endangered or protected species.

For each forest type, three replicates under same previous land-use and similar site conditions (elevation, slope position, aspect, gradient, etc.) were established in 10 m × 10 m plots, resulting in a total of nine sample plots. Within each plot, 20 litter traps measuring 1 m × 1 m with a 1 mm mesh size were installed, totaling 180 traps. In September 2020, fresh leaf litter was collected from the traps and transported to the laboratory. After air-drying at room temperature, the litter samples were placed into

nylon mesh litterbags measuring 15 cm × 10 cm with a 1 mm pore size (Gobiewski et al., 2019; Zeng et al., 2019a), and returned back to their own plots. To compare litter weight loss rate across forest types, 7.5 g of dried leaf litter was placed in each bag, based on annual litterfall data from pure *R. pseudoacacia* and *P. orientalis* forests in the Loess Plateau region (Zhang et al., 2024).

In October 2020, during the peak leaf fall, the field decomposition experiment was initiated (Fig. 1). Each plot was divided into 20 subplots of 4 m × 5 m, and six litterbags were placed in each subplot to ensure even distribution. Litterbags were positioned within the canopy projection area, placed on bare soil after removing the herbaceous layer, litter layer, and humus. In total, 1080 litterbags were deployed across the three forest types (9 plots × 20 replicates × 6 sampling occasions). Litterbags were secured with U-shaped wire to prevent disturbance from humans or animals and were laid parallel without overlapping. To maintain near-natural conditions, one side of each litterbag was placed in direct contact with the soil surface, while the other side was covered with naturally occurring litter. Based on litter decomposition characteristics and critical decomposition periods, sampling was conducted every three months in the first year and every six months in the second year, at 0 (October 2020), 90 (January 2021), 180 (April 2021), 270 (July 2021), 360 (October 2021), and 540 days (April 2022) (Wang et al., 2020; Zhang et al., 2019). At each sampling event, 20 litterbags per plot were carefully retrieved, and soil, debris, and root systems were removed from the bag surfaces and interiors. Then all litters were homogenized and divided into two subsamples: one for determining moisture content, dry weight, nutrient concentrations,

and organic matter, and the other for microbial DNA extraction and enzyme activity measurement.

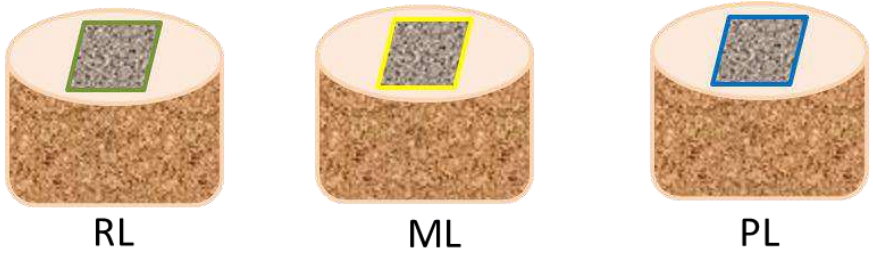


Fig. 1. Schematic representation of the field litter decomposition experiment conducted in pure *R. pseudoacacia* (23 years old; RL), pure *P. orientalis* (25 years old; PL), and mixed *R. pseudoacacia–P. orientalis* (23/25 years old; ML) forest stands.

The litter weight loss rate (R_l) was calculated using the following formula:

$$R_l = \frac{M_0 - M_t}{M_0} \times 100\% \qquad \text{Eq.1}$$

The litter decomposition rate constant (k) was calculated according to Olson model (Olson, 1963):

$$\frac{M_t}{M_0} = exp^{-kt} \qquad \text{Eq.2}$$

$$t_{50\%} = -In0.5/k \qquad \text{Eq.3}$$

$$t_{95\%} = -In0.05/k \qquad \text{Eq.4}$$

Where M_0 indicates the initial dry weight of litter (7.5g in our study), and M_t represents the remaining dry weight of litter collected at time t. $t_{50\%}$ and $t_{95\%}$ represents the time required for 50% and 95% of the litter decomposition.

2.3 Litter chemical analysis

The content of total carbon (TC) was determined using the potassium dichromate-concentrated sulfuric acid oxidation method. Total nitrogen (TN), phosphorus (TP), and potassium (TK) was determined in the H_2SO_4-H_2O_2 digestion, followed by Kjeldahl

nitrogen determination, molybdenum–antimony ascorbic acid colorimetry, and flame emission spectrophotometry, respectively (Yue et al., 2021). Concentrations of calcium (TCa), magnesium (TMg), and manganese (TMn) were analyzed by atomic absorption spectrophotometry following dry ashing (Wang et al., 2020; Zhang et al., 2019).

Cellulose, hemicellulose, and lignin content were determined according to the method established by the National Renewable Energy Laboratory (NREL), which involved Soxhlet extraction with ethanol, subsequent sulfuric acid hydrolysis, and quantification of monosaccharides using high-performance liquid chromatography (Sluiter et al., 2006). Total phenolic content was determined using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1964).

2.4 Enzyme Activity of Litter

First, the crude enzyme extracts were prepared from the litter samples. The activities of endoglucanase, exoglucanase, β-glucosidase, xylanase, and lignin peroxidase were quantified using a UV–visible spectrophotometer, following the methods described by Chen et al. (2014) and Zhang et al. (2008). The enzyme activity units for cellulases (including endoglucanase, exoglucanase, and β-glucosidase), hemicellulases (like xylanase), and lignin enzymes (such as lignin peroxidase) were defined as the amount of enzyme required to release 1 μg of glucose per minute per gram of sample (μg min^{-1} g^{-1}). Parallel controls using heat-inactivated enzyme extracts were processed following the same procedures.

2.5 Determination of Fungi and Bacteria in Litter

Genomic DNA of bacteria and fungi was extracted from 0.5 g of fresh leaf litter using

the CTAB method. DNA concentration and purity were determined through 1% agarose gel analysis. Following concentration quantification, all DNA samples were diluted with sterile water to reach a standardized concentration of 1 ng/μL. The bacterial 16S rRNA gene was amplified using the primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTW--TCTAAT), while the fungal ITS1 region was amplified using primers ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCG--ATGC). For PCR amplification, the reaction system contained 15 μL of Phusion® High-Fidelity PCR Master Mix (supplied by New England Biolabs), 0.2 μM of each primer pair, and 10 ng of the target DNA template. The thermal cycling conditions were set as follows: a first denaturation phase at 98°C lasting 1 min, subsequent 30 cycles of 98°C (10 s) for denaturation, 50°C (30 s) for annealing, and 72°C (30 s) for extension, followed by a final extension step at 72°C for 5 min. Mix PCR products were detected by electrophoresis 2% agarose gel. We constructed the library using the NEBNext® Ultra™ II DNA Library Prep Kit, followed by quantification with Qubit and qPCR. Libraries that passed quality control were sequenced on theIllumina NovaSeq 6000 platform (Majorbio Bio-Pharm Technology Co., Ltd. Shanghai, China).

For sequence processing, FLASH software (V1.2.11, http://ccb.jhu.edu/software/FLASH/) was used to merge paired-end reads, generating raw tags., After quality filtering (Mago and Salzberg, 2011), high-quality sequences were denoised using the DADA2 module in QIIME2, removing ASVs with an abundance below five. Taxonomic assignment of the final amplicon sequence variants

(ASVs) was performed using the classify-sklearn module in QIIME2 against the Silva 138.1 database for 16S sequences and the UNITE v8.2 database for ITS regions.

2.6 Data Analysis

IBM SPSS was used to perform two-way ANOVA to compare differences in litter weight loss rate, organic matter content, enzyme activity, and microbial community abundance and diversity across forest types and sampling times. Prior to analysis, data were tested for normality and homogeneity of variance. Graphical visualizations were generated using Origin 2018. Microbial diversity index, rarefaction curves, and species accumulation boxplots were calculated using QIIME2. The LEfSe (LDA Effect Size) method was applied to identify significantly different microbial taxa across decomposition stages within each forest stand and to construct evolutionary branching diagrams of key microbial groups. Cluster analysis based on the relative abundance of microbial taxa was conducted in R, and heatmaps were generated to illustrate abundance patterns. Pearson correlation analysis was performed to examine relationships among litter weight loss rate, substrate characteristics, microbial communities, organic matter content, enzyme activity, and nutrient levels. Redundancy analysis (RDA) was subsequently conducted to determine the main factors influencing litter weight loss rate and microbial composition. Correlation matrix plots and RDA ordination diagrams were produced using the “ggplot2” package in R.

3 Results

3.1 Litter decomposition rate and initial chemical properties of litter

From 0 to 180 days, R_l increased rapidly, followed by a slower but steady increase after 360 days, with the mixed forest showing the highest litter weight loss rate across all sampling (Fig. 2). The litter weight loss rate and decomposition time fitted Olson's negative exponential model (Table 1). Litter decomposition was the fastest in ML forests, followed by that in RL forests, and the slowest in PL forests. For all three forest stands, the time required for 50% litter decomposition exceeded one year, while at least five years were needed to achieve 95% decomposition.

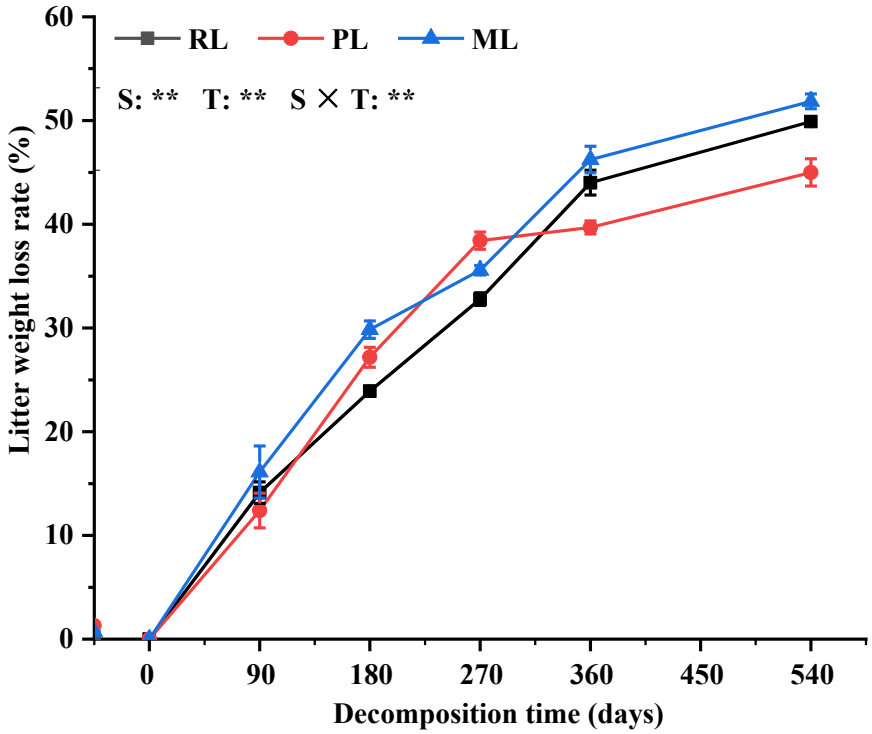


Fig. 2. Dynamic changes of litter weight loss rate (R_l) in different forest stands. S, stand types; T, decomposition time; RL, *R. pseudoacacia* forest; PL, *P. orientalis* forest; ML, mixed forest of *R. pseudoacacia* and *P. orientalis*. **, significant difference, $P<0.01$.

219 Table 1 Olson negative index regression relation between litter weight loss rate and time

Stand types	Fitting function	Fitting coefficient R^2	Decomposition constant k	$T_{50\%}$ (years)	$T_{95\%}$ (years)
RL	$y = exp^{-0.507t}$	0.990	0.507	1.367	5.909
PL	$y = exp^{-0.475t}$	0.978	0.475	1.459	6.307
ML	$y = exp^{-0.550t}$	0.991	0.550	1.260	5.447

220 Key chemical properties of the litter from the three forest stands are shown in Table
221 2. PL litter exhibited the highest contents of TC, C/N, C/P, lignin, and lignin/N, whereas
222 RL litter had the lowest values. In contrast, the concentrations of TCa, TMg, and TMn
223 followed the opposite pattern, and TK was highest in ML. R_l increased throughout the
224 decomposition period and was significantly affected by stand type ($P < 0.01$; Fig. 2).

Table 2 Initial chemical properties of litter in different forest stands. Lowercase letters indicate significant differences among forest stands ($P < 0.05$, Tukey's HSD test). Within each row, values followed by different letters (a, b, c) are significantly different; values sharing the same letter are not significantly different.

Chemical properties	RL	PL	ML
TC (g kg^{-1})	389.44±1.83c	482.29±2.38a	433.53±1.56b
TN (g kg^{-1})	11.79±0.95a	4.12±0.68b	5.51±0.49b
TP (g kg^{-1})	1.02±0.05a	0.84±0.01b	0.96±0a
C/N	33.17±2.70c	119.15±19.45a	79.01±6.49b
C/P	383.16±19.16c	574.29±11.8a	449.46±2.53b
N/P	11.57±0.42a	4.90±0.73b	5.72±0.52b
TK (g kg^{-1})	3.25±0.05b	1.82±0.06c	4.55±0.05a
TCa (mg kg^{-1})	2820.83±33.76a	2451.04±32.29c	2633.33±4.17b
TMg (mg kg^{-1})	192.50±0.83a	107.50±0.83c	175.83±4.17b
TMn (mg kg^{-1})	70.88±0.63a	31.13±1.21c	47.54±0.54b
Total phenol (%)	5.70±1.83b	7.63±2.23b	13.65±3.18a
Cellulose (%)	23.16±0.51a	15.91±0.19b	24.00±0.81a
Hemicellulose (%)	20.10±0.65a	9.78±0.54b	19.11±0.3a
Lignin (%)	20.33±0.19c	27.42±0.12a	21.44±0.45b
Lignocellulose (%)	63.58±0.69a	53.11±0.72b	64.55±1.53a
Lignin/N	17.31±1.38c	67.72±10.91a	39.07±3.3b

3.2 Organic matters during litter decomposition

Organic matters content was significantly influenced by stand types and decomposition time ($P < 0.01$; Fig. 3). Over the 0–540 day period, cellulose and hemicellulose contents in the litter of all three forest stands gradually declined, whereas total phenol content steadily increased. Lignin content initially increased and then decreased after 270 days (Fig. 3). During the early stage of decomposition, cellulose and lignin contents decreased most rapidly in ML, reaching their lowest levels at 270 days (Fig. 3A and B). After 270 days, as lignin content declined, the proportion of total phenols was highest in ML (Fig. 3C and D).

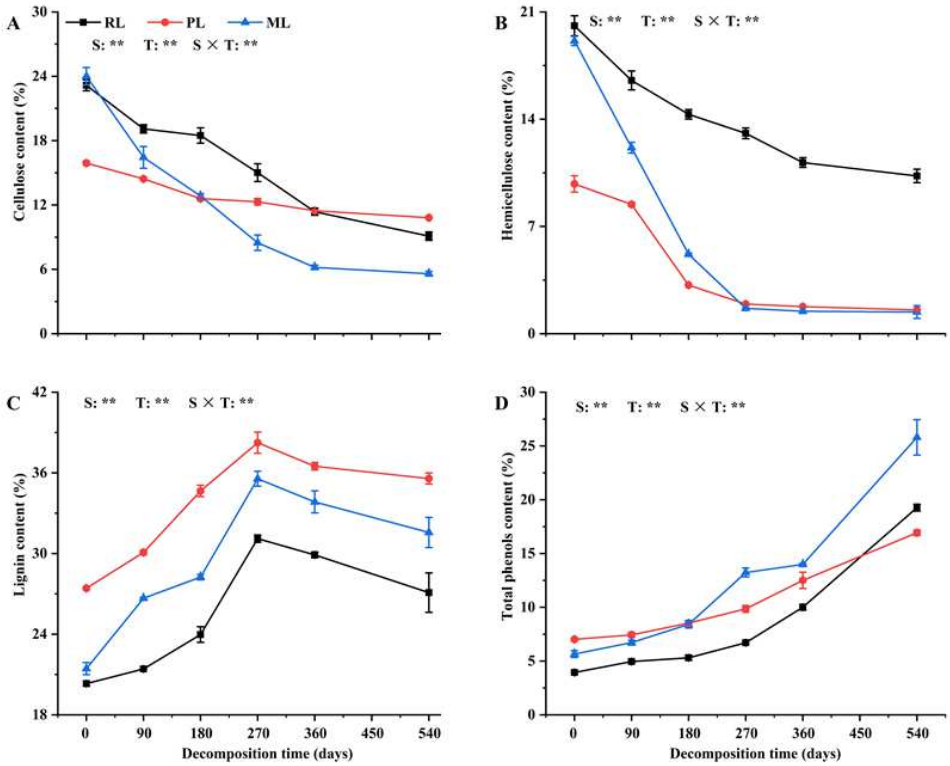
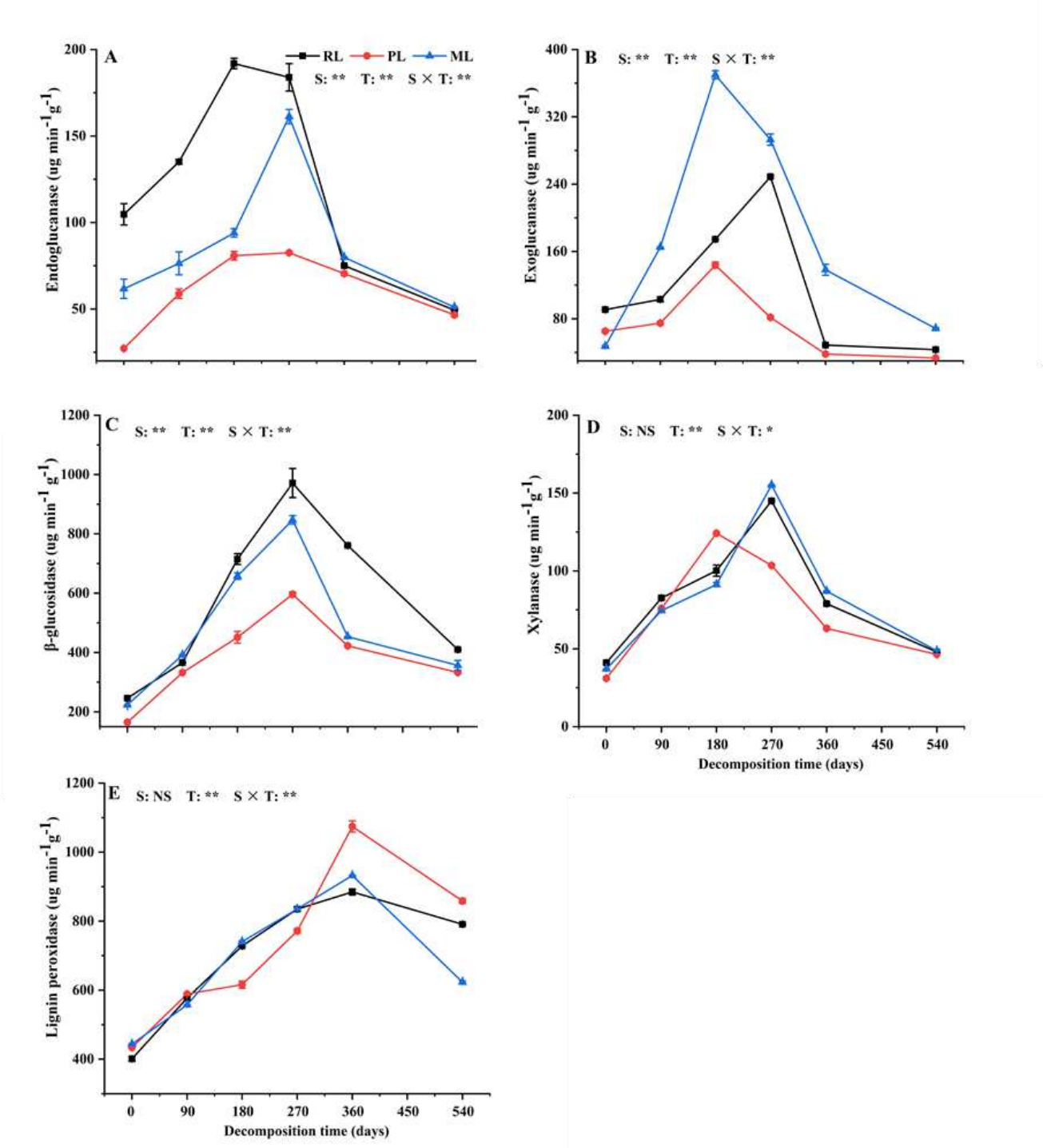


Fig. 3. Dynamic changes in organic matter content during litter decomposition. (A) Cellulose content; (B) Hemicellulose content; (C) Lignin content; (D) Total phenol content. S, stand types; T, decomposition time; RL, *R. pseudoacacia* forest; PL, *P. orientalis* forest; ML, mixed forest of *R. pseudoacacia* and *P. orientalis*. **, significant difference, $P<0.01$； NS, no significant difference.

3.3 Enzymatic activity related to the litter decomposition

Except for xylanase and lignin peroxidase, the activities of endoglucanase, exoglucanase, and β-glucosidase were significantly affected by forest stand type and decomposition time (P<0.05; Fig.4). The activities of all five enzymes initially increased and then declined, peaking at 270 or 360 days (Fig. 4). During the litter decomposition process, endoglucanase, exoglucanase, and β-glucosidase activities were lowest in PL litter, whereas lignin peroxidase activity reached its highest level at 360 days (Fig.4A-C, E). The activities of exoglucanase, xylanase, and endoglucanase in ML gradually exceeded those in RL after 90, 270, and 360 days, respectively (Fig.4A, B, D).



252 Fig. 4. Dynamic changes in enzyme activities during litter decomposition. (A) Endoglucanase; (B)

253 Exoglucanase; (C) β-glucosidase; (D) Xylanase; (E) Lignin peroxidase. S stand types; T,

254 decomposition time; RL, *R. pseudoacacia* forest; PL, *P. orientalis* forest; ML, mixed forest of *R.*

255 *pseudoacacia* and *P. orientalis*. **, significant difference, $P<0.01$； NS, no significant difference.

3.4 Bacterial community diversity and composition during litter decomposition

Bacterial diversity, especially the Pielou-B and Simpson-B indices, showed a fluctuating trend over 0–540 days and was significantly affected by stand type and decomposition time (P<0.05; Fig.5). The initial Chao-B and Shannon-B indices were highest in ML, with the peak values occurring at 360 days (Fig.5A, C). In PL, the lowest Pielou-B and Simpson-B index peaks appeared at 90 days (Fig.5B, D).

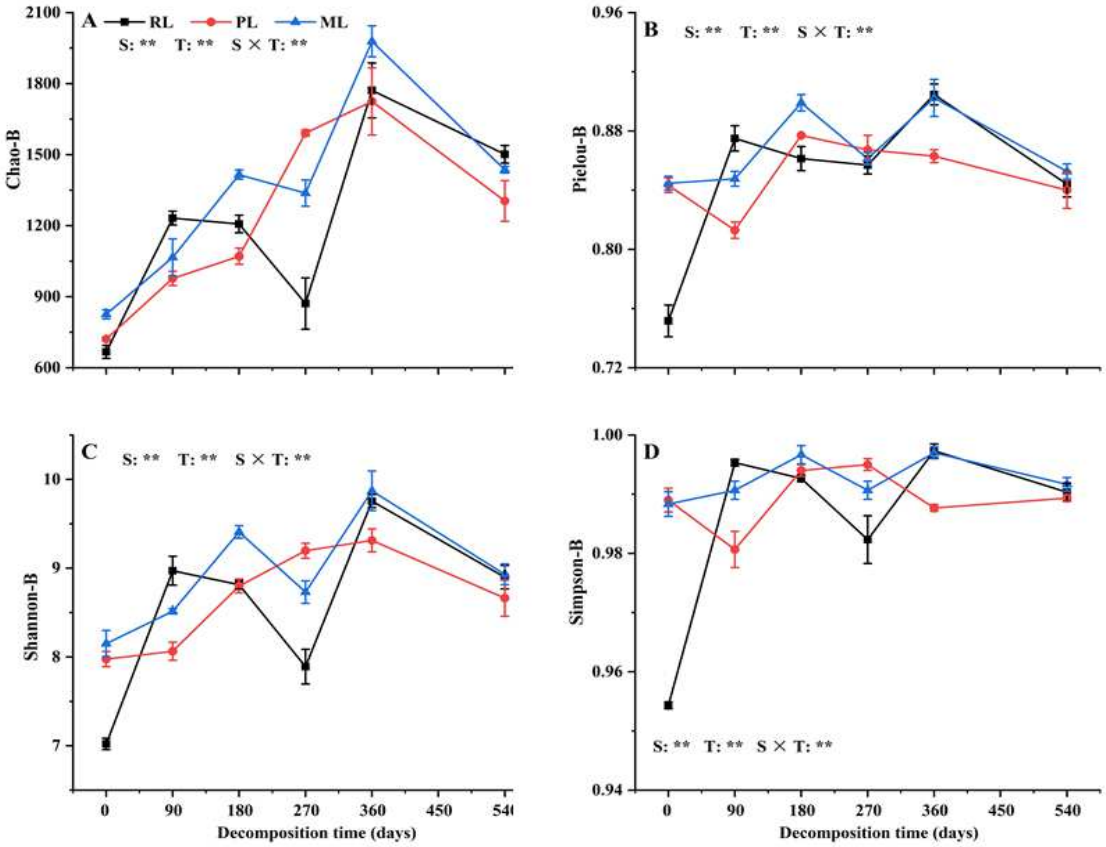


Fig. 5 Variation in bacterial community diversity in litter. S, stand types; T, decomposition time; RL, *R. pseudoacacia* forest; PL, *P. orientalis* forest; ML, mixed forest of *R. pseudoacacia* and *P. orientalis*. **, significant difference (P < 0.01); NS, no significant difference.

Sphingomonadaceae (0 days), Microbacteriaceae (90 days), Chitinophagaceae (360 days), Micromonosporaceae, and Microscillaceae (540 days) were the common and

267 important bacterial family in three forest stands at different decomposition times
268 (Fig.S1). Relative abundance ranking indicated that, *Sphingomonas, Chloroplast,*
269 *Pseudomonas, Methylobacterium-Methylorubrum, Pedobacter, Amycolatopsis,*
270 *Dyadobacter, Taibaiella, Hyme nobacter* and *Promicromonospora* were relatively
271 abundant and important genera in all litters (Fig.6). *Sphingomonas, Pseudomona,*
272 *Muribaculaceae, Spirosoma, Allorhizobium, Brevundimonas,* 0319-6G20, *Devosia,*
273 *Bradyrhizobium, Actinoplanes, Streptomyces, Rhodococcus* and *Novosphingobium* had
274 significant differences among the forest stands (P<0.05; Table S1). In addition, except
275 for *Amycolatopsis, Taibaiella, Promicromonospora, Blastocatella,* and *Aeromonas*,
276 other genera showed significant differences across decomposition times (P<0.05; Table
277 S1).

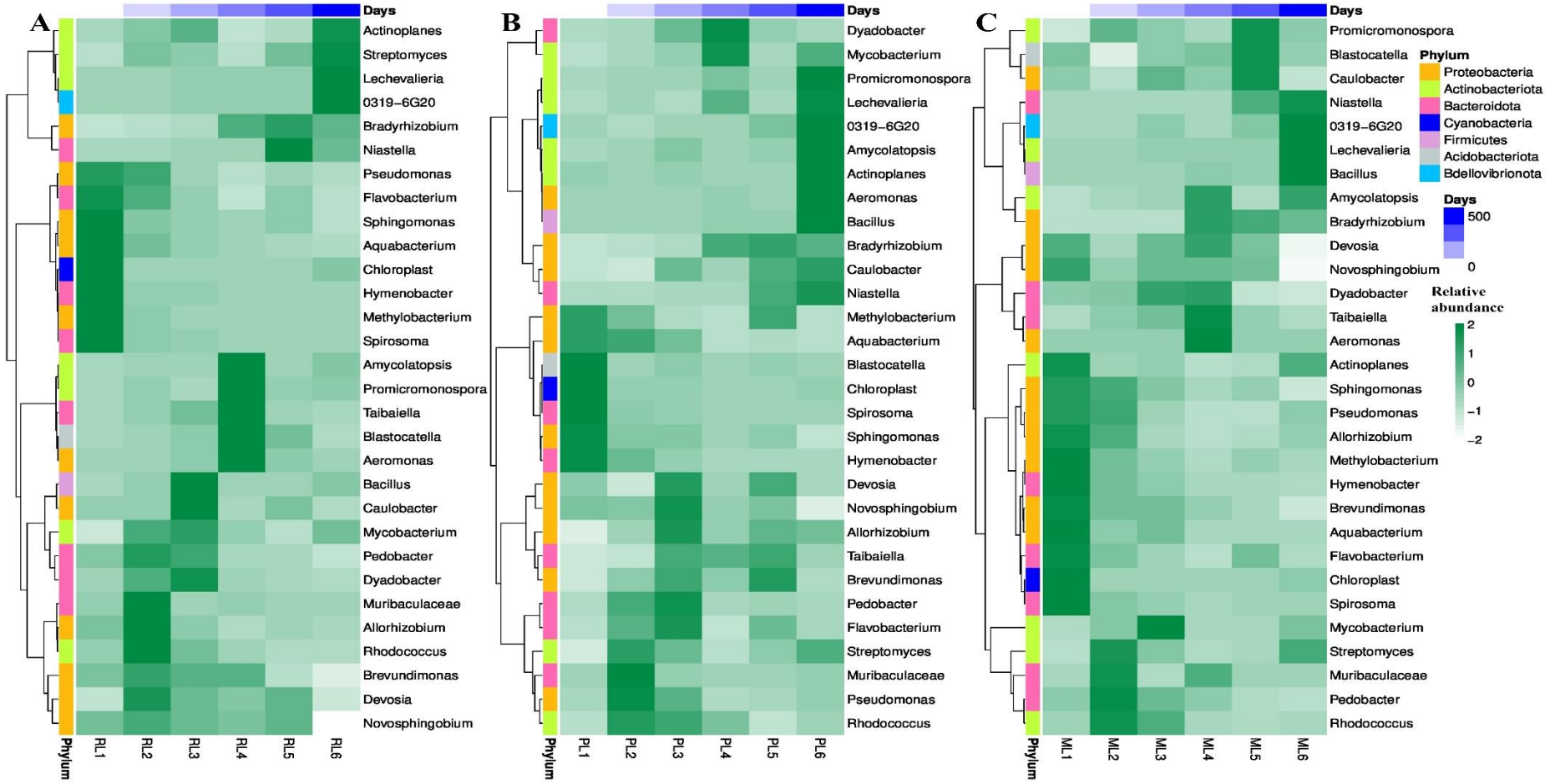


Fig. 6 Relative abundance heatmap of bacterial genera (top 30 in abundance). (A) Robinia pseudoacacia forest litter; (B) Platycladus orientalis forest litter; (C) mixed forest litter.

3.5 Fungal community diversity and composition during litter decomposition

Fungal diversity indices were significantly influenced by forest stand type and decomposition time ($P < 0.05$), and consistently exhibited unimodal temporal dynamics characterized by an initial increase followed by a progressive decline (Fig.7). All fugal diversity indices in ML were higher after 360 days compared with the pure forest stands (Fig.7).

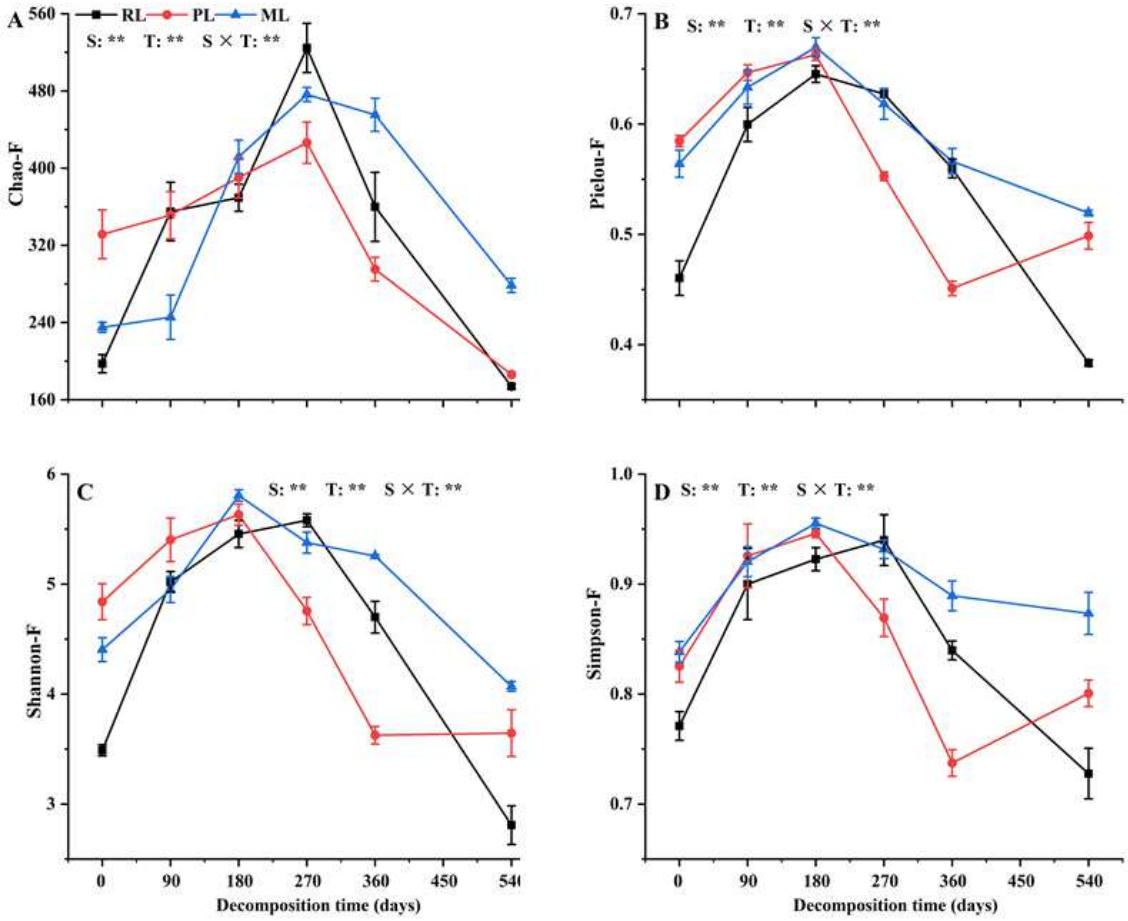
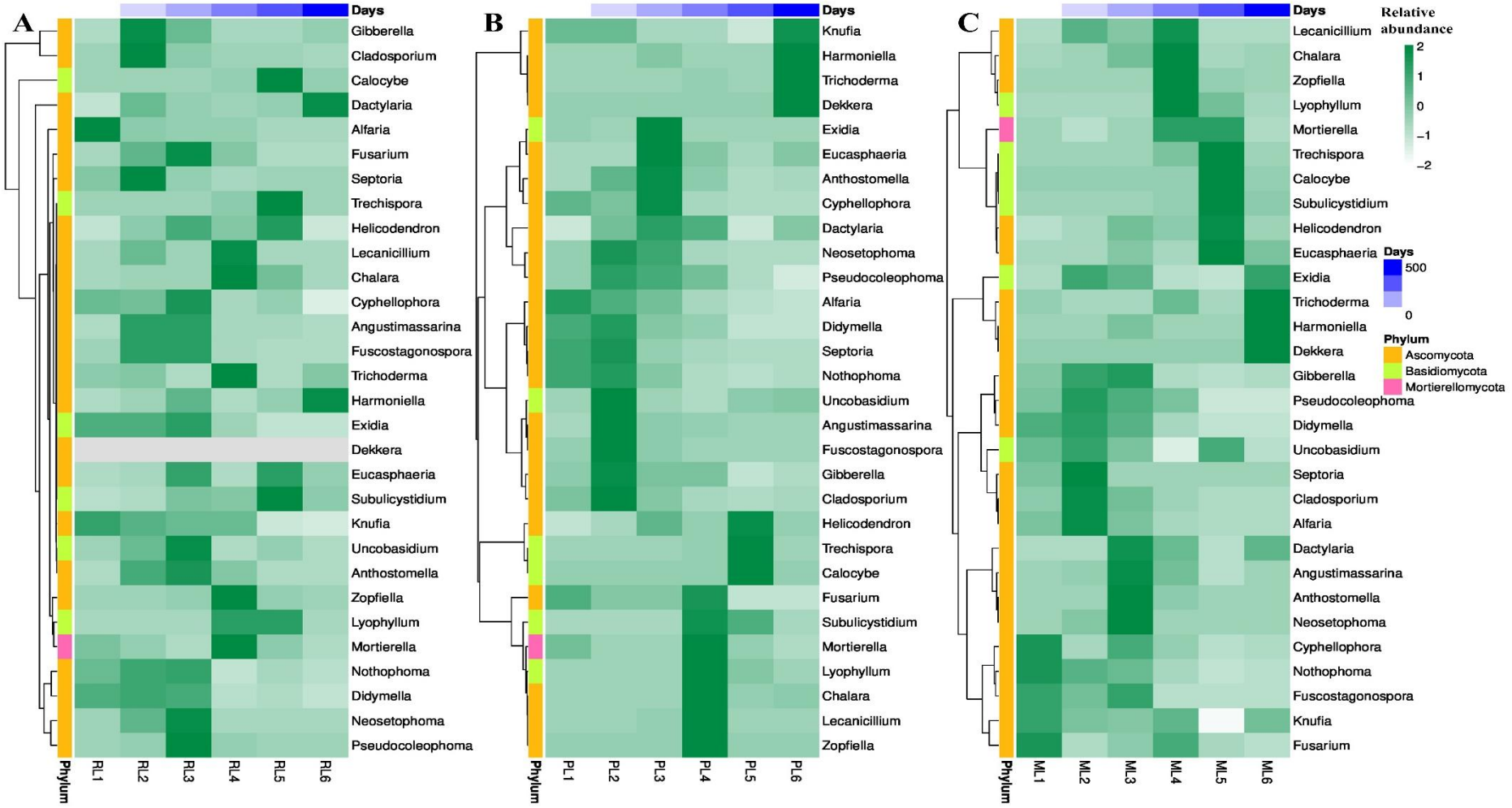


Fig. 7 Variation in fungal community diversity in litter. S, stand types; T, decomposition time; RL, *R. pseudoacacia* forest; PL, *P. orientalis* forest; ML, mixed forest of *R. pseudoacacia* and *P. orientalis*. **, significant difference ($P < 0.01$); NS, no significant difference.

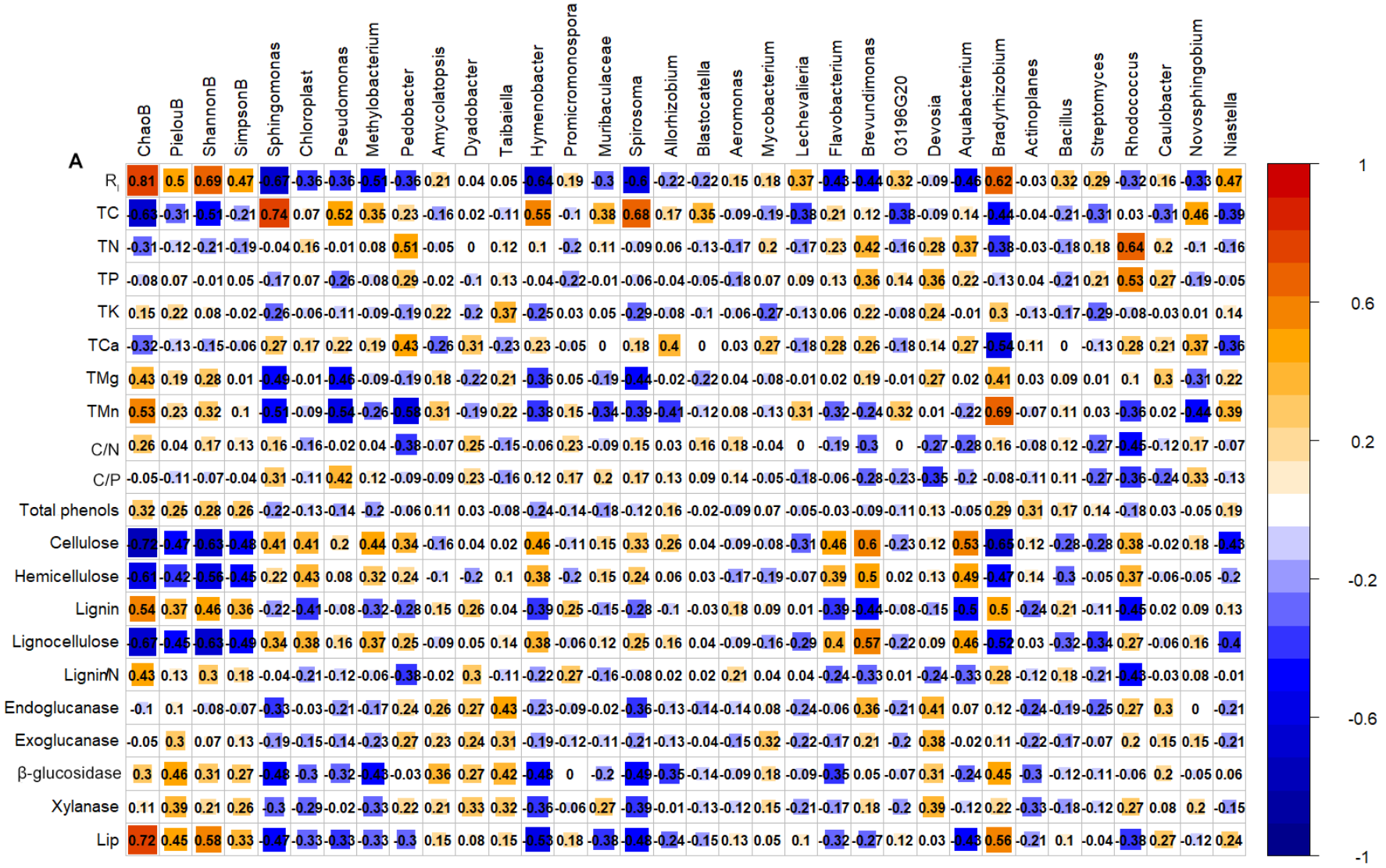
291 *Cladosporiaceae* was the common family across the three forest stands at 90 days,
292 whereas other important families differed among stands at the same decomposition
293 times (Fig. S2). The top ten fungal genera in terms of relative abundance in litters were
294 *Trechispora, Calocybe, Harmoniella, Septoria, Uncobasidium, Gibberella,*
295 *Anthostomella, Dactylaria, Cladosporium,* and *Nothophoma* (Fig. 8), all of which
296 showed significant differences among forest stands ($P < 0.05$; Table S2).

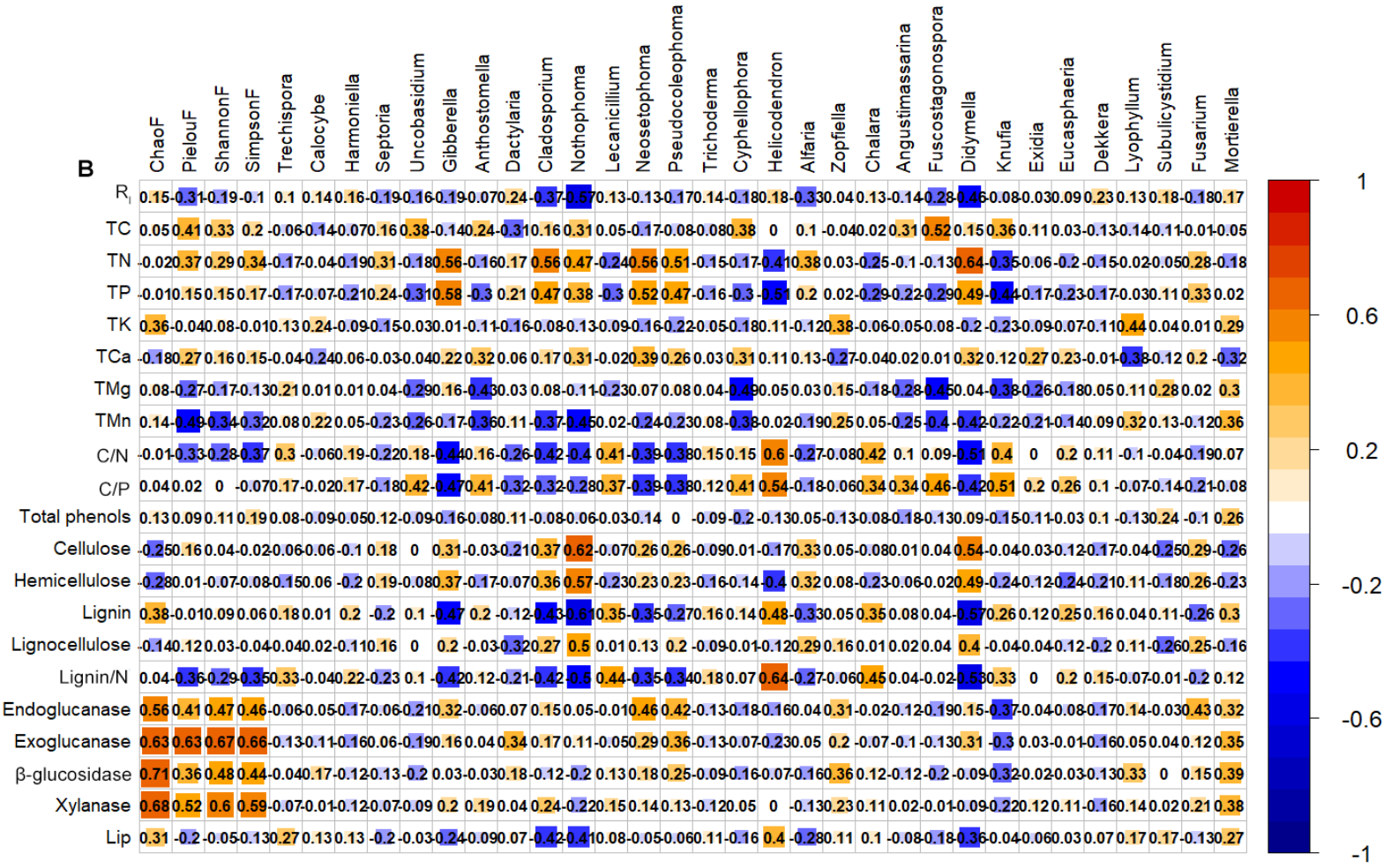


297 Fig. 8 Relative abundance heatmap of fungal genera (top 30 in abundance). (A) Robinia pseudoacacia forest litter; (B) Platycladus orientalis forest litter; (C) mixed
298 forest litter..

3.6 The relationship among litter weight loss rate, organic matters content, enzyme activity, and microorganisms

Bacterial diversity indices (Chao-B and Shannon-B) demonstrated significant positive correlations with R_l, TMn, lignin content, and LiP, showing negative correlations with TC, cellulose, hemicellulose, and lignocellulose concentrations (Fig. 9A). Fungal diversity indices (Chao-F, Pielou-F, and Shannon-F) were positively correlated with TC, TN, and cellulolytic enzymes (endoglucanase, exoglucanase, β-glucosidase, xylanase), but negatively correlated with TMn, C/N, and lignin/N ratios (Fig. 9B). Notably, bacterial diversity exhibited stronger associations with litter weight loss rates than fungal indices (Fig. 9).


A
ChaoB
PielouB
ShannonB
SimpsonB
Sphingomonas
Chloroplast
Pseudomonas
Methylobacterium
Pedobacter
Amycolatopsis
Dyadobacter
Taibaiella
Hymenobacter
Promicromonospora
Muribaculaceae
Spirosoma
Allorhizobium
Blastocatella
Aeromonas
Mycobacterium
Lechevalieria
Flavobacterium
Brevundimonas
03196G20
Devosia
Aquabacterium
Bradyrhizobium
Actinoplanes
Bacillus
Streptomyces
Rhodococcus
Caulobacter
Novosphingobium
Niastella
R
TC
TN
TP
TK
TCa
TMg
TMn
C/N
C/P
Total phenols
Cellulose
Hemicellulose
Lignin
Lignocellulose
Lignin/N
Endoglucanase
Exoglucanase
β-glucosidase
Xylanase
Lip
1
0.6
0.2
-0.2
-0.6
-1



310 Fig. 9 The correlation between microbial diversity and litter weight loss rate, nutrient content, organic matter content and enzyme activity. A, Bacteria; B, fungi.

311 *Brevundimonas* and *Aquabacterium* displayed positive correlations with cellulose
312 and hemicellulose content ($R^2 \geq 0.5$). *Bradyrhizobium* was associated with lignin and
313 showed positive correlations with β-glucosidase and LiP ($R^2 \geq 0.5$). *Chloroplast*,
314 *Hymenobacter,* and *Spirosoma* exhibited negative correlations with β-glucosidase and
315 LiP ($R^2 \geq 0.5$). *Nothophoma* and *Didymella* were linked to cellulose and hemicellulose
316 decomposition, whereas *Helicodendron* correlated positively with lignin. *Gibberella*,
317 *Nothophoma*, and *Didymella* showed negative associations with lignin ($R^2 \geq 0.5$).
318 *Neosetophoma* displayed moderate affinity with endoglucanase ($R^2 \geq 0.5$).
319 Redundancy analysis (RDA) indicated that the bacterial community was strongly
320 influenced by TC, TN, TP, TK, TMg, TCa, TMn, C/P ratio, lignocellulose content,
321 endoglucanase, β-glucosidase, and LiP (Fig. 10A).
322 Among bacterial taxa, *Sphingomonas, Hymenobacter, Methylobacterium,*
323 *Pseudomonas, Pedobacter,* and *Spirosoma* contributed most substantially to cellulose
324 and hemicellulose content. *Chloroplast* significantly influenced hemicellulose content,
325 whereas *Aquabacterium, Muribaculaceae,* and *Flavobacterium* were primary
326 contributors to cellulose content. *Bradyrhizobium, Amycolatopsis,* and *Streptomyces*
327 exhibited the strongest contributions to lignin content and LiP activity. *Pedobacter,*
328 *Dyadobacter,* and *Bradyrhizobium* contributed predominantly to endoglucanase and β-
329 glucanase activities.
330 Nitrogen content primarily regulated *Chloroplast* abundance, whereas TCa had the
331 greatest influence on *Pseudomonas* and *Pedobacter* abundance. TK, TC, C/P ratio, and
332 Mg levels were major regulators of *Pedobacter* and *Dyadobacter* abundance. Mn

concentration exerted the strongest effect on *Lechevalieria, Niastella,* and *03196-G20* abundance, whereas TP primarily affected *Sphingomonas, Hymenobacter, Methylobacterium,* and *Chloroplast* abundance.

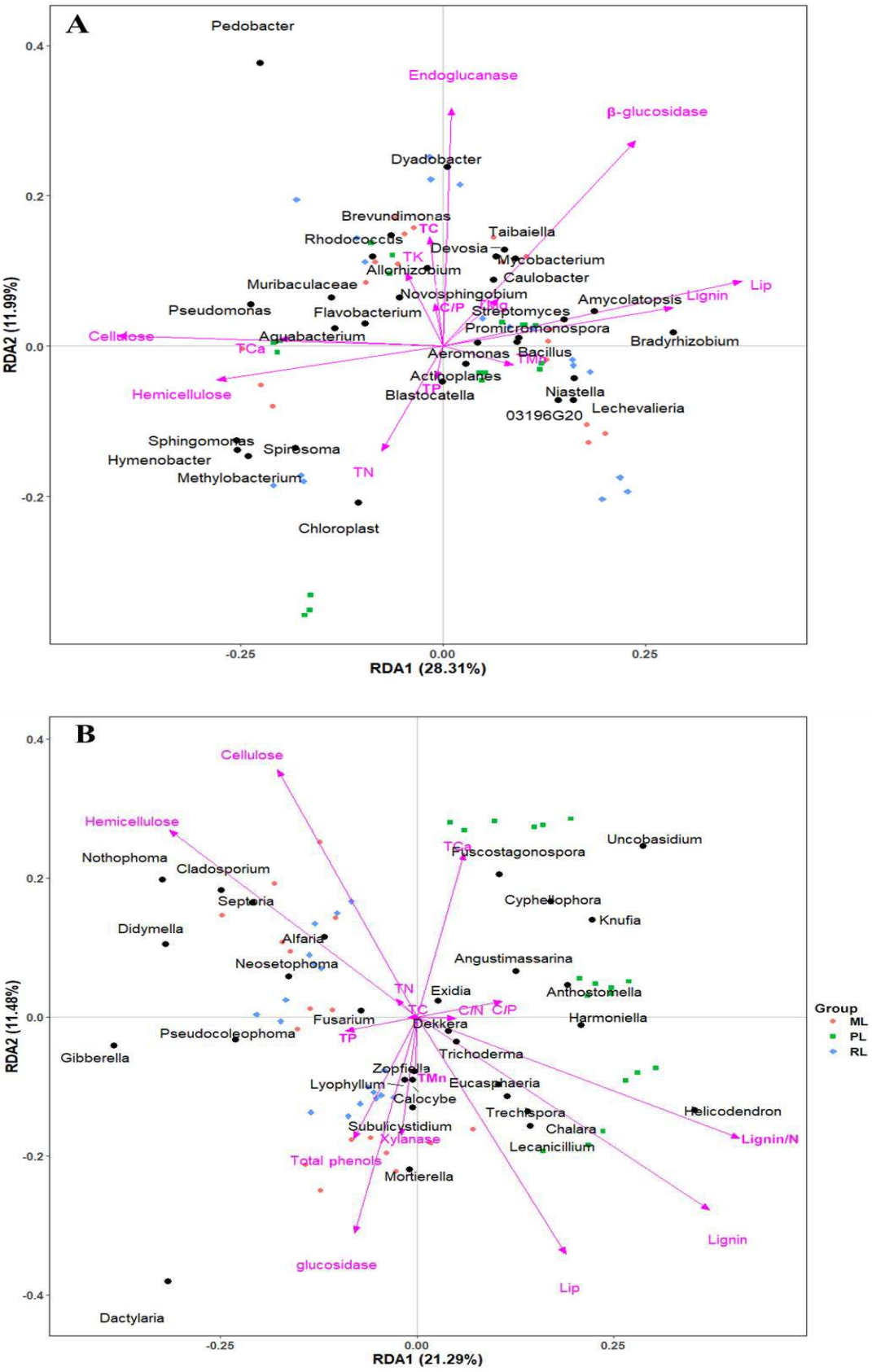


Fig.10 Effects of litter nutrient content, organic matter composition, and enzyme activities on phyllosphere microbial abundance at the order level. (A) Bacterial communities; (B) Fungal communities.

4. Discussion

4.1 Stage-Specific Characteristics of Litter Organic Matter Decomposition across Pure and Mixed Forest Types

The content of major organic components in litter (cellulose, hemicellulose, lignin, and polyphenols) changes dynamically during decomposition. Hemicellulose is considered the most reactive component of lignocellulose, cellulose is relatively easily decomposed in the initial stages, and lignin is recognized as the most recalcitrant carbohydrate, with its resistance determined by the methoxyl group content within its structure (Berg and Bjorn, 2014). Total phenolics, comprising flavonoids, phenolic acids, and tannins, are also difficult to degrade (Berg et al., 1993). Based on the properties of organic compounds, decomposition proceeds through three distinct phases. In the early phase, soluble, low-molecular-weight substances such as mono- and oligosaccharides and hydrolysable tannins are released. The middle phase is characterized by the decomposition of larger molecules, including cellulose and hemicellulose. In the late phase, insoluble and recalcitrant compounds, such as phenolics and lignin, are broken down(Ma et al., 2014).

As ground litter accumulates, soil biota and microorganisms proliferate, leading to the decomposition of hemicellulose and cellulose, thereby decreasing their content in litter from pure *R. pseudoacacia* stands, pure *P. orientalis* stands, and mixed stands, while the relative content of lignin and polyphenols increases (Aerts, 1997; Taylor et al., 1989). At 270 days (mid-phase), a decrease in lignin content indicated that its litter weight loss rate exceeded the overall mass loss rate, signifying that lignin had becoming a primary decomposition substrate. In the late phase, the increasing relative content of

362 lignin alters the microbial environment for phenolic compound decomposition and
363 inhibits polyphenol oxidase and peroxidase activity, resulting in phenolic accumulation,
364 consistent with our findings (Qin et al., 2018). Furthermore, small molecules released
365 from the enzymatic breakdown of cellulose and hemicellulose can complex with
366 phenolics (acting as chelators), forming larger, recalcitrant compounds that inhibit
367 decomposition (Fioretto et al., 2007; Schlesinger and Hasey, 1981). Although lignin
368 decomposition continues, the accumulation of recalcitrant substances such as phenolics
369 reduces bioavailable nutrients, diminishes microbial activity, and slows litter weight
370 loss rate (Qin et al., 2018). These observations underscores the control of late-phase
371 litter weight loss rates by recalcitrant materials like lignin.

372 **4.2 Enzyme-Driven Decomposition of Lignocellulose across Pure and Mixed**
373 **Forest Types**

374 The decomposition of litter organic matter relies on specific enzymatic activities. β-
375 glucosidase activity is a key indicator of cellulose decomposition (Allison and Vitousek,
376 2010; Berlemont and Martiny, 2013; Kang and Freeman, 2009). Hemicellulose
377 degradation involves enzymes such as xylanases, which target xylan and mannan
378 (Vendula et al., 2007). Lignin peroxidase (LiP), often the most common lignin-
379 degrading enzyme, and manganese peroxidase (MnP) initiate lignin degradation via
380 Mn^{3+} chelates (Hofrichter, 2002). We found that cellulase activity (endoglucanase and
381 exoglucanase) were influenced by tree species and decomposition time. Initial activity
382 was higher in *R. pseudoacacia* litter than in *P. orientalis* and mixed stands, suggesting
383 easier cellulose release. Xylanase and LiP activities were not significantly affected by

litter type, indicating similar microbial decomposers for hemicellulose and lignin across the litter types (Zhang et al., 2019). Based on the characteristics of enzyme activity and changes in organic substances, it can be preliminarily inferred that lignocellulose starts to decompose from day 0 and decomposes relatively slowly after 360 days; cellulose decomposes most rapidly between days 180 and 270, and lignin decomposes most rapidly between days 270 and 360. In the mixed stand, cellulose decomposition fastest with the peak at 180–270 days. Hemicellulose decomposition peaked at 0-90 and 180-270 days in *R. pseudoacacia* and mixed stands, but at 90-180 days in *P. orientalis*. Lignin decomposition was fastest at 180-360 days in *P. orientalis* and at 270-360 days in *R. pseudoacacia* and mixed stands. Compared to *P. orientalis,* the mixed stand litter exhibited higher activity for four lignocellulolytic enzymes (excluding LiP). Notably, after 270 days, endoglucanase, exoglucanase, and xylanase activities were higher in the mixed stand than in pure stands, indicating more thorough cellulose and hemicellulose decomposition (Hu et al., 2006; Singh, 2012; Wang et al., 2008). Concurrently, *P. orientalis* litter, which had higher lignin content, showed elevated LiP activity after 270 days.

4.3 Succession of Microbial Communities and Decomposition Functions across Pure and Mixed Forest Types

Proteobacteria, Actinobacteria, and Bacteroidetes were the dominant bacterial phyla throughout decomposition (Purahong et al., 2016; Vojtěch et al., 2016). In the initial phases (0-90 days), taxa rapidly producing proteases and cellulases (e.g., Sphingomonadaceae, Hymenobacteraceae, Pseudomonadaceae; high abundance of

406 *Sphingomonas*, and *Pseudomonas*), were dominant, aligning with our results. Bacteria
407 such as *Sphingomonas, Hymenobacter, Pseudomonas, Pedobacter*, and *Spirosoma*
408 contributed significantly to cellulose and hemicellulose dynamics, suggesting their role
409 as key decomposers, potentially active during 0-90 days. However, correlating enzyme
410 activities with substrate content changes revealed that peak cellulose and hemicellulose
411 decomposition actually occurred at 180-270 days, possibly due to a lag between
412 microbial colonization and peak enzyme production. *Pedobacter* was a key early-stage
413 bacterial decomposer across all litters (Zhang et al., 2019). *Sphingomonas* maintained
414 high abundance, likely due to its broad substrate utilization, including soluble organics,
415 lignin, and aromatics (Fredrickson et al., 1995; Jackrel et al., 2019). *Chloroplast*
416 sequences contributed significantly to hemicellulose content, potentially indicating a
417 role in hemicellulose degradation (Liu et al., 2018). After 270 days (late phase),
418 Micromonosporaceae, Pseudonocardiaceae, Microscillaceae, and Streptomycetaceae
419 (in pure stands) became important. *Amycolatopsis* and *Streptomyces* showed high
420 relative abundance and substantial contributions to lignin dynamics, implicating them
421 as key lignin decomposers (Purahong et al., 2016; Vojtěch et al., 2016). While Wang et
422 al. (2020) reported negative correlations between Actinobacteria abundance and
423 lignocellulose content and enzyme activity, our findings highlight specific
424 actinobacterial genera (*Amycolatopsis* and *Streptomyces*) contributing to lignin
425 degradation, suggesting that phylum-level analyses may mask functional roles of
426 specific taxa (Baldrian et al., 2010).

427 Fungal community composition and function also shifted with litter quality and time

(Jaroslav et al., 2011). Ascomycota dominated the early stages, while the relative abundance of Basidiomycota increased over time (Zhang et al., 2019). Most Ascomycota have limited lignin-degrading capacity and primarily decompose cellulose and hemicellulose (Boberg et al., 2011; Osono and Takeda, 2002), whereas ligninolytic-enzyme-producing Basidiomycota are crucial lignin decomposers (Purahong et al., 2016). At 0-90 days, significant differential families were primarily Ascomycota (Stachybotryaceae, Cladosporiaceae, Mycosphaerellaceae, and Pleosporales). Genera such as *Alfaria, Nothophoma, Cladosporium*, and *Septoria* were abundant and contributed substantially to cellulose and hemicellulose content, indicating their role in decomposing these polymers. Similar to bacteria, fungi appeared to proliferate first before peak enzyme production. After 270 days, significant differential taxa included Basidiomycota (Lyophyllaceae and Hydnodontaceae) and Ascomycota (Helotiaceae, Pyronemataceae, and Hypocreaceae). Genera such as *Helicodendron, Lecanicillium, Chalara*, and *Trechispora* contributed most to lignin content, lignin/N ratio, and LiP activity, suggesting involvement in lignin decomposition. This succession likely accelerated lignocellulose breakdown, supporting the peak decomposition periods: cellulose and hemicellulose at 180-270 days and lignin after 270 days. Findings align with reports of Dothideomycetes and Sordariomycetes as efficient lignocellulose decomposers producing substantial enzymes (Pandit et al., 2016; Yu et al., 2017).

Forest ecosystems can vary due to differences in stand structure, litter properties, and seasonal changes, and these differences may affect the diversity of bacterial and fungal communities (Bai et al., 2024; Chaparro et al., 2014; Ren et al., 2016; Sheng et al.,

2017). In our study, litter chemical property is one of the most factors to microbial communities. C, N, C/P ratio, Mg, and Mn significantly influenced bacterial and fungal abundance. Specifically, N most strongly influenced *Chloroplast* relative abundance; Ca content most affected *Pseudomonas* and *Pedobacter;* K, C, C/P, and Mg most affected *Pedobacter* and *Dyadobacter*; Mn most affected *Lechevalieria*, *Niastella*, and *0319-6G20*; and P content most influenced *Sphingomonas, Hymenobacter, Methylobacterium*, and *Chloroplast*. This reflects the essential role of litter-derived nutrients in microbial growth, metabolism, and community structure, with C and N widely recognized as key drivers (Zeng et al., 2019a; Zhalnina et al., 2015; Zhang et al., 2016). Phosphorus release promotes microbial proliferation and accelerates decomposition (Alfredsson et al., 2016; Pourhassan et al., 2016). Calcium, a vital component of fungal cell walls, influences fungal biomass formation (Yue et al., 2021). Magnesium significantly affects both bacterial and fungal abundance during decomposition, highlighting its crucial regulatory role (Keiluweit et al., 2015; Yue et al., 2021). Manganese, integral to MnP (a key lignin-degrading enzyme), directly regulates the metabolic processes of lignin-degrading microbes (Lovett et al., 2016). Overall, the chemical properties of litter fundamentally govern microbial community dynamics and decomposition processes.

Microbial diversity (Shannon index) initially increased and then decreased (Santonja et al., 2018). Across pure and mixed forest types, early in decomposition, abundant nutrients, especially soluble C and N, supported microbial proliferation and increased diversity (Purahong et al., 2015; Zeng et al., 2019b). Later, depletion of labile substrates

reduced community richness, shifting the community toward specialists decomposing recalcitrant material, which decreased diversity (Alfredsson et al., 2016; Bani et al., 2018).

4.4 Influence of Microbial Communities on Litter Decomposition across Pure and Mixed Forest Types

In forest ecosystems, although the decomposition of litter is affected by various factors, it is mostly a response to the comprehensive effects of many ecological factors under the action of microorganisms (Aerts, 1997; Berg and Bjorn, 2014; Bradford et al., 2016). In this study, we have found that the litter decomposition rate is significantly positively correlated with bacterial community richness index and diversity index (Fig. 9a), indicating that high bacterial richness and diversity promote litter decomposition (Song et al., 2014; Wang et al., 2020). To further elucidate the mechanisms by which microbes drive litter decomposition, our PLS-PM analysis revealed that microorganisms directly influenced litter weight loss rate with a substantial path coefficient (direct effect = 0.79). Additionally, microbes indirectly affected litter weight loss rate by influencing enzyme activities, which subsequently altered litter organic matter composition (Fig. 11). Diversity indices also correlated positively with lignocellulolytic enzyme activities and lignin content, but negatively with cellulose and hemicellulose content. Significant positive correlations between β-glucosidase/LiP activity and litter weight loss rate further indicate that higher microbial diversity and abundance enhance enzyme production, lignocellulose degradation capacity, reduction of cellulose and hemicellulose, accumulation of lignin (relative content), and ultimately

accelerate decomposition (Allison and Vitousek, 2010; Margida et al., 2020; Song et al., 2014).

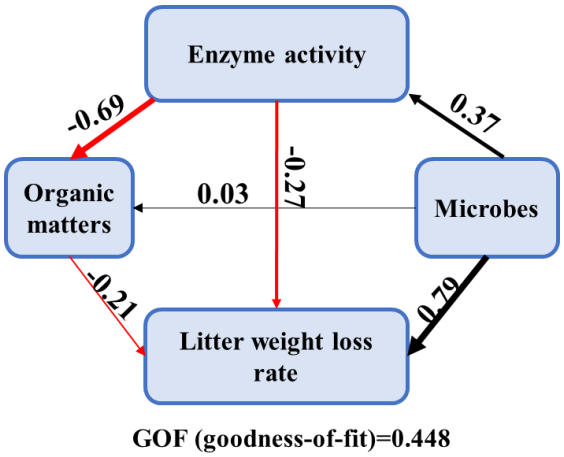


Fig. 11 Effect of microorganisms on litter decomposition.

As a common silvicultural type, mixed forests can improve forest community structure and enhance forest ecosystem service functions; they also induce distinct changes in litter organic carbon, total nitrogen, total phosphorus, as well as soil temperature and soil moisture, which in turn affects microbial diversity and consequently influences litter decomposition rate (Brockett et al., 2012; Liu et al., 2016). Most studies have demonstrated that, compared with pure forests, mixed forests tend to increase environmental heterogeneity and provide diverse substrates for microorganisms, thereby elevating microbial diversity and accelerating litter decomposition rate (Bai et al., 2023; Liu et al., 2016; Porre et al., 2020; Wang et al., 2020; Zhang, 2019). However, some studies found that the mixing of certain litters could exert antagonistic effects on microorganisms, reducing microbial diversity and inhibiting decomposition processes (Hooper et al., 2000; Mukamparirwa et al., 2024). In contrast, mixed forests had no direct impact on microbial diversity, and their

influence on litter decomposition rate was insignificant (Marco et al., 2011; Porre et al., 2020; Zhang et al., 2019). Existing research has indicated that litter decomposition rate in mixed forests is higher than that in pure forests, which might be attributed to differences in the richness and diversity of microbial communities caused by variations in litter quality (Wang et al., 2020; Zhang et al., 2019). Compared with pure forests, mixed forests have more diverse and abundant soluble nitrogen sources. These characteristics can provide rich and varied substrates for microorganisms, increase microbial diversity, and thus promote litter decomposition rate (Bai et al., 2024; Zhang et al., 2019). Consistent with this, the mixed stand exhibited higher endoglucanase, exoglucanase, and xylanase activities, higher microbial diversity indices after 270 days, lower cellulose and hemicellulose contents, and consequently faster litter weight loss rate compared to pure stands.

5 Conclusions

Bacterial and fungal diversity varied significantly with forest type and decomposition time. Dominant bacterial phyla included Proteobacteria, Bacteroidetes, and Actinobacteria, whereas dominant fungal phyla were Ascomycota and Basidiomycota. Cellulose and hemicellulose degrading taxa at the early decomposition stage included *Sphingomonas, Hymenobacter, Pseudomonas*, *Pedobacter, Spirosoma, Nothophoma, Cladosporium, Septoria*, and *Alfaria,* while lignin-degrading taxa after 270 days comprised *Amycolatopsis, Streptomyces, Helicodendron, Lecanicillium, Chalara*, and *Trechispora*. Moreover, total C, N, P, C/P ratio, Ca, and Mn significantly influenced microbial abundance.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Both bacterial and fungal microbiome data have been submitted to the NCBI Sequence Read Archive, with the corresponding accession numbers PRJNA1347395 and PRJNA1347422.

Competing Interest

There are no competing financial interests or personal relationships that could influence this research work.

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Author contributions and acknowledgments

The contributions of each author are as follows:

K.Y. conceived the overall research idea and took charge of revising the manuscript.

Z.L. designed all experimental procedures; collaborated with Z.H. to perform experiments, complete investigative work, and collect samples; additionally analyzed experimental data and drafted the manuscript.

Z.H. participated in experimental execution, investigative work, and sample collection.

W.X., Z.X., L.K., D.K., S.Y., and Z.Q. provided comments and suggestions to optimize the article content.

All authors made contributions to the research and manuscript, and collectively approved the final version submitted for publication.

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