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Temporal dynamics of nutrient release from mulching of legume roots and shoots litter driven by microbial community during decomposition in organic orchards

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Abstract

Grass residue decomposition is crucial for nutrient cycling in agro-ecosystems, enhancing nutrient utilization efficiency and supporting sustainable crop management. While grass mulching has been widely studied for improving orchard soil fertility, the role of soil microbial communities in decomposing different plant organs remains unclear. Before decomposition, the aboveground and belowground plant parts were harvested and placed in separate litterbags, which were later used for evaluating the decomposition rate and chemical characteristics of the shoots and roots for 40 days (at 10 days intervals). The changes in soil fertility, soil microenvironment, soil microbial community were measured after 0, 1 and 3 months, alongside analysis of key microbial taxa under different residues treatments. The remaining mass of root litter treatment was significantly higher than that of other treatments by 72.97%, 17.53% during 1–10 days and 30–40 days, respectively. During the 40-days period, the release of potassium (K) from root litter reached 58.61%, and the decomposition of lignin was recorded at 56.94%, whereas the release of carbon (C), nitrogen (N), and phosphorus (P) remained relatively stable. Despite no significant changes in nodes, edges, and links at 30 and 90 days, the co-occurrence network of root litter exhibited modularity values of 0.774 and 0.773, respectively. The values were higher than those observed in random networks, indicating the presence of functional modules and enhanced stability within the root microbial community. Litter organs enhanced decomposition rates by positively influencing soil fertility and keystone microbial decomposers, while its soil microenvironment affects decomposition rates. Despite its recalcitrance, the chemical composition of root litter plays a key role in regulating soil microbial community structure and improving soil fertility, thereby maintaining orchard ecosystem functionality.

Keywords Litter decomposition, Mulching grass, Soil microbial community, Network analysis, Organic orchards

Introduction

Litter decomposition releases essential nutrients that promote crop growth [1], sustain biodiversity [2, 3], enhance soil fertility [4], and contribute to the global carbon balance [5]. Fine roots and shoots serve as essential elements of plant residual inputs, and are the primary sources returning carbon and nutrients into the topsoil [6, 7], influencing nutrient cycling rates [8] in orchard ecosystems. Fine roots (diameter < 2 mm), being the most important component in

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the biogeochemical cycling and energy turnover with underground agro-ecological systems [9], are highly sensitive to changes in soil environmental parameters, which impact their biomass, growth form, respiration and turnover rate [6]. Roots typically exhibit higher lignin or cellulose concentrations and possess greater specific root length (SLR), resulting in an increased surface area for nutrient absorption [2]. In contrast, shoots generally possess a greater concentration of soluble C compounds and have a higher specific leaf area (SLA) [10]. Due to their accessibility for observation, the decay process of shoots has been more extensively studied [11, 12]. Recent studies have shown that chemical compositions of plants vary between roots and shoots [2, 4], indicating that plant biomass allocation is a significant factor influencing plant decomposition and SOC storage [13, 14]. The combined impact of residual organs on decomposition has not been widely studied, especially regarding the decay patterns of fine roots and their nutrient release, which are less understood compared to shoots.

The decomposition process of grass residues in forest ecosystems has been widely studied [15, 16]; however, it remains relatively underexplored in organic orchards. The release of nutrients from decomposed residues in orchards can fulfill the requirements of sustainable fruit production while reducing dependence on the synthetic fertilizers [17]. Thus, nutrients released from litter decomposition are a crucial addition to organic orchards [18], with their contribution depending on the quantity of nutrients returned to the soil and the dynamics of their release. Mulching leguminous pastures provides a high-quality material characterized by rich nutrients [19] and resilience to drought and cold conditions. Additionally, it promotes root nodules that facilitate nitrogen fixation through symbiotic bacteria, thus minimizing the need for costly chemical fertilizers [18, 20]. Previous studies have shown that leguminous pasture litter, due to their lower C/N ratios, decomposes easily and contributes more nutrients to the soil (particularly nitrogen) than non-leguminous litter in diverse cropping systems [21]. Many researchers investigated the mechanisms of litter decay at the species level in herbaceous ecosystems, including grass-legume intercropping and monocultures of pastures [22, 23]. While these studies have significantly enhanced the scientific understanding of the pattern and fundamentals of species-level decomposition processes, the comprehension of these effects at the organ level remains lacking. Since leaves and stems are the basic components of most herbaceous species, studying the decomposition process at the organ level will significantly enhance the understanding of litter decomposition and nutrient cycling in green manure management practices.

Decomposition is a complex process governed by a number of factors including climate, soil physicochemical properties, decomposing organisms, as well as the quality and chemical traits of litter [4, 11]. Soil micro-environment related factors, such as soil porosity, pH and soil water content, primarily affect residue decomposition and nutrient cycling by regulating microbial succession and functionality in soil [15, 24, 25]. Studies on soil nutrient changes in orchards have mainly focused on macroelements such as C, N, and P, whereas the microelements during decomposition have received less attention. For instance, manganese (Mn) is essential for the synthesis of manganese peroxidase (MnP) [26], which significantly influence the degradation of lignin and humification products. The previous research has demonstrated that the chemical properties of root and shoot litter influence the composition and structure of microbial community during initial decay [27]. Despite progress in understanding litter decomposition, the interactive effects of chemical traits and microbial mechanisms on decomposition remain unclear. Investigating the differences between fine roots and shoots is crucial for understanding how these factors influence nutrient cycling and utilization efficiency in organic orchards, as well as organ-level decomposition mechanisms within orchard ecosystems.

Co-occurrence networks, with dominant OTUs as nodes and microbe–microbe interactions as edges [26], quantify and visualize intricate correlations and assembly patterns with microbial communities, thereby facilitating the evaluation of their complexity and stability. Keystone taxa play a crucial role in modulating bacterial and fungal co-occurrence patterns, thereby driving residue decomposition [29, 30]. Previous research has demonstrated that key species interact with soil nutrients, affecting their availability and distribution, which in turn alter the stability and function of soil microorganisms [16, 31]. However, the significance of keystone taxa in litter decomposition is still not well understood. Despite many studies assessing the role of soil microorganisms in litter decomposition [14, 32], the topological characteristics of microbial co-occurrence networks and the keystone taxa during residue decomposition process remain unclear.

The objectives of this research were to (1) evaluate decomposition rates and litter chemical traits of sweet clover (*Melilotus officinalis*) from root, shoot and their mixed substrates, (2) determine variations in microbial communities among decomposing fine roots, shoots, and mixed litter to assess their roles in decomposition efficiency, and (3) examine the influence of litter chemical traits, soil microenvironment, soil fertility and microbial decomposers on their interrelationships. Therefore, this study systematically examines the differences in litter decomposition dynamics among different litter organs of

sweet clover, focusing on enzyme activities, microenvironment factors, and interactions between litter chemical diversity and the specific pathways through which these litter organs influence decomposition. We hypothesized that (1) the decomposition of sweet clover residues at the organ level affects soil properties, nutrient availability, soil microenvironment, and soil enzymatic activity; (2) microbial community composition and structure exhibit significant temporal variations during the decomposition of fine roots, shoots, and mixed litter; and (3) keystone microbial taxa significantly influence organ-level litter decomposition rates. The findings of this study will provide a robust theoretical basis for promoting sustainable agriculture in local and organic orchards, particularly in the management practices of mulching pastures.

Material and methods

Description of study site and experimental design

The experiment was conducted in the spring of 2023 at Baiheng Organic Orchard Co., Ltd., located in Shangwan Village, Wuquan Town, Yangling, Shaanxi (107°59'E, 34°3'N; altitude, 540.1 m above sea level), within a 12-year-old international standard organic kiwifruit orchard. The region experiences high solar radiation with 2163 h of annual sunshine, and 211 days of frost-free. The annual mean temperature is 12.9 °C, and annual precipitation ranges from 635.1 to 663.9 mm, providing optimal conditions for the growth and development of kiwi trees. The soil at the experimental site is sandy loam (15% sand, 60% silt and 25% clay), classified as Aqualfs according to the USDA textural classification system. Seeds of a locally adapted sweet clover variety in Shaanxi Province were sown at a rate of 45.0 kg ha^{-1} between the rows of kiwi trees. Clover seeds were acquired from Yangling Lvbo Seed and Seedling Company. No fertilizer was applied throughout the growing period, except for the sweet clover litter.

The in-situ litter decomposition experiment

A field-based experiment was conducted to analyze the surface soil decomposition of residues using the litterbag technique. The mixture ratios for shoots and fine roots were determined based on the fresh biomass yield of aboveground and belowground parts from the final cut of sweet clover. This study examined the litter dynamics of mulching grass terminated by mowing, with the objective of simulating agricultural practices to improve soil quality through crop residues. For each treatment, four independent plots, each measuring 2.8 × 50 m, were established and considered as replicates. A 2 m buffer zone was established between adjacent plots, covered with nylon bags. At each sampling interval, twelve litterbags were collected from three plots for analysis.

Fine root samples were collected from the soil using a shovel and subsequently rinsed to remove the adhering soil particles. For stem and leaf litter, samples were collected from fresh aboveground plants. Overall, three grass mulching ratios were used over a duration of 40 days: 100% leaf and stem litter (Shoot litter), 100% fine root litter (Root litter), 80% leaf and stem litter + 20% fine root litter (Mixed litter). Each litter sample was placed in a labeled nylon bag, sealed, and then buried in the soil to represent realistic litter decomposition. Before placing the nylon bags, the soil surface was cleared of dead branches and leaves. The preliminary chemical characteristics of sweet clover residues are presented in Table 1.

The decomposition rate of pasture mulching was considerably faster during the initial 40 days compared to the later stages [17]. Therefore, the decomposition rate of sweet clover litter was evaluated over a 40-day period, divided into four intervals: 1–10 days, 10–20 days, 20–30 days, and 30–40 days. Soil sampling was carried out simultaneously with the sampling of sweet clover litter in the nylon bags, along with an additional sampling at 90 days of decomposition. Soil samples were collected randomly from the soil cores adjacent to each litterbag following a five-point sampling method at 10, 20, 30, 40, and 90 days. Blank soil treatment (CK0) referred to the soil samples collected before the application of mulching. Each soil sample (16 treatments × 4 replicates) was filtered through a 2 mm mesh and then separated into three parts. The first part was dried to assess soil water content (SWC), the second part was stored for analyzing soil enzyme activities and physicochemical properties, and the third part was kept for later high-throughput sequencing.

Table 1 Initial chemical characteristics of the root, shoot and the mixed litter of sweet clover

Litter type	TC mg/g	TN mg/g	TP mg/g	TK (%)	Lignin (mg/g)
Root litter	350.61 ± 14.30a	15.55 ± 0.51c	3.61 ± 0.19a	41.96 ± 2.51a	70.78 ± 4.92a
Shoot litter	390.88 ± 25.02a	38.46 ± 0.79a	4.01 ± 0.09a	46.46 ± 1.10a	53.37 ± 2.86a
Mixed litter	358.58 ± 12.81a	27.64 ± 5.29b	3.48 ± 0.61a	42.27 ± 5.14a	69.17 ± 1.18a

Analysis of soil physicochemical properties and enzymes activity

Soil organic carbon (SOC) was determined following the K_2CrO_7-H_2SO_4 oxidation method. Soil pH was determined according to the potentiometric method, using water as the extraction agent at a soil-to-water ratio of 1:2.5. Bulk density (BD) was calculated as the ratio of the dry soil weight (g) to the volume (cm^3) of the cutting ring. The nitrate nitrogen (NO_3^- -N) and ammonium nitrogen ($NH4^+$-N) were extracted using the AA3 continuous flow analyzer (SEAL Company, Germany), while available phosphorus (AP) was extracted using $NaHCO_3$ and measured with the same analyzer [33].

Soil enzyme activity was determined following the previous method described by Guan [34]. Polyphenol oxidase (PPO) activity was quantified following the iodine titration, cellulase (CEL) activity by nitrosalicylic acid colorimetry, and urease (URE) activity following the indophenol colorimetry with urea. The catalase (CAT) activity was determined by titration with the potassium permanganate, alkaline phosphatase (ALP) with disodium phenyl phosphate colorimetry, and sucrase (SAC) by 3,5-dinitrosalicylic acid colorimetry.

The concentrations of Zn, Cu, Fe, and Mn were quantified using flame atomic absorption spectrophotometry following a 2-h digestion with DTPA-$CaCl_2$-TEA at 35℃ by a Graphite Furnace Atomic Absorption Spectrometer (Perkin Elmer, PinAAcle 900T).

Plant nutrient analysis

The samples of sweet clover in the litterbags were washed and subsequently dried to a constant weight at 75 °C. The residues were crushed and passed through a 0.15 mm sieve, and used for quantifying total carbon (TC), nitrogen (TN), phosphorus (TP), and potassium (TK). The concentrations of TC, TN, TP, and TK were analyzed following the methods outlined by Lu [35]. Lignin concentrations were determined using a Velp-fiwe6 fiber analyzer (FIWE6, VELP, Italy) [36].

High-throughput sequencing

The DNA samples were obtained from 0.5 g of fresh soil at 0, 30, and 90 days, following the instructions of the OMEGA Soil DNA Kit (D5635-02). The V3–V4 region of the bacterial 16S rRNA gene was amplified with the 341F/806R primer set [37], and the ITS1 segment of fungal rRNA gene was amplified with the ITS5/ITS2 primer set [38]. The PCR amplification was conducted following the standard methods described by Ge et al. [39]. Sequencing libraries were performed using the TruSeq Nano DNA LT Library Prep Kit from Illumina. The libraries were subsequently assessed with the Agilent High Sensitivity DNA Kit on an Agilent Bioanalyzer. Raw paired-end sequencing of two regions (2×250 bp) in PCR products was conducted on the Illumina NovaSeq platform to generate raw reads performed by Shanghai Personal Biotechnology Co. Ltd. Sequence processing included primer trimming (qiime cutadapt trim-paired), denoising, and chimera removal (DADA2 via qiime dada2 denoise-paired).

Singleton ASVs were excluded after merging ASV tables. ASVs were classified using the classify-sklearn naive Bayes taxonomy classifier from the feature-classifier plugin, referencing the SILVA Release 132 and UNITE Release 8.0 databases.

Statistical and network analysis

The percentage of remaining mass was calculated after each decay period compared to the initial mass (M_0), with Mt representing the dry mass following the decomposition time, using the formula:

$$Remaining\ mass\ (\%) = M_t/M_0 \times 100\% \quad (1) \qquad (1)$$

The remaining litter mass after each decay period was modeled using a single exponential decay model, where k value representing the decay rate constant [40]:

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

Two-way repeated measures ANOVA was conducted with decomposition time as the repeated factor to assess the effects of litter types (shoot, root, and mixed litter) and their interactions on mass in IBM SPSS 27.0. One-way ANOVA was employed to analyze the litter decomposition rate (k value), soil properties, and plant nutrients, followed by LSD's multiple range test ($\alpha = 0.05$).

The alpha diversity (Shannon index and Pielou's evenness) was calculated in QIIME2. Non-metric multidimensional scaling (NMDS) was used to assess significant differences in microbial community at the phylum level among treatments using permutation test, with the 'adonis' function provided by the 'vegan' R package. Pearson's correlation and the Mantel test were visualized using the 'dplyr', 'ggplot2', and 'linkET' R packages. The co-occurrence network utilized the Pearson correlation coefficient ($P \leq 0.05$) of log-transformed OTU abundances detected from all replicate samples ($n = 4$). A threshold of 0.80 was established for microbial networks in each treatment using the Robust Multiple Threshold (RMT) method [30]. Only correlations with $|r| > 0.8$ or $P < 0.01$ were displayed in the network, ensuring the inclusion of only highly significant and strong

correlations. In the Interactive Gephi platform (version 0.10), topological characteristics of the networks were calculated including the number of nodes, positive/negative links, edges, average clustering coefficient (ACC), density, diameter, average path length (APL) and relative modularity. Node topologies were determined using the 'hmisc' and 'igraph' R packages, applying boundary values for Zi and Pi (2.5 and 0.62, respectively) [31]. The random networks were performed by using the ' igraph ' package, which involved iterating the nodes and links of the empirical network 1000 times and comparing the results with those of the empirical networks. Redundancy analysis (RDA) was conducted using Canoco 4.5, with Monte Carlo permutation test. PLS-SEM models were developed using the 'plspm' package to identify potential pathways associated with litter decomposition effects. RDA combined with the Mantel test was conducted to identify key variables influencing the decomposition rate for inclusion in the PLS-SEM. The total, direct and indirect effects of environmental factors on decomposition rate were calculated. Graphs were generated using Origin 2024 (Origin Lab Corp. USA).

Results

Decomposition and nutrient release from different residue organs of sweet clover

The ANOVA results showed significant effects of the litter composition on the remaining litter mass(Fig. 1). At 0–10 d and 30–40 d, the decomposition rate of root litter was significantly lower ($P<0.05$) compared to that of shoot and mixed litter. At the final decomposition stage (40 days), the remaining mass proportions of root, shoot and mixed litter were 29.77%, 17.53% and 17.70%, respectively. Following the natural logarithm of remaining mass and decomposing time, the decomposition rates for different litter organs were calculated, and the time required to achieve 95% ($T_{0.95}$) and 50% ($T_{0.5}$) decomposition of litter was determined. The duration required for 50% ($T_{0.5}$) and 95% decomposition ($T_{0.95}$) of root litter was 19.25 and 66.48 days, whereas the duration for shoot litter was 15.73 and 62.47 days, while for mixed litter was 15.07 and 61.58 days, respectively.

The three litter types exhibited significant variations in nutrient concentration and initial organic chemical composition (Table 1), particularly in total nitrogen content. Statistically, no significant difference in C, P, K and lignin content was observed between root litter and shoot litter. Table S1 represents the details regarding nutrient release rates. During the 0–40 days of decomposition, the total stocks of C, N, P, K and lignin in different plant organ litter decreased over time (Table S2). The C:N ratio of shoot and root litter revealed a predominantly increasing trend over the decomposition period, whereas a decreasing trend was observed in the mixed litter.

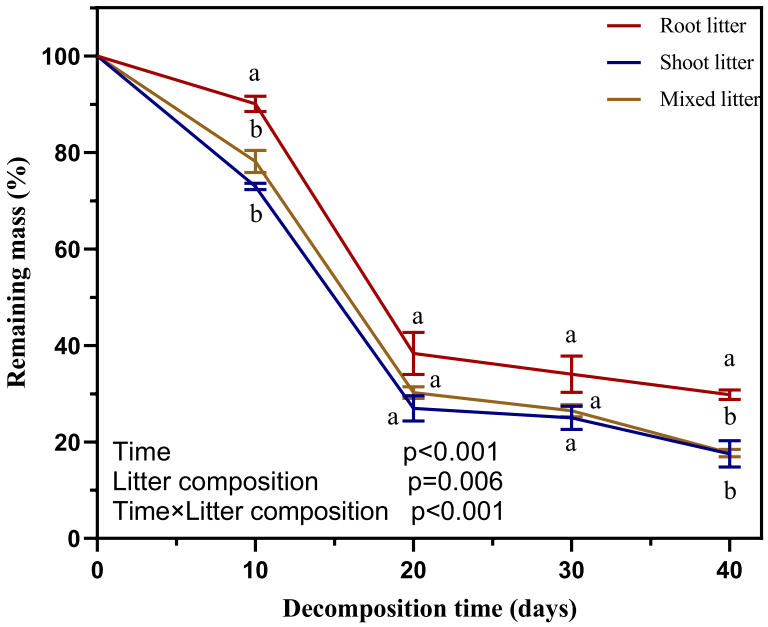

Fig. 1 The remaining mass proportion for the three litter types through time (days). Significant differences among treatments at each decomposition time are denoted by different lowercase letters according to the LSD multiple range test ($P<0.05$)

Effects of litter from different sweet clover parts on soil physicochemical properties and enzyme activity

The application of sweet clover litter significantly improved soil biochemical properties ($P < 0.05$; Table S3). Throughout the litter decomposition period, soil pH in both shoot litter and mixed litter treatments exhibited a consistent trend over time, reaching peak values of 8.39 and 8.36 during 31–40 days, respectively. Soil water content in mixed litter was higher than that in root litter during the 0–20 day period, with a significant difference also observed at 90 days. The NO_3^- -N and NH_4^+-N content increased with root litter and was greater than that in shoot litter and mixed litter at 0–10 days. In contrast, the concentrations of AP content in shoot litter and mixed litter exhibited a similar trend over time; however, AP content was observed to accumulate more rapidly in shoot litter than in mixed litter. The TC content remained relatively stable across different litter organs throughout the decomposition period.

The Mantel test was conducted to identify the primary factors affecting the structure of microbial community (Fig. 2). At the two decomposition sampling intervals (30 days and 90 days), the soil pH, SWC, and SAC significantly affected the composition of bacterial community($P < 0.01$), whereas no critical environmental factors exhibited a strong relationship with fungal community composition ($P < 0.01$). Furthermore, the PPO, SOC and NO_3^- -N showed significant impact on the dynamics of bacterial community composition($P < 0.05$), whereas BD and NO_3^--N had strong effect on the fungal community ($P < 0.05$). The bacterial community showed a stronger association with soil environmental factors than the fungal community composition. At 30 and 90 days of decomposition, a consistent correlation between microbial community composition and soil properties was evident.

Effects of litter from different sweet clover organs on the microbial diversity and community composition

The structure and composition of soil microbial decomposer communities, shaped by litter organs and decomposing time, significantly impact their functional capabilities and associated enzymatic activities. After normalizing the raw sequence reads, 93.2% (2,804,672/3,010,561) of bacterial and 92.3% (3,512,350/3,804,336) of fungal sequencing were accepted for microbiome data analyses, with means of 100,166 ± 38,302 and 125,441 ± 13,589 sequences for each sample, respectively. A total of 71,668 bacterial and 3,377 fungal OTUs were identified across all treatments based on high-quality readings. The alpha diversity analysis, using Pielou's evenness and Shannon index revealed an increase in the microbial diversity of bacteria and fungi during the decomposition of litter organs (Fig. S3). The mixed litter markedly increased the alpha richness of both bacteria and fungi ($P < 0.05$). Specifically, the Simpson index for fungal and bacterial communities was notably higher at 90 days, while fungal alpha diversity showed a significant increase at 30 days compared to the CK0.

The litter types and decomposition time both significantly influenced the composition of soil microbial communities (Fig. 3). The dominant phyla and community structure of litter fungi and bacteria exhibited significant changes with increasing decomposition

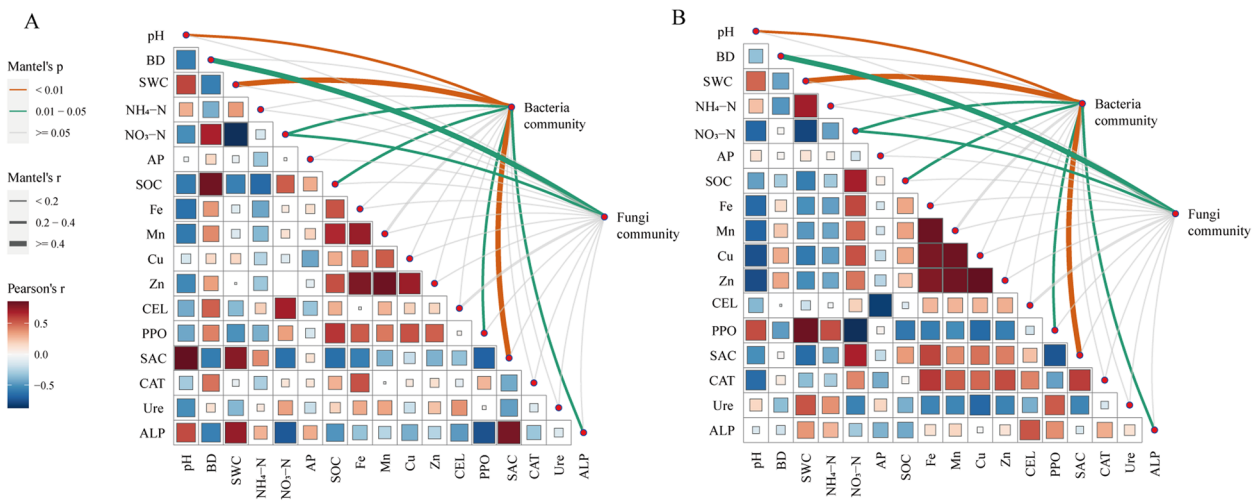

Fig. 2 Mantel test between microbial community and environmental factors at 30 days (**A**) and 90 days (**B**). Line width reflects the Mantel's r statistic, while line color indicates statistical significance (orange for $P < 0.01$; green for $0.01 < P < 0.05$; gray for $0.05 \leq P$). Environmental factors are compared pairwise, with the Pearson's correlation coefficient indicated by the color gradient and the size of the squares

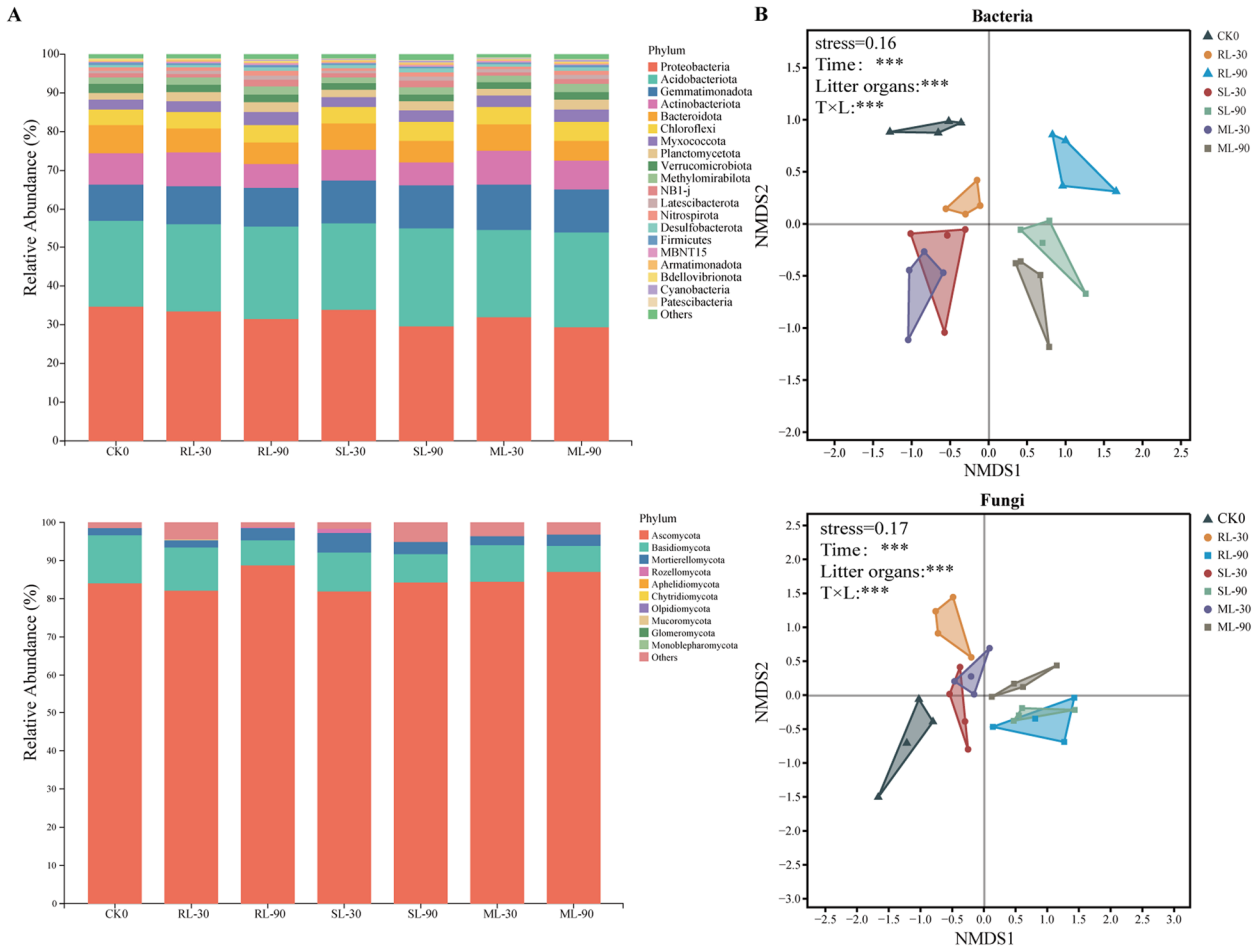

Fig. 3 Composition of bacterial and fungal taxa (**A**) at the phylum level and NMDS of community (**B**). *** indicates significant impacts on microbial community structure due to litter organs, decomposition time, or their interactions ($P < 0.001$, Adonis). Treatments including CK0 (the blank soil), RL (root litter), SL (shoot litter) and ML (mixed litter). Soil samples were taken at 0, 30 and 90 days post-residue addition

time (Fig. 3A). Throughout the decomposition period, Proteobacteria, Gemmatimonadota and Acidobacteria dominated the soil bacterial communities (Fig. 3A, B), accounting for about 70% of the total abundance across treatments. The analysis of fungal community revealed a decline in the relative abundance of Ascomycota decreased at 30 days, with values of 81.22% in shoot and 81.90% in root litter, compared to 84.07% in CK0. In contrast, Basidiomycota significantly decreased from 12.49% (CK0) to 6.67% (ML-90 and RL-90). Beta-diversity was analyzed using NMDS scatter plots, which indicated the differences in community structure among treatments (Fig. 3B). The NMDS scatter plots revealed significant clustering of bacterial and fungal community structures based on the litter types and decomposition time.

Effects of litter from different sweet clover organs on co-occurrence networks and keystone taxa

Networks were built based on the community succession at 0, 30 and 90 days, and subsequently clustered into modules to represent shifts in the co-occurrence patterns of microbial communities with the decomposition (Fig. 4). All nodes were categorized into seven major modules across different species, indicating that significant modularity within the network. The application of litter resulted in a significant increase in the number of nodes and edges within microbial networks as compared to the CK0 (Fig. 4A). The comparison of co-occurrence networks among treatments indicated that litter-induced microbial changes resulted in an increase in the number of nodes and edges (Fig. 4A). The shoot litter exhibited the highest number of nodes and edges on the 90th day,

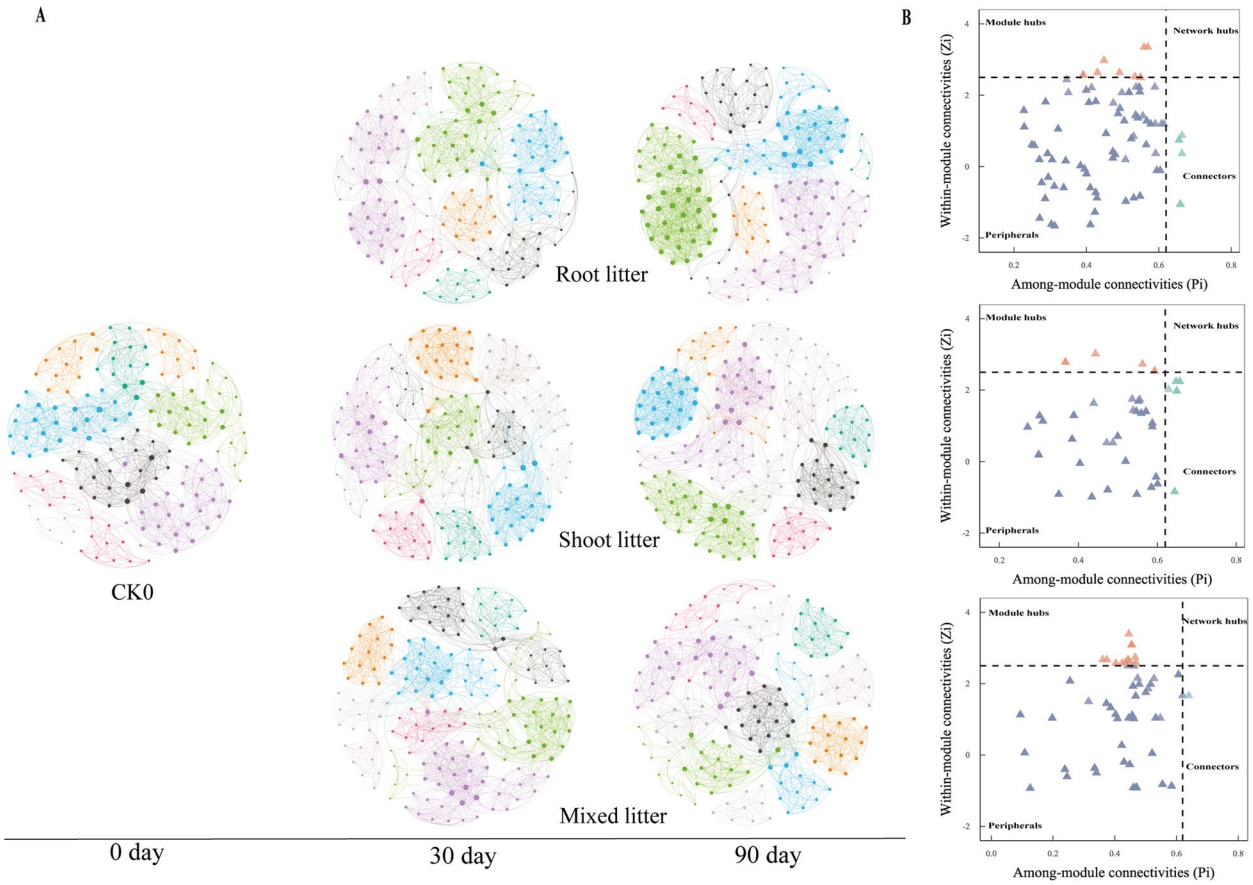

Fig. 4 **A** Co-occurrence networks of microbial communities and **B** Identification of keystone taxa in litter organs ($n=4$). Different colors in microbial networks indicate different modules; the node size reflects its degree of connectivity. The comprehensive details are available in Table S4-S6

particularly the positive edges (Table 2). Moreover, the majority of keystone species in shoot litter treatment were identified as bacterial phyla such as Acidobacteriota, Gemmatimonadota, Chloroflexi, Myxococcota and Proteobacteria, with fungi remaining unassigned (Table. S5).

Zi-Pi plots were used to determine the topological functions of nodes in the co-occurrence network, categorized as network hubs, module hubs, and connectors, all exhibiting high connectivity with other network modules (Fig. 4B). Additionally, the peripherals included most OTUs. The module hubs and connectors were identified in three litter types based on their topological roles; however, no network hubs were detected (Fig. 4B). The taxonomic information for the keystone taxa is provided in Supporting Material (Table S4-6). The connectors and most of the module hubs were identified as bacteria, highlighting the dominance of bacteria in the decomposition of pasture residues within farmland soil.

The topological features of the networks are presented in Table 2. The average path length (APL) ranged from 2.900 to 4.667, approximately corresponding to the logarithm of the total number of nodes in the networks. This indicates that the microbial ecological networks (MENs) in both bacterial and fungal communities exhibit small-world properties, signifying that network nodes are generally closely interconnected. Additionally, the ACC and APL for the empirical networks significantly differed from those of the randomized networks. The higher values observed for the empirical networks highlight their small-world, typical hierarchical and modular characteristics (Table 2). Overall, the topological properties revealed that the microbial network indices for soil with root and shoot litter differed at 30 and 90 days.

Path analysis for decomposition of litter from different sweet clover organs

The RDA analysis was conducted to identify key factors influencing the decomposition rate, followed by path analysis, focusing on the interactions among soil microbial communities, enzyme activities, soil properties, litter quality, and decomposition rate (k value) (Fig. 5). The RDA analysis identified six dominant bacterial phyla in the soil samples, including Chloroflexi,

Table 2 Topological features of empirical and randomized networks from microbial communities

Microbial community		Empirical networks									Random networks		
Time	Treatments	Nodes	Edges	Positive links	Negative links	ACC	APL	Diameter	Density	Modularity	ACC	APL	Modularity
0d	CK0	161	1086	856 (78.82%)	203 (21.18%)	0.720	4.172	10	0.091	0.690	0.077 ± 0.004	2.283 ± 0.004	0.233 ± 0.006
30d	Root litter	168	1177	942 (80.02%)	235 (19.98%)	0.816	4.136	8	0.090	0.744	0.080 ± 0.004	2.238 ± 0.004	0.222 ± 0.006
	Shoot litter	183	1341	1086 (81.28%)	255 (18.72%)	0.860	4.548	11	0.086	0.794	0.079 ± 0.003	2.215 ± 0.003	0.213 ± 0.005
	Mixed litter	183	1354	1106 (81.73%)	248 (18.27%)	0.852	4.427	10	0.087	0.773	0.077 ± 0.003	2.237 ± 0.003	0.217 ± 0.006
90d	Root litter	168	1142	878 (76.92%)	264 (23.08%)	0.863	4.478	11	0.087	0.773	0.075 ± 0.007	2.293 ± 0.004	0.232 ± 0.007
	Shoot litter	195	1547	1284 (82.97%)	263 (17.03%)	0.427	2.900	6	0.085	0.460	0.077 ± 0.003	2.213 ± 0.003	0.209 ± 0.005
	Mixed litter	174	1457	1145 (77.66%)	330 (22.34%)	0.843	4.667	11	0.103	0.689	0.092 ± 0.003	2.120 ± 0.003	0.199 ± 0.005

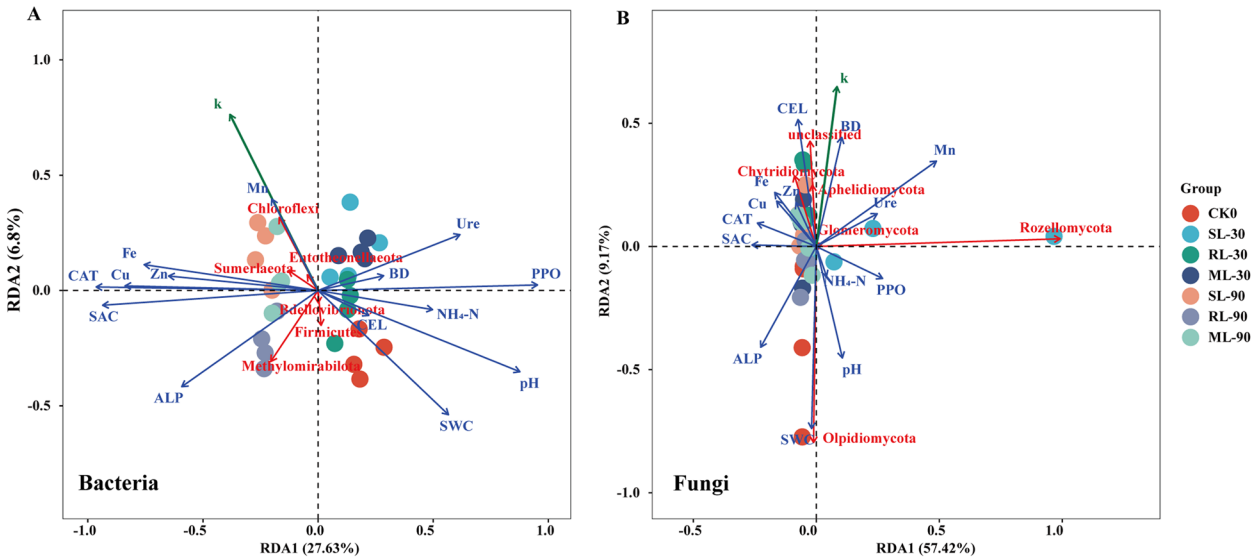

Fig. 5 Redundancy analysis (RDA) of soil bacterial (**A**) and fungal (**B**) community. Treatments include CK0 (blank soil), RL (root litter), SL (shoot litter), and ML (mixed litter), with soil samples collected at 0, 30, and 90 days post-residue addition. PPO: Polyphenol oxidase, CEL: Cellulase, Ure: Urease, CAT: Catalase, ALP: Alkaline phosphatase, SAC: Sucrase, SWC: Soil water content, BD: Bulk density

Methylomirabilota, Sumerlaeota, Firmicutes, Entotheonellaeota and Bdellovibrionota. In addition, Rozellomycota, Olpidiomycota, Chytridiomycota, Aphelidiomycota, Glomeromycota and unclassified taxa in the fungal phyla were also identified in soil sample. The results revealed that the k value, SAC, PPO, CAT, SWC, pH and ALP exhibited a positive correlation with the bacterial community, while only Mn, CEL and the k value were positively associated with the fungal community. The most effective bacterial indicators were the decomposition rate (k), pH, and the activities of CAT, and PPO. The decomposition rate exhibited a positive correlation with soil microelements (Mn, Fe, Zn, and Cu).

The decomposition rate significantly influenced the community composition of litter microbes, especially within the fungal community. The Chloroflexi within the bacterial community showed a strong positive correlation with the k value and Mn, while showing a negative correlation with SWC, ALP and CEL. Methylomirabilota had significant positive correlations with ALP, SAC, SWC, CAT, Cu, Fe, and Zn, while showing negative relationship with pH, PPO, BD, k value, Ure, and Mn. Among fungi, Aphelidiomycota and unclassified fungi were positively linked to the k value and CEL, while negatively associated with pH and SWC. RDA1 and RDA2 explained 35.4% and 26.11% of the total variation of fungal community, indicating that RDA effectively illustrates the relationship between decomposition rate and microbial community, soil physicochemical properties and enzyme activity (Fig. 5B).

The PLS-SEM indicates that the decomposition of litter was primarily affected by different litter organs through three main pathways (Fig. 6). First, litter organs of sweet clover exhibited a positive effect on litter chemical diversity ($r=1.072$), which positively impacted the soil fertilization ($r=0.307$). Thus, different litter from sweet clover parts positively influenced the litter decomposition rate through their chemical diversity. Therefore, the combination of shoot and root litter increased chemical diversity, thereby enhancing the synergistic effects on decomposition and directly accelerating litter decay. Furthermore, different litter types negatively impacted the soil microenvironment ($r=-0.500$), which indirectly reduced the litter decomposition rates through soil enzymatic activity ($r=0.197$) and keystone microbial decomposers ($r=-0.589$). The duration of litter decomposition negatively impacts litter diversity and the soil microenvironment, while positively influencing soil fertility. The community of keystone microbes directly influenced the decomposition rate ($r=0.998$), and reduced the activity of soil enzymes, which in turn enhanced the decomposition rate ($r=0.704$).

Discussion

Dynamics of residues decomposition and litter nutrients release

In this study, shoot litter exhibited a more rapid decay rate than fine root litter in organic orchards (Table S2), partially corroborating findings from previous studies on farmland ecosystems [6, 17]. Moreover, the synergistic

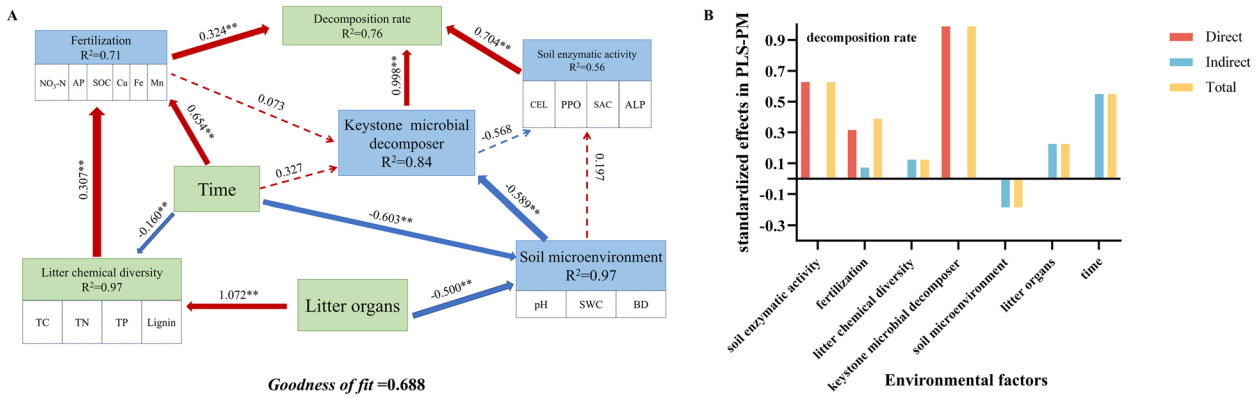

Fig. 6 The PLS-SEM analysis (**A**) and the effects of different factors on decomposition rate (**B**). Positive and negative effects are indicated by red and blue, respectively. Significance is denoted by stars at probability levels of $P < 0.05$ and $P < 0.01$ (* and **, respectively). The PLS-SEM model includes gray pathways

effect of mixed litter on decomposition was observed through changes in litter chemical properties (Fig. 1), which improved the microenvironment and niche complementarity among microbial decomposers (Fig. 5), thereby promoting microbial diversity and accelerating the decomposition rate [25, 30]. Nutrient release from sweet clover residues was rapid within the first 20 days. Throughout the experimental period, the mass of shoot residues consistently decreased, while residues of fine root mass stabilized after 30 days. Previous studies have shown that slower decay rates of roots compared to shoots are primarily due to their higher lignin concentrations [6, 12]. High soil relative humidity is recognized to spur the process of residual decomposition [8, 41]; however, root decomposition in wetter environments occurs at a slower rate than that of shoots. Our study corroborates this observation by emphasizing the higher soil water content in fine roots compared to shoots, especially during the first month of rapid decomposition (Table S3).

Potassium (K) showed the highest leaching rate in the early stages for both shoots and mixed litter (Table S2-3). This phenomenon has been documented in numerous studies on shoot and root decomposition [42, 43], which explains the high mobility of K by its minimal incorporation into organic structures. The slower release of N and P in fine roots litter is attributed to their lower initial concentrations compared to shoots. After 40 days, the concentrations of N and P in three litters were comparable, despite the constant loss in mass. The slower rate of N release in both roots and mixed litter compared to shoots during decomposition is generally ascribed to a higher C: N or lignin concentrations [11], potentially facilitating the recruitment and reproduction of a specific group of microbes. At specified carbon loss in litter, a stronger correlation between C and N losses in legume litter was demonstrated by a previous study [12], which is also supported by our present experiment. The present study demonstrated that fine roots significantly contribute to nitrogen retention through active uptake and assimilation of nitrogen from the soil, closely aligning with previous research findings [14, 43]. Overall, our findings highlighted the significant influence of the initial chemical properties of litter on nutrient release dynamics decay rates.

Effects of litter decomposition on soil nutrient cycling and microbial composition

Litter from different organs of sweet clover, comprising shoots and roots, significantly enhanced soil quality by providing abundant C and N substrates, thereby promoting microbial activity and nutrient cycling (Table S3). The availability of these nutrients is regulated by decomposition dynamics, which mediate their gradual release and subsequent incorporation into soil biochemical processes. As litter decomposes, sufficient time is required for soil nutrient cycling to be fully realized [17, 44]. In this study, nutritional indicators including SOC, NO_3-N, AP, and BD markedly increased in litter treatments during 40 days compared to the CK0. Differences in biomass between shoots and fine roots are recognized as a critical factor in evaluating the overall influence of litter input on soil nutrient cycling in organic orchard ecosystems. Results from the present study indicated that soil with fine roots and shoots litter exhibited a notable difference in N concentration, with fine roots presenting a higher C/N ratio, suggesting a greater potential to meet the N demands of the microbial community [14, 45]. While root litter may serve as a slow-release fertilizer, continuously supplying nutrients, shoot litter

is likely to contribute to nutrient availability through rapid decomposition [12]. Additionally, the Mantel test results indicated that NO_3-N significantly influenced both bacterial and fungal communities, highlighting its role as a key driver of microbial dynamics (Fig. 2). Tanikawa et al. [32] found that changes in the structure of bacterial community correlated with NO_3^--N concentration and pH, suggesting that the N dynamics of shoots and fine roots litter are significantly affected by their chemical properties and the microbes they recruit. Remarkably, the synergistic impact of increased N content at the organ level in the grassland ecosystem was primarily driven by pathways other than the enhancement of litter quality on decomposition [45]. These findings support our results indicating that NO_3^--N content significantly influences the microbial community structure.

Similarly, the present study revealed that the temporal dynamics of nutrient release from litter inputs varied, with root litter facilitating a more prolonged release of nutrients compared to the more immediate release from shoots litter [41]. This result corroborates earlier studies suggesting that changes in soil nutrient status are not the primary factors influencing the colonization and decomposition functions of various microbial types within litter [15] (Fig. 6; Table S3). This may be linked with the higher nutrient content and labile organic matter content in the litter relative to the soil, which significantly reduces the influence of soil nutrient status on soil fungi once these microbes begin to utilize litter substrates. It was observed that the relative abundance of Chloroflexi and Rozellomycota markedly increased during the decomposition process, showing a positive correlation with the decomposition rate (Fig. 5). Additionally, Anaerolineae, Dehalococcoidia, and KD4-96, which belong to Chloroflexi, were identified as keystone taxa in root, shoot, and mixed litter (Fig. 4B). This was further supported by the influence of various environmental factors, including pH, ALP, SWC, and SAC, which likely affect the composition and function of the microbial decomposer. Previous studies reported comparable findings, revealing that the keystone order Rhizobiales was dominant within the Alphaproteobacteria class, which contained the functional genes for extracellular enzymes [42]. Nutrient levels in litter govern the composition and distribution of bacterial communities, leading to deterministic assembly. In contrast, soil fungi, which are less sensitive to nutrient variations, exhibit a broader niche distribution [16]. Thus, changes in the fine roots and shoots litter, driven by plant responses to environmental factors, may affect nutrient transfers through plant–soil–microbe interactions [15].

Temporal dynamics of soil microbial networks and keystone taxa during litter decomposition

The incorporation of pasture residues in mulch-orchards affects the colonization and turnover of microorganisms, leading to temporal shifts in microbial diversity and community structure driven by key species throughout the decomposition process. The alpha diversity indices of soil bacteria in organic orchards indicated that bacterial community diversity was higher in the mixed litter during decomposition, followed by fine roots and shoots treatments. Overall, all litter treatments had higher diversity than the CK0 treatment (Table S2). Diversity indices suggested that fine roots and mixed litter harbored more taxa than shoots litter, and microbial abundance after 90 days of decomposition notably surpassed the initial levels. The composition of microbial communities was primarily regulated by their specific affinity for and proficient ability to decompose the substrates [27]. Thus, despite similar decomposition rates, significant shifts in microbial abundance and community structure across different litter types suggest functional redundancy within microbial systems [28].

The dynamics of microbial interactions play a vital role in determining microbial structure and their impact on soil function sustainability may outweigh that of species richness and abundance [46], particularly in organic farming. The addition of pasture litter resulted in a notable increase in microbial abundance, enhancing both the dominance and complexity of the bacterial community in the field by alleviating nutrient limitations that constrain bacterial functions [47]. Recent studies are increasingly focusing on analyzing the relationship between co-occurrence networks and the chemical properties of shoot litter [11, 17]. In this study, we explore the relationship between the temporal dynamics of microbial community structure and litter decomposition, employing an RMT-based network method to analyze microbial responses and interactions. In the co-occurrence network of the litter decomposition process, microbial community structure of sweet clover showed different symbiotic patterns at the organ level (Table 2; Fig. 4A). Fine root litter networks exhibited "small world" traits at both 30 and 90 days, strengthening resistance to environmental factors while simultaneously increasing susceptibility to the removal of closely related keystone species [48]. Moreover, compared to CK0, the networks with greater relative modularity were associated with improved stability and persistence. This implies that co-occurrence networks of root litter with persistence and stability generally demonstrate higher modularity, resulting from more robust interactions both within and between groups [49]. The stability of the microbial community, driven by its modular structure and keystone taxa, was modulated by

variations in the surrounding microenvironment [50]. These findings align with prior research, suggesting that increased network complexity in root litter enhances stability during decomposition, as intensified interspecific interactions are recognized to improve community stability and resistance [45, 49].

Keystone taxa identified through microbial network analysis influence community composition by affecting subsidiary taxa through strong biotic interactions rather than through high abundance [29]. Consequently, their removal can cause substantial alterations in microbiome structure and functionality. Several studies have shown that the dynamic interactions between keystone species and decay processes are essential for decomposition, and incorporating these taxa into models may improve predictions of overall microbial community changes [45, 49]. For fungi, our results indicate that Ascomycetes were fundamental for the decomposition of mixed litter compared to single-organ litter. Ascomycota is among the primary players in litter decomposition [46], which includes cellulolytic groups identified within this phylum in the litter layer. Additionally, high number of connectors and module hubs were discovered in shoot networks. Several keystone species discovered in shoot litter belonged to the phyla Gemmatimonadota, Proteobacteria, Acidobacteriota, Bacteroidota, and Myxococcota (Fig. 4; Table S4), which play critical roles in C cycling and N fixation [52]. The unassigned genus (OTU_59814) and Phycisphaeraceae, identified as crucial keystone taxa in root and mixed litter, were categorized as Planctomycetota. Despite their distinct spatial differentiation characteristics, Planctomycetota, as eco-friendly microorganisms, have been found to be specialized in the chitin degradation and to indirectly promote plant growth [53]. Previous studies have shown that during the early stages of decomposition, bacterial keystone taxa significantly influence extracellular enzyme activity and impact diverse litter chemistry [51]. Previously, it was reported that the decomposition rate of soil organic matter was primarily governed by the abundance of specific keystone taxa, rather than the overall microbial diversity [28]. For future studies, it is essential to validate the taxonomic and functional roles of keystone taxa in litter decomposition through further culturing and additional approaches such as metagenomics.

In this study, litter used for decomposition experiments was collected from an organic orchard of Xu Xiang kiwi (*Actinidia chinensis*), ensuring that the associated decomposition environment—including the microenvironment, soil nutrients, and resident decomposer communities—was well represented. This supports our first hypothesis that litter composition and plant organ type influence the decomposition dynamics. Over a period of 40 days, mixed litter exhibited higher nutrient release rates and improved soil fertility compared to shoot and root litter. The fungal and bacterial communities, compared to the blank soil (CK0), increased from 0 to 90 days following litter input, indicating significant clustering of microbial community characteristics based on litter types and decomposition time, thereby corroborating our second hypothesis. Furthermore, over the 90-day period, we identified key species within the fungal-bacterial co-occurrence networks associated with different litter types. The third hypothesis was also validated, as key species positively influenced the decomposition of different litter types. This highlights their importance within microbial communities and confirms their potential to enhance decomposition efficiency and nutrient release.

Considering that environmental factors are key determinants of litter decomposition, our study cannot entirely rule out the possibility that the observed results were influenced by the home-field advantage effect. Therefore, we prioritized investigating the linkages between litter characteristics and decomposition processes at the organ level, especially for potential microbiological decomposition mechanisms.

Conclusions

In conclusion, this study revealed that root and shoot litter decompose at different rates and release nutrients over different temporal scales, primarily due to initial variations in nitrogen-related chemical traits. The mixing of roots and shoots improved the synergistic effect, leading to an increase in soil α- and β- microbial diversity within the first 30 days of decomposition. This process also increased soil TOC and NO_3^--N concentrations, while reducing BD and SWC. The NO_3^--N, SOC and PPO showed strong correlations with bacterial community, whereas NO_3^--N and BD were correlations with the fungal community. The co-occurrence network within the fine root litter of sweet clover exhibited the highest modular characteristics and average clustering coefficient by the end of the 90-day decomposition period. The keystone taxa of microorganisms affecting the decomposition of sweet clover litter at the organ level were identified through co-occurrence networks. The PLS-SEM analysis integrated both direct and indirect effects, emphasizing that the chemical diversity at litter organ level significantly affected the decomposition rate, with both direct and indirect contributions from soil microbial decomposers. Overall, this study highlighted the significant role of fine roots, in soil fertility and microbial community succession.

Abbreviations

MnP	Synthesize manganese peroxidase
SRA	Sequence read archive

PCR	Polymerase chain reaction
PCoA	The principal coordinates analysis
ANOVA	Analysis of variance
NCBI	National Center for Biotechnology Information
DNA	Deoxyribonucleic acid
ITS	Internal transcribed spacer
rRNA	Ribosomal ribonucleic acid
SWC	Soil water content
AP	Available phosphorus
SOC	Soil organic carbon
BD	Bulk density
PPO	Polyphenol oxidase
URE	Urease
CAT	Catalase
ALP	Alkaline phosphatase
SAC	Sucrose
NMDS	Non-metric multidimensional scaling
RDA	Redundancy analysis
PLS-SEM	Partial least squares structural equation modeling
ACC	Average clustering coefficient
APL	Average path length
RMT	Random matrix theory

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06392-2.

Supplementary Material 1.

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Not applicable.

Authors' contributions
ML designed the methodology of this study. HT, HC, and JC conducted the field work and collected the data. MC and HT analyzed the data. MC wrote the manuscript. SH provided guidance and support throughout the study.

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Data availability
The datasets generated during and/or analyses during the current study are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (BioProject ID: PRJNA1140730; PRJNA1140841).

Declarations

Ethics approval and consent to participate
All methods and experimental research were carried out in compliance with local and national guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Hobbie SE. Plant species effects on nutrient cycling: revisiting litter feedbacks. Trends Ecol Evol. 2015;30(6):357–63.
2. Bardgett RD, Mommer L, De Vries FT. Going underground: root traits as drivers of ecosystem processes. Trends Ecol Evol. 2014;29(12):692–9.
3. Zhang W-P, Fornara D, Yang H, Yu R-P, Callaway RM, Li L. Plant litter strengthens positive biodiversity–ecosystem functioning relationships over time. Trends Ecol Evol. 2023;38(5):473–84.
4. Cotrufo MF, Soong JL, Horton AJ, Campbell EE, Haddix Michelle L, Wall DH, Parton WJ. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat Geosci. 2015;8(10):776–9.
5. Gaudaré U, Kuhnert M, Smith P, Martin M, Barbieri P, Pellerin S, Nesme T. Soil organic carbon stocks potentially at risk of decline with organic farming expansion. Nat Clim Chang. 2023;13(7):719–25.
6. Sun T, Hobbie SE, Berg B, Zhang H, Wang Q, Wang Z, Hättenschwiler S. Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. Proc Natl Acad Sci. 2018;115(41):10392–7.
7. Ventura M, Scandellari F, Bonora E, Tagliavini M. Nutrient release during decomposition of leaf litter in a peach (Prunus persica L.) orchard. Nutr Cycling Agroecosyst. 2009;87(1):115–25.
8. Dong N, Hu G, Zhang Y, Qi J, Chen Y, Hao Y. Effects of green-manure and tillage management on soil microbial community composition, nutrients and tree growth in a walnut orchard. Sci Rep. 2021;11(1):16882.
9. Liu Q, Zhuang L, Yin R, Ni X, You C, Yue K, Tan B, Liu Y, Zhang L, Xu Z. Root diameter controls the accumulation of humic substances in decomposing root litter. Geoderma. 2019;348:68–75.
10. Tjoelker MG, Craine JM, Wedin D, Reich PB, Tilman D. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytol. 2005;167(2):493–508.
11. Cuchietti A, Marcotti E, Gurvich DE, Cingolani AM, Pérez HN. Leaf litter mixtures and neighbour effects: Low-nitrogen and high-lignin species increase decomposition rate of high-nitrogen and low-lignin neighbours. Appl Soil Ecol. 2014;82:44–51.
12. Erdenebileg E, Wang C, Yu W, Ye X, Pan X, Huang Z, Liu G, Cornelissen JHC. Carbon versus nitrogen release from root and leaf litter is modulated by litter position and plant functional type. J Ecol. 2022;111(1):198–213.
13. Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 2011;193(1):30–50.
14. Versini A, Laclau J-P, Mareschal L, Plassard C, Diamesso LA, Ranger J, Zeller B. Nitrogen dynamics within and between decomposing leaves, bark and branches in Eucalyptus planted forests. Soil Biol Biochem. 2016;101:55–64.
15. Zhang X, Hu J, Wang L, Liu K, Tian S, Zhou W. Alterations in litter chemical traits and soil environmental properties limit the litter decomposition of near-mature Robinia pseudoacacia plantations. Geoderma. 2023;439:116668.
16. Hao H, Yue Y, Chen Q, Yang Y, Kuai B, Wang Q, Xiao T, Chen H, Zhang J. Effects of an efficient straw decomposition system mediated by Stropharia rugosoannulata on soil properties and microbial communities in forestland. Sci Total Environ. 2024;916: 170226.
17. He H-B, Li W-X, Zhang Y-W, Cheng J-K, Jia X-Y, Li S, Yang H-R, Chen B-M, Xin G-R. Effects of Italian ryegrass residues as green manure on soil properties and bacterial communities under an Italian ryegrass (Lolium multiflorum L.)-rice (Oryza sativa L.) rotation. Soil Tillage Res. 2020;196:104487–104487.
18. Lu Y, Zhou J, Sun L, Gao J, Raza S. Long-term land-use change from cropland to kiwifruit orchard increases nitrogen load to the environment: A substance flow analysis. Agr Ecosyst Environ. 2022;335: 108013.
19. Mortenson MC, Schuman GE, Ingram LJ. Carbon Sequestration in Rangelands Interseeded with Yellow-Flowering Alfalfa (Medicago sativa ssp. falcata). Environ Manage. 2004;33(S1):S475–81.
20. Duchene O, Vian J-F, Celette F. Intercropping with legume for agroecological cropping systems: complementarity and facilitation processes and the importance of soil microorganisms. A review. Agric, Ecosyst Environ. 2017;240:148–61.
21. Virk AL, Lin B-J, Kan Z-R, Qi J-Y, Dang YP, Lal R, Zhao X, Zhang H-L. Simultaneous effects of legume cultivation on carbon and nitrogen accumulation in soil. Adv Agron. 2022;171:75–110.

22. Siqueira DP, de Carvalho GCMW, de Souza Silva JG, Caldeira MVW, Barroso DG. Litter decomposition and nutrient release for two tropical N-fixing species in Rio de Janeiro. Brazil J Forest Res. 2021;33(2):487–96.
23. Dorissant L, Brym ZT, Swartz S. Residue decomposition dynamics in mixed ratios of two warm-season cover crops. Agrosyst, Geosci Environ. 2022;5(4): e20311.
24. Zhang S, Fang Y, Kawasaki A, Tavakkoli E, Cai Y, Wang H, Ge T, Zhou J, Yu B, Li Y. Biochar significantly reduced nutrient-induced positive priming in a subtropical forest soil. Biol Fertil Soils. 2023;59(6):589–607.
25. Zhang S, Huang F, Guo S, Luo Y, Zhang L, Zhang L, Li Z, Ge S, Wang H, Fu J. Mitigation of soil organic carbon mineralization in tea plantations through replacement of pruning litter additions with pruning litter derived biochar and organic fertilizer. Ind Crops Prod. 2025;225:120518.
26. Bani A, Pioli S, Ventura M, Panzacchi P, Borruso L, Tognetti R, Tonon G, Brusetti L. The role of microbial community in the decomposition of leaf litter and deadwood. Appl Soil Ecol. 2018;126:75–84.
27. Feng J, He K, Zhang Q, Han M, Zhu B. Changes in plant inputs alter soil carbon and microbial communities in forest ecosystems. Glob Change Biol. 2022;28(10):3426–40.
28. Banerjee S, Kirkby CA, Schmutter D, Bissett A, Kirkegaard JA, Richardson AE. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biol Biochem. 2016;97:188–98.
29. Banerjee S, Schlaeppi K, van der Heijden MGA. Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol. 2018;16(9):567–76.
30. Deng Y, Jiang Y-H, Yang Y, He Z, Luo F, Zhou J. Molecular ecological network analyses. BMC Bioinformatics. 2012;13(1): 113.
31. Han Z, Xu P, Li Z, Lin H, Zhu C, Wang J, Zou J. Microbial diversity and the abundance of keystone species drive the response of soil multifunctionality to organic substitution and biochar amendment in a tea plantation. GCB Bioenergy. 2022;14(4):481–95.
32. Tanikawa T, Maie N, Fujii S, Sun L, Hirano Y, Mizoguchi T, Matsuda Y. Contrasting patterns of nitrogen release from fine roots and leaves driven by microbial communities during decomposition. Sci Total Environ. 2023;855: 158809.
33. Bao S. Soil and agricultural chemistry analysis, ed. Beijing: China Agriculture Press China Agriculture Press; 2000:178–200.
34. Guan SY, Zhang D, Zhang Z. Soil enzyme and its research methods, ed. Beijing: Chinese Agricultural Press; 1986. p. 274–97.
35. Lu RK. Agriculture chemical analysis methods of soil, ed. Beijing: China Agricultural Science and Technology Press; 2000.
36. Luxhøi J, Magid J, Tscherko D, Kandeler E. Dynamics of invertase, xylanase and coupled quality indices of decomposing green and brown plant residues. Soil Biol Biochem. 2002;34(4):501–8.
37. Cm J, Os O, Wang Q, Nikkilä J, Marchesi JR, Smidt H, de Vos WM, Ross RP, O'Toole PW. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. Plos One. 2009;4(8): e6669.
38. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal Rna genes for phylogenetics. 1989.
39. Ge Z, Li S, Bol R, Zhu P, Peng C, An T, Cheng N, Liu X, Li T, Xu Z. Differential long-term fertilization alters residue-derived labile organic carbon fractions and microbial community during straw residue decomposition. Soil Tillage Res. 2021;213: 105120.
40. Kenneth P, Burnham David RA. Model selection and multimodel inference, ed.: Springer, New York, NY;Springer New York; 2004.
41. Sun Z, Tian P, Zhao X, Wang Y, Wang S, Fang X, Wang Q, Liu S. Temporal shifts in the explanatory power and relative importance of litter traits in regulating litter decomposition. For Ecosyst. 2022;9:755–63.
42. Naeem I, Asif T, Zhang T, Guan Y, Wu X, Tariq H, Wang D. Mixing effects of three Eurasian plants on root decomposition in the existence of living plant community in a meadow steppe. Sci Total Environ. 2022;811: 151400.
43. Wang Y, Wang H, Dai X, Kou L, Meng S, Fu X. Decoupled responses of leaf and root decomposition to nutrient deposition in a subtropical plantation. Soil Biol Biochem. 2022;168: 108643.
44. Almagro M, Ruiz-Navarro A, Díaz-Pereira E, Albaladejo J, Martínez-Mena M. Plant residue chemical quality modulates the soil microbial response related to decomposition and soil organic carbon and nitrogen stabilization in a rainfed Mediterranean agroecosystem. Soil Biol Biochem. 2021;156:108198.
45. Hou S-L, Lü X-T. Mixing effects of litter decomposition at plant organ and species levels in a temperate grassland. Plant Soil. 2020;459(1–2):387–96.
46. Zheng H, Yang T, Bao Y, He P, Yang K, Mei X, Wei Z, Xu Y, Shen Q, Banerjee S. Network analysis and subsequent culturing reveal keystone taxa involved in microbial litter decomposition dynamics. Soil Biol Biochem. 2021;157: 108230.
47. Hu Y, Jin J, Ding K, Ye Z, Wang X, Palansooriya KN, Fu W, Wu J. Long-term cover cropping improved soil bacterial community and soil multifunctionality in a Carya cathayensis plantation. Agr Ecosyst Environ. 2023;347: 108372.
48. Comte J, Lovejoy C, Crevecoeur S, Vincent WF. Co-occurrence patterns in aquatic bacterial communities across changing permafrost landscapes. Biogeosciences. 2016;13(1):175–90.
49. Liu S, Yu H, Yu Y, Huang J, Zhou Z, Zeng J, Chen P, Xiao F, He Z, Yan Q. Ecological stability of microbial communities in Lake Donghu regulated by keystone taxa. Ecol Ind. 2022;136: 108695.
50. Guo L, Deng M, Yang S, Liu W, Wang X, Wang J, Liu L, Wang Z. The coordination between leaf and fine root litter decomposition and the difference in their controlling factors. Glob Ecol Biogeogr. 2021;30(11):2286–96.
51. Wang X, Liang C, Mao J, Jiang Y, Bian Q, Liang Y, Chen Y, Sun B. Microbial keystone taxa drive succession of plant residue chemistry. ISME J. 2023;17(5):748–57.
52. Shao P, Lynch L, Xie H, Bao X, Liang C. Tradeoffs among microbial life history strategies influence the fate of microbial residues in subtropical forest soils. Soil Biol Biochem. 2021;153: 108112.
53. Shi Y, Delgado-Baquerizo M, Li Y, Yang Y, Zhu Y-G, Peñuelas J, Chu H. Abundance of kinless hubs within soil microbial networks are associated with high functional potential in agricultural ecosystems. Environ Int. 2020;142: 105869.

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