Leaf litter mixtures alter decomposition rate, nutrient retention, and bacterial community composition in a temperate forest

Kun Li[1,3], Ying Lu[1,3], Qing-Wei Wang[2], Ruiqiang Ni[1,3], Rongchu Han[1,3], Chuanrong Li[1,3*], Caihong Zhang[1,3*], Weixing Shen[4], Qi Yao[4], Yueyin Gao[5] and Sergio de-Miguel[6,7]

[1] *Mountain Tai Forest Ecosystem Research Station of State Forestry Administration/Key Laboratory of State Forestry Administration for Silviculture of the Lower Yellow River, Tai'an 271018, Shandong, PR China;*
[2] *CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology Chinese Academy of Sciences, Shenyang 110016, PR China;*
[3] *Research Center for Forest Carbon Neutrality Engineering of Shandong Higher Education Institutions/Key Laboratory of Ecological Protection and Security Control of the Lower Yellow River of Shandong Higher Education Institutions, Tai'an 271018, Shandong, PR China ;*
[4] *Mount Tai Scenic Spot Management Committee, Tai'an 271000, Shandong, PR China*
[5] *State-owned Guangping Forest Farm, Chiping District, Liaocheng 252100, Shandong, PR China*
[6] *Department of Crop and Forest Sciences, University of Lleida, Av. Alcalde Rovira Roure 191, E-25198 Lleida, Spain*
[7] *Joint Research Unit CTFC–AGROTECNIO-CERCA, E-25280 Solsona, Spain*
* Corresponding authors, E-mail: chrli@sdau.edu.cn; zhangcaihong78@163.com

Abstract

Litter decomposition is a key step in global biogeochemical cycling. In forest ecosystems, litter from different tree spec1ies often decompose together. Although species diversity is widely acknowledged to accelerate decomposition through the regulation of nutrient transfer between litter and decomposer communities, the underlying mechanism remains unclear. To explore the association between the bacterial community and mixed-litter chemical transformation, we conducted a one-year litter mixing decomposition experiment using leaf litter from four dominant tree species in Mount Tai (Eastern China), *Robinia pseudoacacia*, *Quercus acutissima*, *Pinus tabulaeformis*, and *Pinus densiflora*. Our results showed that: 1) Mass loss of leaf litter mixtures was significantly faster than that of leaf litter monocultures, except for *R. pseudoacacia*. Litter mixtures without *R. pseudoacacia* showed non-additive synergistic effects, whereas litter mixtures with *R. pseudoacacia* exerted additive effects; 2) Litter species in the absence of *R. pseudoacacia* significantly decreased the nutrient retention rates of litter mixtures compared to those of monocultures; 3) Litter mixtures with or without *R. pseudoacacia* showing additive and non-additive effects in monocultures had a distinct bacterial community structure; 4) Bacterial community structure was also modified by initial litter traits; carbon (C), nitrogen (N), and phosphorus (P) concentrations in monocultures; N/P and C/N ratios of mixtures with *R. pseudoacacia*; and the lignin/N ratio of mixtures without *R. pseudoacacia*. Overall, these findings indicate that tree species diversity controls decomposition and nutrient cycling, implying that an appropriate species community composition is beneficial to maintaining forest ecosystems.

Introduction

Decomposition of litter is crucial for terrestrial biogeochemical cycles, and it is widely acknowledged that the litter decomposition rate is regulated by climate, litter quality, and decomposer communities[1–4]. Litter quality is the primary factor governing the rates at which litter decomposes across different biomes globally[5,6]. The decomposition rates vary greatly depending on the substrates, which can simultaneously cause the rapid decline in easily accessible nutrients and the accumulation of more resistant compounds[7,8]. Microorganisms are important components of biogeochemical cycling and ecosystem functioning[9]. In ecosystems, microorganisms are the primary decomposers of plant litter, and they are more important than detritivores in litter mixtures[10,11]. Although the litter decomposition rate and its regulatory factors have been intensely investigated, most of our current knowledge is derived from litter monoculture studies[12,13]. Thus, most findings are not necessarily applicable to natural ecosystems, where different types of litter decompose together.

Much attention has been given to species diversity and its effects on litter decomposition and terrestrial nutrient cycles[14,15] because litter type, species richness, and litter–species interactions significantly drive litter decomposition[16,17]. Studies have been reported that decomposition rates increase with greater litter diversity[18,19], whereas no relationship or an opposite trend was observed by others[20,21]. This discrepancy may be due to interactions among litters comprised of different tree species, which can affect decomposer communities[8,19,22]. Compared with monospecific litter, mixed litters may alter the physical and chemical properties of litters as well as decomposer abundance and activity, leading to considerable mixing effects on decomposition[10,19]. Although litter quality may affect decomposition dynamics[23], the mechanism responsible for the mixing effect on nutrient cycling and decomposer communities still remains unclear.

The composition of the microbial community determines the rate of litter decomposition[24,25]. Generally, the composition of microbes shifts from being dominated by fungi at the early stages of decomposition to being dominated by bacteria at the

later stages[26]. These changes can be attributed to the different structures and functions of the microbial communities[27,28]. In terrestrial ecosystems, fungi outperform bacteria in the utilization of more complex and various carbon sources, while bacteria are often superior to fungi in using more labile carbon sources[29]. However, a recent study has indicated that diverse bacteria and fungi coexist and interact throughout decomposition[30]. Bacteria not only support fungal decomposers by supplying electrons or essential micronutrients but also establish themselves at the soil-litter interface during the breakdown of complex macromolecules by extracellular fungal enzymes[31]. These findings imply that bacterial communities may serve as the primary drivers of litter decomposition by modulating patterns of mass loss and contributing to nutrient cycles[32].

In mixed forests, litter mixtures may favor bacterial communities, which depends on the respective biological functions[33], given that the trophic complexity of a decomposer community is crucial for litter decomposition[34]. Litter mixtures not only increase the complementary resource utilization by decomposer communities[19], but also provide diverse substrates and niches for microorganisms[35], which can accelerate the litter decomposition rate[15,36,37]. Furthermore, microorganisms secrete extracellular enzymes to decompose substrates into smaller compounds for plant nourishment and development[38]. The transfer of nutrients from superior to inferior quality litter can occur through either active microbial transfer or passive leaching, as postulated by the nutrient transfer theory[19]. Therefore, knowledge on the effects of litter mixtures can contribute to a better understanding of nutrient cycling and feedback mechanisms that regulate species diversity[39]. To date, however, few studies have examined the microbial community in terms of the litter decomposition process[40]. Although an earlier report has indicated that litter diversity accelerates litter decomposition by increasing the abundance of microorganisms and detritivores[10], empirical studies are rare because of limitations in measurement techniques[20]. As a result, how litter mixtures drive microbial community structure and composition remains unclear.

To clarify the effects of litter mixtures on decomposition, we quantified the effects of leaf litter mixtures on litter mass, nutrient loss, and bacterial community structure through a litter-bag experiment in Mount Tai, East China. Our hypotheses were as follows: (1) Litter mixtures can show non-additive effects on litter mass loss due to greater chemical dissimilarity among litters; (2) Decomposition of litter mixtures significantly increases nutrient retention of specific litters, because according to the nutrient transfer theory, nutrients from higher quality litter species are generally transferred to lower quality litter species; and (3) litter mixtures significantly affect the structure and composition of bacterial communities compared with litter monocultures, and the change in litter decomposition rates are due to litter species diversity increases complementary among different decomposers.

Materials and methods

Study site

An experiment focusing on leaf litter decomposition was carried out at the Yaoxiang Forest Ecosystem National Positioning Observation Research Station located in Mount Tai in East China. The study area encompassed a total area of 1210.2 hectares, with geographic coordinates of 117°10'E and 36°17'N. This region has a warm, temperate continental monsoon climate, characterized by an annual average temperature of 10.8 °C. The maximum temperature recorded is 34 °C, while the minimum temperature recorded is −24 °C. The average annual precipitation is 950 mm. The soil is typical of mountain brown terrain, with the soil depth ranging from 15 to 90 cm. The forest vegetation mostly consists of planted stands, which were established in the 1950s. The main tree species include *Robinia pseudoacacia*, *Quercus acutissima*, *Pinus tabulaeformis*, and *Pinus densiflora* after decades of reforestation efforts. These species always occur in monospecific stands, with low biodiversity, weak stability, and high vulnerability to pests and diseases, which are unfavorable for ecosystem energy flow and nutrient cycling[41].

Experimental design

Four litter species, *R. pseudoacacia*, *Q. acutissima*, *P. densiflora*, and *P. tabuliformis*, were collected from four monospecific plantations using litter boxes in the study area in September 2015. The leaf litter was air-dried to constant mass, and a portion of each air-dried litter was oven-dried (65 °C, 48 h) to measure the air- and oven-dried mass ratios. The decomposition experiment was conducted in July 2016 as follows: i) one treatment contained a litter monoculture of four species; 6 g of air-dried leaf litter per species were placed in nylon mesh bags (25 cm × 25 cm, 1-mm mesh size), and ii) the other treatment contained a mixture of two species in all possible pair-wise combinations from four species in equal proportions (six types in total), and each nylon mesh bag (25 cm × 25 cm, 1-mm mesh size) contained two small bags (15 cm × 10 cm, 3 g of air-dried litter per bag) to easily distinguish the litter mixtures. Thus, there were 10 types of litter bags (four monocultures + six mixtures), and each litter type had six replicates. To avoid the effect of home-field advantage on decomposition[42], the litter bags were placed in an area devoid of forests of the Mount Tai Forest Ecosystem Observation and Research Station (36°20'3″N,117°7'11″E). The information, study site climate, and initial litter properties are shown in Supplemental Table S1, Supplemental Fig. S1, and Supplemental Table S2, respectively.

We adopted a randomized, complete block design experiment with six blocks (10 cm × 10 m) that were separated 5 m from each other with similar environmental conditions. Each block included 10 litter bag types, which were pinned to the ground surface.

Litter bags were retrieved in July 2017 after one year of decomposition. Three replicates of each litter type were stored in cryotubes in a liquid nitrogen tank and transferred to the lab for determining the bacterial community composition and structure. The other three replicates were dried at 65 °C for 48 h and weighed after removing adhering soil particles and living plants. Samples were ground for chemical analyses.

The concentrations of C and nitrogen (N) were determined with an elemental analyzer (Costech ECS4010, Costech Analytical Technologies, Valencia CA, Italy), and that of phosphorus (P) by the Mo-Sb antispetrophotography method[43]. The lignin concentration was determined by the acid detergent lignin method[44].

Bacterial DNA extraction and 16S rDNA amplification

The CTAB method[45] was used to extract genomic DNA from the samples, and DNA concentration and purity were assessed

by spectroscopy and 1% agarose gel electrophoresis. DNA was diluted to 1 ng/μL with sterile water, and thirty samples served as templates for high throughput sequencing (HTS) analysis, which was conducted by Novogene (Tianjin, China).

To amplify the 16S rDNA genes of distinct regions (16SV4-V5), we employed specific primers [515F (5′-GTGCCAGCMGC-CGCGGTAA-3′) and 907R (5′-CCGTCAATTCCTTTGAGTTT-3′)], along with the addition of barcode sequences. All polymerase chain reactions (PCR) were performed using the Phusin High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). Subsequently, PCR products were combined with an equal volume of 1× loading buffer (containing SYB green) and subjected to 2% agarose gel electrophoresis. Samples displaying a clear, prominent band of 400–450 bp were selected for further study. To ensure a balanced representation, the PCR products were combined according to equal density ratios. Combined PCR products were purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). For library construction, the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) was used according to the manufacturer's instructions, in addition to the incorporation of index codes. The quality of the library was evaluated using the Qubit@2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and the Agilent Bioanalyzer 2,100 instrument (Santa Clara, CA, USA). Finally, the library was sequenced on the Illumina HiSeq 2500 platform to generate 250-bp paired-end reads.

Statistical analysis

The percentage of litter mass remaining (percentage of initial mass) (D) and the nutrient retention rate (R) were calculated as follows[16]:

$$D(\%) = M_t/M_0 \times 100\% \qquad (1)$$

$$R(\%) = (C_t \times M_t)/(C_0 \times M_0) \times 100\% \qquad (2)$$

Where M_0 and M_t are the oven-dried weights of leaf litter before and after decomposition, respectively; C_0 is the initial concentration of C, N, P, or lignin; and C_t is the concentration of these elements as a percentage of litter mass at each sampling event.

The predictive mass of the litter mixtures was calculated as follows[46]:

$$\text{Predictive value} = [M_1/(M_1 + M_2)] \times R_1 + [M_2/(M_1 + M_2)] \times R_2 \qquad (3)$$

Where R_1 and R_2 are the percentages of the mass remaining in the single species litter-bag of species 1 and 2, respectively, and M_1 and M_2 are the estimated initial litter dry masses of these species in the mixture.

Sequencing reads from the dataset were trimmed, quality-controlled, and aligned. Operational taxonomic units (OTUs) were clustered at 97% identity using Uparse (v7.0.1001, http://drive5.com/uparse/). Taxonomic classification was conducted using RDP classifier (v2.2, http://sourceforge.net/projects/rdp-classifier/). Alpha diversity was used to analyze the diversity of bacterial species for each sample based on three different diversity indices, Chao 1, Shannon, and ACE, which were calculated with QIIME (v1.9.1) and displayed using R software (v2.15.3, https://cran.r-project.org/).

To clarify the mixing effects, significance of the differences between the observed and predicted decomposition values was assessed by a single sample t-test for each mixture treatment. A non-additive effect was defined as a significant difference between the observed and predicted decomposition values; otherwise the effect was considered to be additive. Nonmetric multidimensional scaling (NMDS) was used to

examine the differences in bacterial community structure between the litter-bag types using Bray–Curtis distances[47]. Analysis of similarities (ANOSIM) was used to examine the significance of bacterial community structure on litter types[48]. Pearson's correlation analysis was conducted to explore relationships between the litter nutrient retention and alpha diversity. Spearman's correlation analysis and redundancy analysis (RDA) were applied to determine the main factors driving litter decomposition and bacterial community structure[24]. Correlation analysis and one-way analysis of variance (ANOVA) were conducted in SPSS 26.0 (IBM Armonk, NY, USA). NMDS and RDA were performed using R (v2.15.3) to examine relationships between the bacterial community structure and initial nutrient concentration of litter.

Results

Differences in litter mass remaining of mixtures

After one year, except for *R. pseudoacacia*, the litter mass remaining in monocultures (33.61%–8.90%) was significantly higher than that in mixtures (21.21%–48.05%) with no significant difference among the three mixtures (Fig. 1). For *R. pseudoacacia*, there was no difference between the monocultures and mixtures, except for the *P. tabuliformis* mixture (Fig. 1). The litter mass remaining for mixtures without *R. pseudoacacia* [*P. tabuliformis* × *Q. acutissima* (38.17%), *P. densiflora* × *Q. acutissima* (26.72%), and *P. densiflora* × *P. tabuliformis* (41.00%)] was significantly lower than the predicted values (*p* < 0.05) (57.15%, 51.73%, and 53.48, respectively), suggesting non-additive synergistic effects. On the other hand, the litter mass remaining for the three mixtures with *R. pseudoacacia* had no significant difference in the predicted values (*p* > 0.05), suggesting additive effects (Fig. 2).
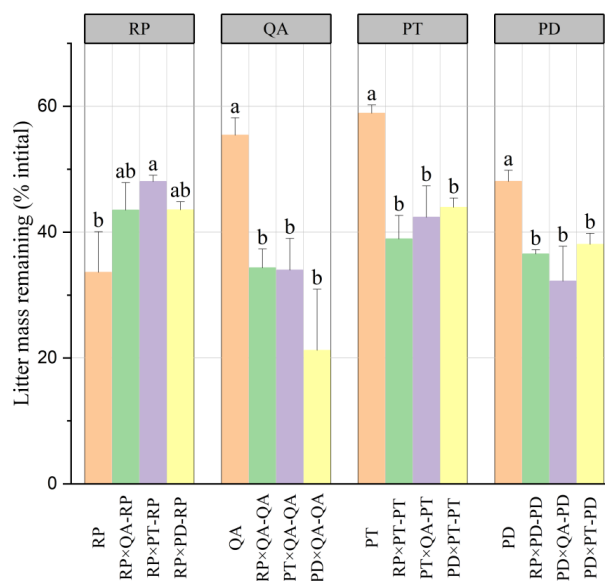

Fig. 1 Litter mass remaining in decomposing monocultures and mixtures. × represents mixed decomposition. A×B-A and A×B-B represent decomposition characteristics of A and B in mixed decomposition, respectively. Mean ± standard error (SE), n = 3. Error bars represent SE. Different lowercase letters indicate significant differences (*p* < 0.05) among different types of litter-bag. RP, *Robinia pseudoacacia*; QA, *Quercus acutissima*; PT, *Pinus tabulaeformis*; and PD, *Pinus densiflora*.
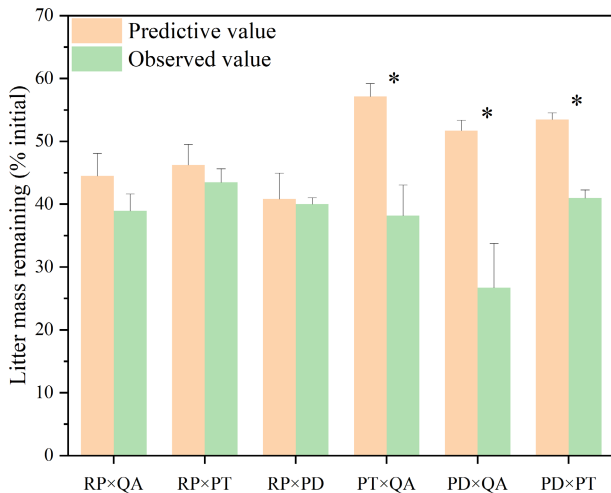
Fig. 2 Observed and predicted litter mass remaining for litter mixtures. × represents mixed decomposition. Mean ± SE, n = 3. * indicates significant differences (*p* < 0.05) between predictive and observed values among different types of litter-bag. RP, *Robinia pseucdoacacia*; QA, *Quercus acutissima*; PT, *Pinus tabulaeformis*; and PD, *Pinus densiflora*.

Differential litter nutrient retention among different litter types

Litter decomposition significantly decreased the retention rates of litter C and N in both monocultures and mixtures

(Fig. 3a, b). The P retention rate showed a similar decreasing trend in two conifers (*P. densiflora* and *P. tabuliformis*) but a dissimilar trend in two broadleaves species (*R. pseudoacacia* and *Q. acutissima*). For *R. pseudoacacia*, the P retention rate increased in the *P. tabuliformis* mixtures and decreased in the monocultures. For *Q. acutissima*, the P retention rate increased in the monocultures (Fig. 3c). Furthermore, the nutrient retention rate in monocultures was higher than that in mixtures, except for *R. pseudoacacia* (Fig. 3). For *R. pseudoacacia*, the retention rate of litter C, N, and lignin in monocultures were similar with those in mixtures, except for the *P. tabuliformis* mixture (Fig. 3a, b, d), and it showed a similar trend with the litter mass remaining (Fig. 1). Compared with the litter monocultures, the decomposition of litter mixtures significantly increased the P retention rate of *R. pseudoacacia*, irrespective of the mixture type (Fig. 3c).

Composition and diversity of bacterial communities

In total, 52,953 effective tags were used for analyzing the composition and diversity of bacterial communities, and they were clustered into 2,275 OTUs at a 97% similarity level. The sequences were assigned to 36 phyla and 447 genera (Fig. 4). Proteobacteria and Actinobacteria were the main phyla across all samples after decomposition for one year, accounting for 57.5%–64.8% and 12.5%–17.3% of the total valid reads, respectively (Fig. 4a). At the genus level, the groups with average relative abundance higher than 2% were *Bradyrhizobium* (3.8%), *Burkholderia-Paraburkholderia* (3.6%), *Sphingomonas* (3.2%), *Rhizomicrobium* (3.6%), and *Rhizobium* (2.5%) (Fig. 4b).
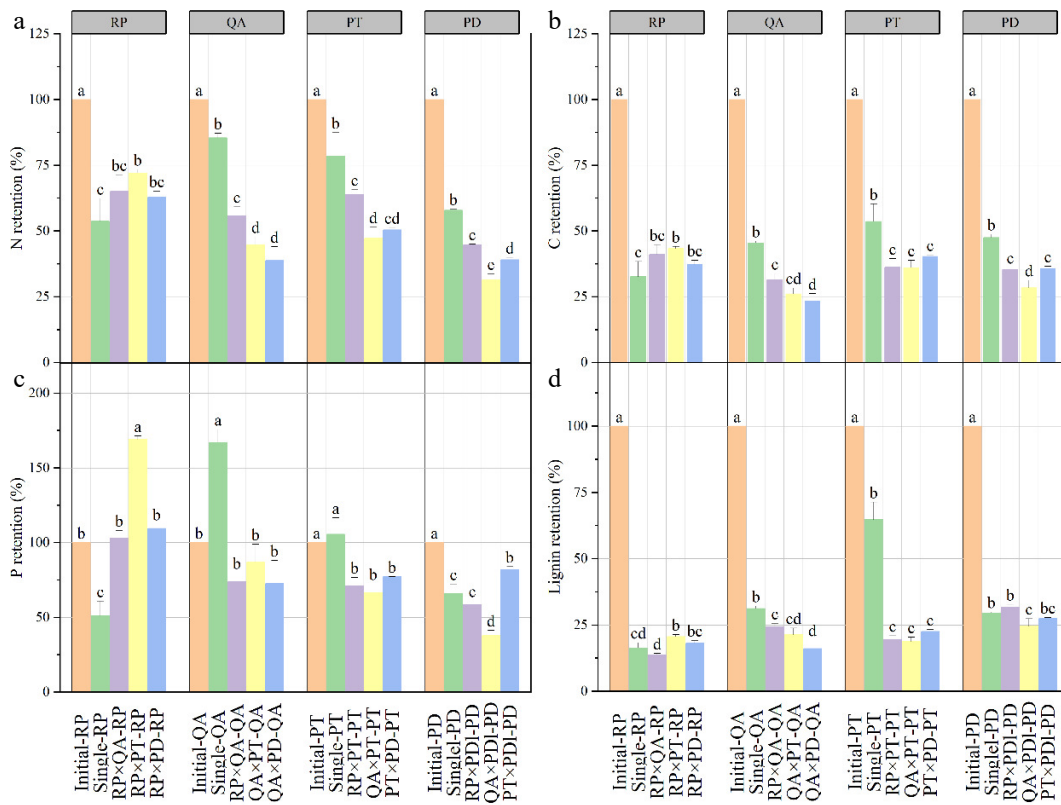

Fig. 3 Nutrient retention rates of litter in monocultures and mixtures after one year's decomposition. × represents mixed decomposition. Error bars represent standard errors. Different lowercase letters above the bars indicate significant differences (*p* < 0.05) between the different litter-bag types. A, B, C, and D represent N, C, P, and lignin retention, respectively. RP, *Robinia pseucdoacacia*; QA, *Quercus acutissima*; PT, *Pinus tabulaeformis*; and PD, *Pinus densiflora*.
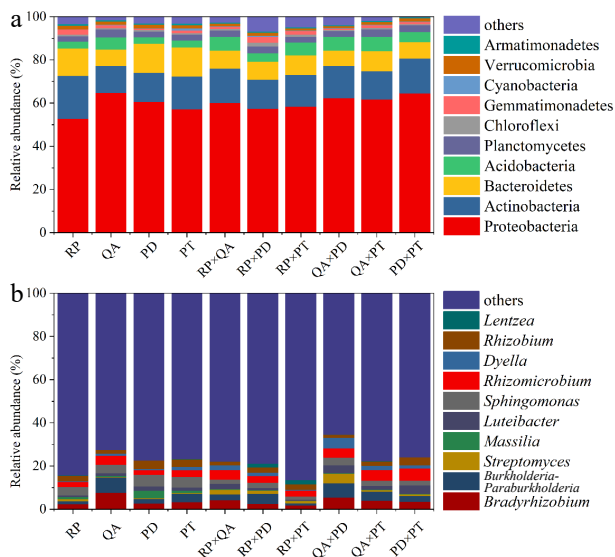
Fig. 4 Composition of the 10 most abundant taxonomic groups according to the mean relative abundances of bacterial assemblages, (a) at the phylum level and (b) at the genus level. × represents mixed decomposition. RP, *Robinia pseucdoacacia*; QA, *Quercus acutissima*; PT, *Pinus tabulaeformis*; and PD, *Pinus densiflora*.

For the monocultures, the bacterial species richness (Chao1 and Ace) of the two broadleaves (*R. pseudoacacia* and *Q. acutissima*) was higher than that of the two conifers (*P. densiflora* and *P. tabuliformis*). The Shannon index of the *Q. acutissima* litter was significantly lower than that of the other three litter types (*p* < 0.05) (Supplemental Table 3). For the mixtures, the bacterial α-diversity of the three mixtures with *P. densiflora* (*P. densiflora* × *R. pseudoacacia*, *P. densiflora* × *Q. acutissima*, and *P. densiflora* × *P. tabuliformis*) was lower than that of the other mixtures. However, the bacterial α-diversity of the mixtures of broad-leaved tree species (*R. pseudoacacia* or *Q. acutissima*) with *P. tabuliformis* was significantly higher than that of the other mixtures, especially for *R. pseudoacacia* × *P. tabuliformis* (Supplemental Table 4). Both NMDS (Fig. 5) and ANOSIM revealed that mixtures with *R. pseudoacacia* (additive effects), mixtures without *R. pseudoacacia* (non-additive effects), and those in monocultures had distinct bacterial community structures (ANOSIM *R* = 0.369, *p* = 0.022).

Linking litter mass remaining to bacterial community and initial litter properties

The relative abundance of the bacterial community was mainly correlated with the initial litter C/N, N/P and lignin/N ratios (Supplemental Tables 5 & 6), especially at the genus level. The relative abundance was positively associated with the initial litter C/N and lignin/N ratios and negatively associated with the N/P ratio (*p* < 0.05, Supplemental Table 6). Bacterial community structure in both litter monocultures and mixtures was driven by initial litter properties (Fig. 6). In monocultures, the bacterial community structure mainly depended on initial C, N, and P concentrations, and there was great difference between high quality litters (N concentrations) and low quality litters (C and P concentrations). In mixtures, the bacterial community structure in mixtures with *R. pseudoacacia* (additive effects) was mainly determined by the initial N/P ratio, and mixtures without *R. pseudoacacia* (non-additive effects) were mainly determined by C/N and lignin/N ratios (Fig. 6, Supplemental Table 7).
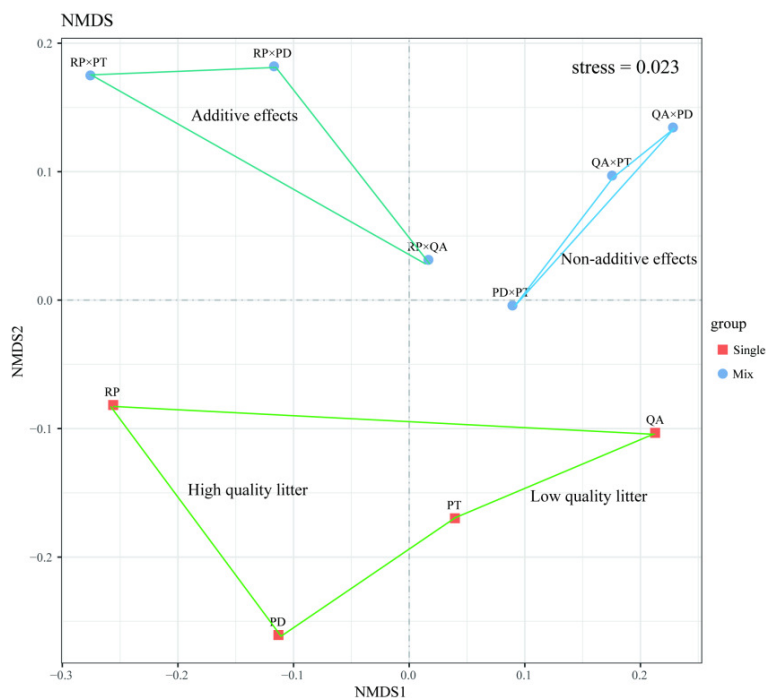

Fig. 5 Bacterial community structure in leaf litter samples decomposed for one year using Bray–Curtis distances. Squares and circles show bacterial community structures of litter monocultures and mixtures, respectively. The stress value was 0.023. RP, *Robinia pseudoacacia*; QA, *Quercus acutissima*; PT, *Pinus tabulaeformis*; and PD, *Pinus densiflora*.
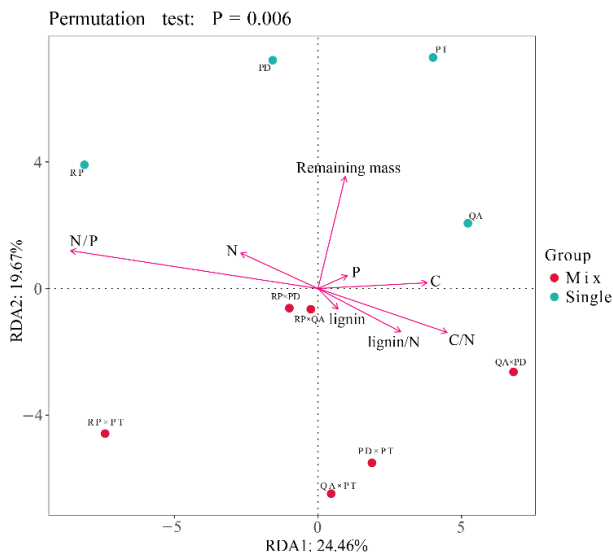
Permutation test: P = 0.006

Fig. 6 Redundancy analysis (RDA) of bacterial community structure, litter mass remaining, and initial litter properties. Red and blue circles represent bacterial community structure of litter monocultures and mixtures, respectively. Red lines represent the litter mass remaining and initial litter properties. RP, *Robinia pseucdoacacia*; QA, *Quercus acutissima*; PT, *Pinus tabulaeformis*; and PD, *Pinus densiflora*.

Discussion

Non-additive and additive effects occur concurrently in decomposition of mixed litters

We reveal that litter decomposition is influenced by the specific characteristics of each litter species within a mixture. The different litter species included in the mixtures exhibited varying responses to decomposition[49]. As hypothesized, the addition or exclusion of *R. pseudoacacia* in litter mixtures resulted in additive or non-additive synergistic effects on litter decomposition, respectively. These outcomes are consistent with previous research, which indicated that litter types influence litter mixing effects[19,50]. The mixture of three native species (*Q. acutissima*, *P. densiflora*, and *P. tabuliformis*) showed synergistic effects on litter decomposition. This observation may be attributed to the long-term adaptation of local decomposer communities, which favor synergistic effects that originated from the combination of different litter types[49]. Our study provides further evidence that the composition of species greatly impacts the intensity of non-additive effects in temperate plantations.

Great dissimilarities in litter compounds can produce synergistic effects on the decomposition of mixed litters. For instance, N is transferred from the N-rich to the N-poor litter to consequently enhance the microbial decomposition of poor-quality litter[19,51]. Thus, litter mixtures with higher N concentrations are expected to yield synergistic effects on litter decomposition. However, we found that litter mixtures with high-N concentrations containing *R. pseudoacacia* exerted additive effects (Fig. 2), which is inconsistent with previous findings[17,47]. One explanation could be that plant litter containing specific compounds may inhibit microbial activities and produce antagonistic effects on adjacent component litters[52]. The low decomposition rate of litter mixtures containing *R. pseudoacacia* can be detrimental to nutrient release[6,53].

Furthermore, *R. pseudoacacia*, an invasive N-fixing species, does not require much surrounding nutrient[54,55]. Therefore, *R. pseudoacacia* can reduce the competitiveness of native plants by inhibiting the litter decomposition rate during the decomposition of mixed litter. From this perspective, the litter mixing effects may explain the phenomenon of biological invasion.

Effects of mixed litter decomposition on nutrient retention

The decomposition of mixed litter significantly changed the nutrient retention rate of specific litters in the mixtures, supporting our second hypothesis that decomposition of litter mixtures significantly increases nutrient retention of specific litters. The decomposition rate of *R. pseudoacacia* in the litter mixtures showed no significant difference from that in the monocultures, and the nutrient retention rate, especially that of P, increased mostly in the mixtures, consistent with previous findings[6,51]. Invasion of *R. pseudoacacia* into grassland ecosystems can have a significant impact on temperature and light conditions in the understory[56]. This invasion has been shown to decrease the abundance and richness of microarthropods and nematodes, as well as plant diversity[54]. It may also be related to the presence of allelochemicals such as acacetin and quercetin in *R. pseudoacacia* litter[57]. These findings suggest that nutrient cycling in mixed plantations can be negatively affected by *R. pseudoacacia* litter. These findings also highlight the importance of understanding how plants acquire nutrients, as this may play a key role in the decomposition of mixed litter in temperate forest plantations. Therefore, it is important to decrease *R. pseudoacacia* expansion to maintain temperate forest ecosystems.

Compared with monocultures, the presence of *Q. acutissima*, *P. densiflora*, and *P. tabuliformis* in mixtures significantly decreased the litter mass remaining and the nutrient retention rate, suggesting a strong synergistic effect when these species are combined. A previous study has shown that the presence of invasive species in litter can increase the rates of decomposition and release of C, N, and P from the litter of native species[58]. The synergistic effects observed in litter mixtures without *R. pseudoacacia* may be attributed to the nutrient transfer theory. According to this theory, nutrients are transferred from higher quality litter to lower quality litter through either active microbial transfer or passive leaching[19]. The other reason may be the home-field advantage of local microclimatic conditions and decomposer communities for native rather than invasive species[49]. Therefore, mixed litters comprised of native species promote its own decomposition as well as that of other litters, consequently accelerating nutrient cycling. These findings may explain why mixed plantation systems are relatively stable. These synergistic effects suggest that species combinations can be applied to mixed plantations.

Structure and composition of the bacterial community significantly affects the mixing effects through nutrient links

Litter monocultures and mixtures distinctly drove the litter bacterial community structure due to differences in the initial litter properties. Bacterial community structure was determined by initial litter C, N, and P concentrations of monocultures, and C/N, N/P, and lignin/N ratios of mixtures. These findings suggest that chemical elements, as fundamental resources for decomposition in the food web, are indispensable for

microbial reproduction[59,60]. Chemical element ratios in litter materials shape the structure of decomposer communities[61]. Microbes have specific nutrient requirements for their metabolic processes related to energy and growth, which can alter decomposition processes. Our findings demonstrated that the bacterial diversity (α-diversity) of the broadleaved litter was considerably greater than that of the coniferous litter. The broadleaved litter, which is characterized by its high-quality composition and rapid decomposition, releases significant amounts of basic cations such as Ca and Mg that may contribute to maintaining a lower soil acidification, which changes the bacterial community composition and activity[37,62,63]. In addition, bacterial α-diversity was generally higher in litter mixtures than in litter monocultures because mixtures can combine complementary resources to meet the requirements of various bacteria[64].

Changes in bacterial community composition may also be driven by litter matrix nutrient availability[27]. Greater nitrogen (N) and lower lignin contents (indicated by a lower lignin/N ratio) result in the higher availability of energy and nutrients, along with reduced resistance to decomposition, thereby accelerating microbial activities[65]. Our results showed that Proteobacteria and Actinobacteria were the main phyla involved in litter decomposition, accounting for 57.5%–64.8% and 12.5%–17.3% of the total valid reads, respectively. Furthermore, *R. pseudoacacia* in monocultures and mixtures had a lower abundance of Proteobacteria than other treatments. The relative abundance of Proteobacteria exhibited a positive correlation with the initial carbon-to-nitrogen (C/N) ratio and a negative correlation with the initial nitrogen-to-phosphorus (N/P) ratio because Proteobacteria is the dominant phylum in litter decomposition processes and conducive to C and N cyclings[66]. Furthermore, Actinobacteria produces multiple degradation enzymes[67]. Although the abundance of Actinobacteria was higher in the *R. pseudoacacia* litter, its abundance was lower in litter mixtures with *R. pseudoacacia*. Therefore, the decline in the population of Proteobacteria and Actinobacteria when combined with *R. pseudoacacia* could be the primary reason for the additional impact on the decomposition of mixed litter.

At the genus level, microbes are sensitive to initial litter properties[27]. Our results showed that *Bradyrhizobium* was positively correlated with the C/N ratio, which is consistent with a report by Janssens et al.[68], who showed that a lower *Bradyrhizobium* abundance significantly reduced N fixation and increased decomposition. *Burkholderia* is negatively correlated with the N/P ratio because *Burkholderia* is a P solubilizer. Among the various microorganisms, only *Sphingomonas* exhibits a positive correlation with the remaining litter mass, as it can regulate C and N metabolism and degrade aromatic organic compounds[69]. During the entire process, litter decomposition selectively stimulates and increases the abundance of *Sphingomonas*[70]. Therefore, the relatively lower abundance of *Sphingomonas* in litter mixtures compared to littler monocultures may explain the non-additive effects observed in the decomposition of mixed litter. However, because of the short decomposition time, there is still an urgent need for more observational data to support the findings of this study.

Conclusions

Our study provides direct field evidence that the decomposition of mixed litter is influenced by the type of litter mixture,

which is mediated by the interactive modulation of litter properties and the composition of bacterial communities. Litter mixtures without *Robinia pseudoacacia* showed non-additive synergistic effects on litter decomposition, whereas litter mixtures with *R. pseudoacacia* exerted additive effects. These results indicate that nutrient release in mixtures was faster than that in monocultures, except for those with *R. pseudoacacia*. *R. pseudoacacia* may slow down ecosystem nutrient cycling, thus facilitating its invasion. Litter mixtures significantly modified the structure and composition of the bacterial community through nutritional links with litter traits. These findings have expanded our understanding of the mixing effects and microbial mechanisms underlying the acceleration of litter decomposition in mixed plantations compared with monoculture plantations, which indicate that the non-additive effects from mixed plantations are crucial for forest restoration and ecosystem health.

Author Contributions

The authors confirm contribution to the paper as follows: study conception and design: C.H. Zhang; data collection: Lu Y, Han R, Shen W, Yao Q, Gao Y; analysis and interpretation of results: Zhang C, Li C, Li K, Ni R, de-Miguel S; draft manuscript preparation: Li K, Wang Q, Zhang C. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

Conflict of interest

TThe authors declare that they have no conflict of interest.

Supplementary Information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/FR-2023-0022)

Dates

References

1. Tresch S, Frey D, Le Bayon RC, Zanetta A, Rasche F, et al. 2019. Litter decomposition driven by soil fauna, plant diversity and soil management in urban gardens. *Science of The Total Environment* 658:1614−29

2. Dighton J, Walsh E, Groben G, Zhang N. 2021. Influence of southern pine beetle on fungal communities of wood and bark decomposition of coarse woody debris in the New Jersey pine barrens. *Forestry Research* 1:17

3. Deng J, Fang S, Fang X, Jin Y, Kuang Y, et al. 2023. Forest understory vegetation study: current status and future trends. *Forestry Research* 3:6

4. Murúa JM, Gaxiola A. 2023. Variability in terrestrial litter decomposition can be explained by nutrient allocation strategies among soil decomposer communities. *Functional Ecology* 37:1642−52

5. Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, et al. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* 11:1065−71

6. Yang K, Zhu J, Zhang W, Zhang Q, Lu D, et al. 2022. Litter decomposition and nutrient release from monospecific and mixed litters: comparisons of litter quality, fauna and decomposition site effects. *Journal of Ecology* 110:1673−86

7. Grossman JJ, Cavender-Bares J, Hobbie SE. 2020. Functional diversity of leaf litter mixtures slows decomposition of labile but not recalcitrant carbon over two years. *Ecological Monographs* 90:e01407

8. Porre RJ, van der Werf W, De Deyn GB, Stomph TJ, Hoffland E. 2020. Is litter decomposition enhanced in species mixtures? A meta-analysis *Soil Biology & Biochemistry* 145:107791

9. Shi L, Xu J, Gui H. 2021. Effects of vegetation type and soil horizon on soil bacterial and fungal communities in a dry–hot valley. *Circular Agricultural Systems* 1:10

10. Tonin AM, Boyero L, Monroy S, Basaguren A, Pérez J, et al. 2017. Stream nitrogen concentration, but not plant N-fixing capacity, modulates litter diversity effects on decomposition. *Functional Ecology* 31:1471−81

11. Ullah MR, Carrillo Y, Dijkstra FA. 2023. Relative contributions of fungi and bacteria to litter decomposition under low and high soil moisture in an Australian grassland. *Applied Soil Ecology* 182:104737

12. Sun T, Hobbie SE, Berg B, Zhang H, Wang Q, et al. 2018. Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. *Proceedings of the National Academy of Sciences of the United States of America* 115:10392−97

13. Tao J, Zuo J, He Z, Wang Y, Liu J, et al. 2019. Traits including leaf dry matter content and leaf pH dominate over forest soil pH as drivers of litter decomposition among 60 species. *Functional Ecology* 33:1798−810

14. Santschi F, Gounand I, Harvey E, Altermatt F. 2018. Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems. *Functional Ecology* 32:522−32

15. Xiao W, Chen H, Kumar P, Chen C, Guan Q. 2019. Multiple interactions between tree composition and diversity and microbial diversity underly litter decomposition. *Geoderma* 341:161−71

16. Lu W, Liu N, Zhang Y, Zhou J, Guo Y, et al. 2017. Impact of vegetation community on litter decomposition: evidence from a reciprocal transplant study with ^{13}C labeled plant litter. *Soil Biology and Biochemistry* 112:248−57

17. Yang X, Qu Y, Yang N, Zhao H, Wang J, et al. 2019. Litter species diversity is more important than genotypic diversity of dominant grass species *Stipa grandis* in influencing litter decomposition in a bare field. *Science of The Total Environment* 666:490−98

18. Frainer A, Moretti MS, Xu WJ, Gessner MO. 2015. No evidence for leaf-trait dissimilarity effects on litter decomposition, fungal decomposers, and nutrient dynamics. *Ecology* 96:550−61

19. Liu J, Liu X, Song Q, Compson ZG, LeRoy CJ, et al. 2020. Synergistic effects: a common theme in mixed-species litter decomposition. *New Phytologist* 227:757−65

20. Gripp AR, de Assis Esteves F, Carneiro LS, Guariento RD, Figueiredo-Barros MP, et al. 2018. Weak to no effects of litter biomass and mixing on litter decomposition in a seasonally dry tropical forest. *Pedobiologia* 68:20−23

21. Desie E, Zuo J, Verheyen K, Djukic I, Van Meerbeek K, et al. 2023. Disentangling drivers of litter decomposition in a multi-continent network of tree diversity experiments. *Science of The Total Environment* 857:159717

22. Zhang L, Li J, Wang Z, Zhang D, Liu H, et al. 2023. Litter mixing promoted decomposition and altered microbial community in common bean root litter. *BMC Microbiology* 23:148

23. Zeng L, He W, Teng M, Luo X, Yan Z, et al. 2018. Effects of mixed leaf litter from predominant afforestation tree species on decomposition rates in the Three Gorges Reservoir, China. *Science of The Total Environment* 639:679−86

24. He Z, Yu Z, Huang Z, Davis M, Yang Y. 2016. Litter decomposition, residue chemistry and microbial community structure under two subtropical forest plantations: a reciprocal litter transplant study. *Applied Soil Ecology* 101:84−92

25. Su Z, Su B, Wu Y, Zhang Y, Wang J, et al. 2023. A less complex but more specialized microbial network resulted in faster fine-root decomposition in young stands of *Robinia pseudoacacia*. *Applied Soil Ecology* 182:104735

26. Hu Z, Xu C, McDowell NG, Johnson DJ, Wang MH, et al. 2017. Linking microbial community composition to C loss rates during wood decomposition. *Soil Biology and Biochemistry* 104:108−16

27. Barel JM, Kuyper TW, Paul J, de Boer W, Cornelissen JHC, et al. 2019. Winter cover crop legacy effects on litter decomposition act through litter quality and microbial community changes. *Journal of Applied Ecology* 56:132−43

28. Zhang W, Yang K, Lyu Z, Zhu J. 2019. Microbial groups and their functions control the decomposition of coniferous litter: a comparison with broadleaved tree litters. *Soil Biology and Biochemistry* 133:196−207

29. Žifčáková L, Větrovský T, Lombard V, Henrissat B, Howe A, et al. 2017. Feed in summer, rest in winter: microbial carbon utilization in forest topsoil. *Microbiome* 5:122

30. Purahong W, Kapturska D, Pecyna MJ, Jariyavidyanont K, Kaunzner J, et al. 2015. Effects of forest management practices in temperate beech forests on bacterial and fungal communities involved in leaf litter degradation. *Microbial Ecology* 69:905−13

31. Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, et al. 2011. Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology and Molecular Biology Reviews* 75:583−609

32. Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, et al. 2016. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular Ecology* 25:4059−74

33. Yan J, Wang L, Hu Y, Tsang Y, Zhang Y, et al. 2018. Plant litter composition selects different soil microbial structures and in turn drives different litter decomposition pattern and soil carbon sequestration capability. *Geoderma* 319:194−203

34. Stoker D, Falkner AJ, Murray KM, Lang AK, Barnum TR, et al. 2017. Decomposition of terrestrial resource subsidies in headwater streams: does consumer diversity matter? *Ecosphere* 8:e01868

35. Fanin N, Bertrand I. 2016. Aboveground litter quality is a better predictor than belowground microbial communities when estimating carbon mineralization along a land-use gradient. *Soil Biology and Biochemistry* 94:48−60

36. Sun H, Wang Q, Liu N, Li L, Zhang C, et al. 2017. Effects of different leaf litters on the physicochemical properties and bacterial communities in *Panax ginseng*-growing soil. *Applied Soil Ecology* 111:17−24

37. Jin X, Wang Z, Wu F, Li X, Zhou X. 2022. Litter mixing alters microbial decomposer community to accelerate tomato root litter decomposition. *Microbiology Spectrum* 10:e00186-22

Forestry
Research

38. Bai X, Dippold MA, An S, Wang B, Zhang H, et al. 2021. Extracellular enzyme activity and stoichiometry: the effect of soil microbial element limitation during leaf litter decomposition. *Ecological Indicators* 121:107200

39. Handa IT, Aerts R, Berendse F, Berg MP, Bruder A, et al. 2014. Consequences of biodiversity loss for litter decomposition across biomes. *Nature* 509:218–21

40. Dong X, Gao P, Zhou R, Li C, Dun X, et al. 2021. Changing characteristics and influencing factors of the soil microbial community during litter decomposition in a mixed *Quercus acutissima* Carruth. and *Robinia pseudoacacia* L. forest in Northern China. *CATENA* 196:104811

41. Marron N, Epron D. 2019. Are mixed-tree plantations including a nitrogen-fixing species more productive than monocultures? *Forest Ecology and Management* 441:242–52

42. Wang X, Lin D, Zhao L, Michalet R. 2023. The relative importance of coarse-scale climate and fine-scale nitrogen availability contrasts in driving home-field advantage effects in litter decomposition. *Ecosystems*

43. Lin C, Yang Y, Guo J, Chen G, Xie J. 2011. Fine root decomposition of evergreen broadleaved and coniferous tree species in mid-subtropical China: dynamics of dry mass, nutrient and organic fractions. *Plant and Soil* 338:311–27

44. Vanderbilt KL, White CS, Hopkins O, Craig JA. 2008. Aboveground decomposition in arid environments: results of a long-term study in central New Mexico. *Journal of Arid Environments* 72:696–709

45. Hultman J, Waldrop MP, Mackelprang R, David MM, McFarland J, et al. 2015. Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes. *Nature* 521:208–12

46. Hoorens B, Aerts R, Stroetenga M. 2003. Does initial litter chemistry explain litter mixture effects on decomposition? *Oecologia* 137:578–86

47. Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR. 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nature Communications* 8:14213

48. He Q, Wang S, Hou W, Feng K, Li F, et al. 2021. Temperature and microbial interactions drive the deterministic assembly processes in sediments of hot springs. *Science of The Total Environment* 772:145465

49. Zhou S, Butenschoen O, Barantal S, Handa IT, Makkonen M, et al. 2020. Decomposition of leaf litter mixtures across biomes: the role of litter identity, diversity and soil fauna. *Journal of Ecology* 108:2283–97

50. Chen Y, Ma S, Jiang H, Yangzom D, Cheng G, et al. 2021. Decomposition time, chemical traits and climatic factors determine litter-mixing effects on decomposition in an alpine steppe ecosystem in Northern Tibet. *Plant and Soil* 459:23–35

51. Zhang C, Li S, Zhang L, Xin X, Liu X. 2014. Litter mixing significantly affects decomposition in the Hulun Buir meadow steppe of Inner Mongolia, China. *Journal of Plant Ecology* 7:59–67

52. Zhang X, Wang Y, Jiang W, Mao R. 2020. Effect of expanded shrub litter on decomposition of graminoid litter in a temperate freshwater marsh. *Plant and Soil* 451:409–18

53. Pausas JG, Bond WJ. 2020. On the three major recycling pathways in terrestrial ecosystems. *Trends in Ecology & Evolution* 35:767–75

54. Lazzaro L, Mazza G, d'Errico G, Fabiani A, Giuliani C, et al. 2018. How ecosystems change following invasion by *Robinia pseudoacacia*: insights from soil chemical properties and soil microbial, nematode, microarthropod and plant communities. *Science of The Total Environment* 622–623:1509–18

55. Liu J, Zhang Z, Li Y, Han J, Si H, et al. 2022. Effects of the vegetative propagation method on juvenility in *Robinia pseudoacacia* L. *Forestry Research* 2:17

56. Chikowore G, Martin GD, Chidawanyika F. 2021. An assessment of the invasive alien tree, *Robinia pseudoacacia* canopy traits and its effect on grassland microclimates and subsequent arthropod assemblages. *Journal of Insect Conservation* 25:429–39

57. Nasir H, Iqbal Z, Hiradate S, Fujii Y. 2005. Allelopathic potential of *Robinia pseudo-acacia* L. *Journal of Chemical Ecology* 31:2179–92

58. Wang C, Wang W, Sardans J, Ouyang L, Tong C, et al. 2020. Higher fluxes of C, N and P in plant/soil cycles associated with plant invasion in a subtropical estuarine wetland in China. *Science of The Total Environment* 730:139124

59. Polyakova O, Billor N. 2007. Impact of deciduous tree species on litterfall quality, decomposition rates and nutrient circulation in pine stands. *Forest Ecology and Management* 253:11–18

60. Ren C, Zhao F, Kang D, Yang G, Han X, et al. 2016. Linkages of C:N:P stoichiometry and bacterial community in soil following afforestation of former farmland. *Forest Ecology and Management* 376:59–66

61. Krashevska V, Malysheva E, Klarner B, Mazei Y, Maraun M, et al. 2018. Micro-decomposer communities and decomposition processes in tropical lowlands as affected by land use and litter type. *Oecologia* 187:255–66

62. Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, et al. 2012. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions *The ISME Journal* 6:1749–62

63. Lladó S, López-Mondéjar R, Baldrian P. 2017. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. *Microbiology and Molecular Biology Reviews* 81:e00063-16

64. Niu X, Sun X, Chen D, Zhang S. 2020. Mixing litter from *Larix kaempferi* (lamb.) Carr. and broad-leaved trees enhances decomposition by different mechanisms in temperate and subtropical alpine regions of China. *Plant and Soil* 452:43–60

65. Sanaullah M, Chabbi A, Girardin C, Durand JL, Poirier M, et al. 2014. Effects of drought and elevated temperature on biochemical composition of forage plants and their impact on carbon storage in grassland soil. *Plant and Soil* 374:767–78

66. Ashworth AJ, DeBruyn JM, Allen FL, Radosevich M, Owens PR. 2017. Microbial community structure is affected by cropping sequences and poultry litter under long-term no-tillage. *Soil Biology and Biochemistry* 114:210–19

67. Sauvadet M, Chauvat M, Cluzeau D, Maron PA, Villenave C, et al. 2016. The dynamics of soil micro-food web structure and functions vary according to litter quality. *Soil Biology and Biochemistry* 95:262–74

68. Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, et al. 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience* 3:315–22

69. Chen L, Redmile-Gordon M, Li J, Zhang J, Xin X, et al. 2019. Linking cropland ecosystem services to microbiome taxonomic composition and functional composition in a sandy loam soil with 28-year organic and inorganic fertilizer regimes. *Applied Soil Ecology* 139:1–9

70. Zeng Q, Liu Y, Zhang H, An S. 2019. Fast bacterial succession associated with the decomposition of *Quercus wutaishanica* litter on the Loess Plateau. *Biogeochemistry* 144:119–31