Dynamic Characteristics of Dissolved Organic Carbon Release During Mixed Decomposition of Coniferous and Broadleaf Litter (Postprint)

Authors: Li Xun, Zhang Yan, SONG Simeng, Zhou Yang, Zhang Jian

Date: 2023-12-24T00:00:00+00:00

Abstract

To adjust the stand structure of low-efficiency Pinus massoniana (P) pure plantations and clarify the release patterns of dissolved organic carbon (DOC) during the mixed decomposition of its leaf litter with that of native broadleaf tree species, this study utilized leaf litter from Pinus massoniana, Cinnamomum camphora (C), and Toona sinensis (T) as research subjects. The litters were combined into 15 treatments (3 single-species treatments + 12 mixed treatments) according to different tree species and mass ratios for a field litter decomposition experiment, aiming to investigate the optimal tree species combination and mixing ratio for DOC release. The results showed that: (1) The DOC content in Pinus massoniana and most mixed treatment litters (except PT64) significantly increased during the early decomposition stage (0–6 months), exhibiting an enrichment phenomenon, and subsequently decreased with prolonged decomposition time, with a minor carbon enrichment phenomenon reoccurring in the middle-to-late decomposition stage (12–18 months) or at the decomposition end stage (18–24 months). Higher proportions of broadleaf litter corresponded to lower DOC content in later stages. (2) During the early decomposition stage (0–6 months), antagonistic effects on DOC release from mixed litters were strong (58.33%), with only 8.33% (1/12) of the mixed treatments exhibiting synergistic effects. Subsequently (6–18 months), synergistic effects gradually intensified (91.67% at 18 months), while synergistic effects weakened during the decomposition end stage (18–24 months) (66.67%). Among all mixed treatments, PT64 consistently showed synergistic effects throughout the entire decomposition period, followed by PT73, PCT622, and PCT613, which exhibited synergistic effects during most decomposition periods (3/4). (3) Partial Least Squares (PLS) regression analysis indicated that among the initial quality factors of leaf litter, N content, P content, lignin content, condensed tannin content, C/N, C/N, lignin/N, and lignin/P are important factors influencing DOC release from litter in the study area. Overall, DOC release from mixed Pinus massoniana and broadleaf litters was jointly influenced by tree species, mixing ratio, and decomposition time. Compared with other mixed treatments, mixed litter combinations with broadleaf proportions $ $30% and containing Toona sinensis (T) (PT64, PCT613, PCT622, and PCT613) were more effective in promoting DOC release.

Full Text

Dynamic Characteristics of Dissolved Organic Carbon Release During Mixed Decomposition of Coniferous and Broadleaf Litter

LI Xun[1], **ZHANG Yan**[1], **SONG Simeng**[1], **ZHOU Yang**[1], **ZHANG Jian**[2*]

[1]Research Center for Ecological Restoration and Characteristic Industry Cultivation in Hengduan Mountains Region, Sichuan Minzu College, Kangding 626001, Sichuan, China
[2]Key Laboratory of Forestry Ecological Engineering in Sichuan, Collaborative Innovation Center of Ecological Security in the Upper Reaches of Yangtze River, Sichuan Agricultural University, Chengdu 611130, China

Abstract: To adjust the stand structure of inefficient *Pinus massoniana* plantations and elucidate the release patterns of dissolved organic carbon (DOC) during mixed decomposition with native broadleaf species, this study examined leaf litter from *P. massoniana*, *Cinnamomum camphora* (C), and *Toona sinensis* (T). Fifteen treatments were established (three single-species treatments and twelve mixed treatments) based on different species combinations and mass ratios, and field decomposition experiments were conducted to identify optimal combinations for DOC release. The results showed that: (1) DOC content in *P. massoniana* and most mixed treatments (except PT64) significantly increased during early decomposition (0–6 months), exhibiting carbon enrichment, then decreased with decomposition time, with minor carbon enrichment reappearing in middle-to-late stages (12–18 months) or final stages (18–24 months). Higher broadleaf proportions correlated with lower DOC content in later stages. (2) Antagonistic effects on DOC release were strong in early decomposition (0–6 months) (58.33%), with only 8.33% (1/12) of mixed treatments showing synergistic effects. Synergistic effects gradually strengthened during 6–18 months (91.67% at 18 months) but weakened in final stages (18–24 months) (66.67%). Among all mixed treatments, PT64 exhibited synergistic effects throughout the entire decomposition period, followed by PT73, PCT622, and PCT613 which showed synergistic effects during most decomposition periods (3/4). (3) Partial least squares (PLS) regression analysis indicated that initial litter quality factors including N content, P content, lignin content, condensed tannin content, C/N, C/P, lignin/N, and lignin/P were important factors affecting DOC release in this study area. Overall, DOC release from mixed *P. massoniana* and broadleaf litter was jointly influenced by tree species, mixing ratio, and decomposition time. Compared with other mixed treatments, combinations with broadleaf proportion $ $30% containing *T. sinensis* (PT64, PCT613, PCT622, and PCT613) better promoted DOC release.

Keywords: *Pinus massoniana*; native broadleaf tree species; mixed leaf litter; dissolved organic carbon; synergistic effects; antagonistic effects

---

Litter-derived dissolved organic carbon (DOC) constitutes an important component of forest material cycling and a significant source of soil active carbon pools, playing a crucial role in maintaining forest ecosystem stability and organic matter production. Investigating DOC release characteristics during litter decomposition is essential for maintaining forest ecosystem stability and accurately assessing ecosystem carbon cycling. Forest species composition directly affects litter types, thereby influencing soil material composition and nutrient accumulation. Broadleaf species generally release more DOC than conifers due to higher concentrations of soluble compounds such as sugars, low-molecular-weight phenolics, and secondary metabolites. For instance, broadleaf litter can release high concentrations of soluble nitrogen (N) and phosphorus (P), resulting in higher DOC-to-soluble-nutrient ratios in leachates. Litter structure characteristics, including surface wax and trichomes, and water-holding capacity also significantly affect DOC quantity. Conifers possess thicker epidermal and subcutaneous tissues that protect litter tissues and substances, whereas broadleaf litter is less rigid, more fragile, and more conducive to leaching soluble substances, making broadleaf-derived soluble compounds more readily released. Thus, conifer DOC release and production are constrained by inherent "substrate quality," and enhancing decomposition rates and DOC production is crucial for forest ecosystem nutrient cycling.

Recent research has extensively examined mixed decomposition characteristics and nutrient dynamics of coniferous-broadleaf litter. For example, Zhang et al. (2019) found that mixing *Picea asperata* and *Betula albo-sinensis* litter enhanced mutual decomposition rates by 8.67% and 8.11%, respectively. Xiao (2015) reported that carbon release rates in mixed *Eucalyptus urophylla* × *E. grandis* and *Robinia pseudoacacia* litter were significantly higher than in single-species eucalyptus litter during late decomposition stages. Zhang et al. (2022) observed that mixing *P. massoniana* and *T. sinensis* litter promoted cellobiohydrolase and β-glucosidase activities, while mixing with *Sassafras tzumu* and *C. camphora* enhanced polyphenol oxidase and peroxidase activities. Mixed litter decomposition exhibits mixing effects, and DOC from different species can produce non-additive effects on biodegradability. Non-additive effects are classified as synergistic (observed value significantly exceeds expected value by >0) or antagonistic (observed value significantly below expected value). Some plant litter leachates, rich in phenolics and tannins, inhibit bacterial growth and metabolism, and mixing such litter with others can reduce DOC biodegradability, demonstrating antagonistic effects. Forest litter typically exists as mixtures on the forest floor, and mixed litter facilitates nutrient transfer between species (e.g., high-quality DOC nutrients transferring to low-quality DOC), creating complementary effects in nutrient utilization. Therefore, single-species DOC decomposition characteristics are insufficient to accurately reflect carbon and nutrient cycling in forest ecosystems, making studies of DOC release from mixed litter decomposition crucial for ecosystem stability and carbon cycle assessment.

Pinus massoniana is widely planted in southern China due to its adaptability to drought and infertile soils and its versatile applications. However, as plantations age, pure *P. massoniana* stands face declining soil fertility, increased pest and disease pressure, and reduced plant diversity, threatening forest health. Mixed-species planting represents an effective near-natural management approach to promote soil organic carbon formation and stability. Different litter quantities and qualities in mixed forests alter mixed litter substrate quality, thereby affecting DOC release and surface soil carbon and nitrogen pools. Previous research indicates that harder, thicker, and coarser-textured litter decomposes more slowly. Compared with conifers, broadleaf litter has larger surface areas, softer texture, lower C/N, lignin/N, and phenolic content, but higher DOC, N, and P content, which benefits soil fertility and microbial communities. *Toona sinensis* and *Cinnamomum camphora* are common native broadleaf species in southwestern *P. massoniana* mixed forests. *T. sinensis*, a valuable fast-growing timber species with edible and medicinal properties, is widely distributed in southwestern provinces. *C. camphora*, a precious timber and ornamental species, is commonly planted as a companion species in ecological restoration and landscaping. However, research is lacking on DOC release characteristics when *P. massoniana* litter is mixed with these native broadleaf litters at different ratios. This study selected *P. massoniana* litter and two native broadleaf litters (*T. sinensis* and *C. camphora*) for field decomposition experiments, mixing them in one-conifer-one-broadleaf and one-conifer-two-broadleaf combinations at various ratios to investigate DOC release characteristics and provide theoretical guidance for species selection and "mixed-plantation" transformation of pure *P. massoniana* forests in southwestern China.

1.1 Study Area Description

The study was conducted at the Sichuan Agricultural University Field Experimental Base in Dujiangyan City, Sichuan Province (103°34 –103°36 E, 31°01 –31°02 N). The region features a typical subtropical monsoon humid climate with mean annual precipitation of approximately 1,243.8 mm and mean annual temperature of 15.2 °C. Elevation ranges from 805 to 850 m. Dominant tree species include *P. massoniana*, *Cunninghamia lanceolata*, *C. camphora*, and *Camptotheca acuminata*. In early August 2016, three 30 m × 30 m plots were established within the experimental base using fences, selecting mixed forests of *P. massoniana* and *C. camphora* with similar topography, elevation, aspect, soil type, species composition, and stand density for field decomposition experiments. Basic plot information is presented in Table 1 .

Table 1 Basic information regarding the three plots (n=3)

Altitude (m)	Slope (°)	Aspect	Total carbon ($g \cdot kg^{-1}$)	Total nitrogen ($g \cdot kg^{-1}$)	Soil bulk density ($g \cdot cm^{-3}$)
811.22$±13.35	10.67±5.31	*S*	12.27±2.01	0.73±0.15	1.42±0.03
824.94±11.45	13.11±6.31	*SE*	13.67±2.11	0.70±	

Note: S = South; SE = Southeast.

1.2 Sample Preparation

From April to June 2016, leaf litter of *P. massoniana* (P), *C. camphora* (C), and *T. sinensis* (T) was collected at Laifu State Forest Farm, Gaoxian County, Yibin City, Sichuan Province (104°48 E, 28°11 N). Fresh, damaged, and partially decomposed leaves were removed, and the litter was air-dried at room temperature for two weeks. Samples weighing 15.00 ± 0.05 g were placed in decomposition bags measuring 23 cm × 20 cm (internal dimensions) with 3.00 mm mesh on the upper layer and 0.04 mm nylon mesh on the lower layer. Based on the principle that "the proportion of main tree species in mixed forests should not be less than 60%" [Lü SY, 2001; Yang XF and Ye JS, 2001], fifteen litter combinations were established (Table 2), totaling 240 bags = [(3 single-species treatments + 12 mixed treatments) × 4 sampling periods × 3 plots + 60 (backup for potential loss or damage)]. In mid-August 2016, all decomposition bags were carefully transferred to the three pre-established 30 m × 30 m plots and randomly placed on the soil surface with 2–5 cm spacing between adjacent bags to avoid mutual influence. After placement, three bags were randomly collected to calculate transport/placement loss rates and determine air-dry moisture content. iButton temperature loggers (iButton DS1921G, Maxim Integrated, USA) were installed in each plot, recording temperature every 2 hours.

Table 2 Detailed description of the different treatments

Mixed treatment	Abbreviation	Mixed proportion
Contrast	P/C/T	Single species
P. massoniana + one broadleaf tree species	PC/PT	8:2, 7:3, 6:4
P. massoniana + two broadleaf tree species	PCT	8:1:1, 7:2:1, 7:1:2, 6:3:1, 6:2:2, 6:1:3

Note: P = Pinus massoniana*; C =* Cinnamomum camphora*; T =* Toona sinensis*; PC =* P. massoniana* + *C. camphora*; PT = *P. massoniana* + *T. sinensis*; PCT = *P. massoniana* + *C. camphora* + *T. sinensis*. The same below.*

1.3 Sample Collection

Field decomposition experiments began in August 2016, with samples collected every 6 months for a total of four collections: February 2017 (6 months), August

2017 (12 months), February 2018 (18 months), and August 2018 (24 months). During each collection, three bags of each of the 15 treatments were randomly sampled from the three plots and transported to the laboratory, where soil particles, arthropods, and foreign plant roots were carefully removed with tweezers before measuring dry mass.

1.4 Sample Analysis

Litter samples were dried in a forced-air oven at 65 °C for 48 h and weighed to determine dry mass for calculating remaining mass. Dried samples were ground, passed through a 0.25 mm sieve, and stored in sealed bags. DOC content was determined by weighing 1 g of ground sample into a centrifuge tube with 50 mL deionized water, shaking at 180 $r \cdot min^{-1}$ for 4 h, centrifuging at 6,000 $r \cdot min^{-1}$ for 10 min, and filtering the supernatant through a 0.45 m membrane (filtration pressure -0.09 MPa). DOC concentration was measured using a total organic carbon analyzer (Vario TOC cube; Elementar Analysis system GmbH, Langenselbold, Germany). Initial quality parameters were measured as follows: total carbon by TOC analyzer; total nitrogen by Kjeldahl method (LY/T 1269—1999); total phosphorus by molybdenum-antimony colorimetry (LY/T 1270—1999); total phenols by Folin-Ciocalteu method; condensed tannins by vanillin-HCl method [Schofield et al., 1998]; lignin and cellulose by Van Soest detergent fiber method [Vanderbilt et al., 2008].

1.5 Data Processing and Statistical Analysis

The DOC release rate was calculated using the following formula and variables: R represents the DOC release rate (%) at each sampling; Mt is the remaining litter mass (g) at sampling; M0 is the initial litter mass (g) before decomposition; Ct is the DOC content ($g \cdot kg^{-1}$) at sampling; C0 is the initial DOC content ($g \cdot kg^{-1}$) before decomposition. The release rate was calculated as: $R = (1 - (Mt \times Ct)/(M0 \times C0)) \times 100\%$.

Expected values were calculated as: $A \times n1 + B \times n2 + C \times n3$, where A, B, and C represent DOC release rates from species A, B, and C in single-species decomposition, and n1, n2, and n3 represent their respective proportions in the mixture.

One-way ANOVA with Turkey's test was used to compare DOC release rates among three single species (*P. massoniana*, *C. camphora*, and *T. sinensis*), different mixed treatments, and different decomposition periods for the same treatment. Levene's test assessed homogeneity of variance, with log transformation applied when assumptions were violated. Expected values represented theoretical DOC release rates under mixed decomposition, while observed values represented actual rates. Independent t-tests ($\alpha = 0.05$) distinguished between additive effects (no significant difference between observed and expected values) and non-additive effects (significant difference). Non-additive effects were further classified as synergistic (observed - expected > 0, $P < 0.05$) or antagonistic (observed - expected < 0, $P < 0.05$).

Partial least squares (PLS) regression analyzed the relative importance of initial litter chemistry and stoichiometry on DOC release rate mixing effects (observed - expected). Variable importance in projection (VIP) values indicated factor contributions, with VIP > 1 representing significant contributions to DOC release rate variation. SPSS 26.0 and Excel 2013 were used for statistical analysis and graphing.

Results

Initial Quality Characteristics

Initial quality characteristics of the three single-species litters are shown in Table 3 . The two native broadleaf species exhibited significantly lower C content, lignin content, cellulose content, total phenol content, condensed tannin content, C/N, C/P, lignin/N, and lignin/P than *P. massoniana*. N and P contents were highest in *T. sinensis*, intermediate in *C. camphora*, and lowest in *P. massoniana*. No significant differences were found in N/P among the three species. Overall, mixed litters with higher *P. massoniana* proportions had higher C, lignin, cellulose, total phenol, condensed tannin, lignin/N, and lignin/P contents, while those with greater *T. sinensis* and *C. camphora* proportions had higher N and P contents (Table 4).

Table 3 Initial quality characteristics of leaf litter from three single tree species (n=3)

Parameter	*Pinus massoniana*	*Cinnamomum camphora*	*Toona sinensis*
Total carbon ($g \cdot kg^{-1}$)	452.71 (6.27) A	420.77 (6.32) B	378.95 (2.42) C
Total nitrogen ($g \cdot kg^{-1}$)	6.07 (0.41) C	8.22 (0.47) B	11.46 (0.40) A
Total phosphorus ($g \cdot kg^{-1}$)	0.92 (0.02) B	1.11 (0.07) B	1.41 (0.06) A
Lignin ($g \cdot kg^{-1}$)	351.07 (8.64) A	149.63 (4.16) B	134.4 (6.02) B
Cellulose ($g \cdot kg^{-1}$)	136.46 (12.72) A	144.72 (5.81) A	99.19 (3.51) B
Total phenol ($g \cdot kg^{-1}$)	54.17 (2.10) A	14.82 (0.30) C	29.05 (0.44) B
Condensed tannin ($g \cdot kg^{-1}$)	24.75 (0.77) A	13.38 (0.07) B	3.67 (0.01) C
C/N	75.34 (5.43) A	51.52 (2.81) B	33.16 (1.33) C
C/P	492.79 (16.40) A	380.54 (21.27) B	269.45 (8.99) C
N/P	6.59 (0.38) A	7.47 (0.76) A	8.17 (0.54) A
Lignin/N	58.24 (2.86) A	18.38 (1.52) B	11.76 (0.67) B
Lignin/P	381.87 (10.95) A	135.45 (9.37) B	95.84 (7.30) B

Note: Values are means (standard error). Different capital letters indicate significant differences in initial substance content or stoichiometric ratios among tree species (P < 0.05). The same below.

Table 4 Initial quality characteristics of mixed leaf litter (n=3)

Mixed treatment	Total carbon (g · kg^{-1})	Total nitrogen (g · kg^{-1})	Total phosphorus (g · kg^{-1})	Lignin (g · kg^{-1})	Cellulose (g · kg^{-1})	Total phenol (g · kg^{-1})	Condensed tannin (g · kg^{-1})	C/N	Lignin/N	Lignin/P
PC82	437.96 (7.26) ABC	7.15 (0.35) AB	1.02 (0.03) BCD	307.74 (9.2) A	129.01 (15.28) A	49.15 (2.26) A	20.54 (0.87) ABC	61.44 (3.07) ABC	43.11 (0.93) ABC	302.29 (4.35) ABC
PC73	430.58 (6.49) ABC	7.68 (0.23) A	1.07 (0.03) ABC	286.07 (7.85) AB	125.28 (13.93) A	46.63 (1.91) ABC	18.43 (0.76) CDE	56.08 (1.72) ABC	37.23 (0.16) CDEF	268.10 (0.52) CDEF
PC64	423.2 (5.76) C	8.22 (0.12) B	1.12 (0.03) A	264.40 (6.69) B	121.55 (12.59) A	44.12 (1.56) ABCD	16.32 (0.66) E	51.47 (0.83) C	32.15 (0.36) F	236.96 (2.50) G
PT82	446.32 (5.31) A	6.5 (0.58) AB	0.96 (0.03) D	310.78 (9.70) A	138.11 (13.46) A	46.30 (2.45) AB	22.48 (0.85) A	69.29 (6.65) A	48.13 (3.43) A	324.62 (17.54) A
PT73	443.13 (3.53) AB	6.71 (0.58) AB	0.98 (0.03) CD	290.64 (8.51) AB	138.94 (11.27) A	42.37 (2.20) ABCD	21.34 (0.74) AB	66.56 (6.21) AB	43.57 (3.19) ABC	297.75 (18.24) ABCD
PT64	439.93 (1.75) ABC	6.93 (0.59) AB	1.00 (0.04) BCD	270.50 (7.41) B	139.77 (9.18) A	38.43 (1.94) D	20.21 (0.63) ABC	64.01 (5.81) AB	39.30 (3.00) BCDE	271.95 (18.56) CDEFG
PCT811	442.14 (6.28) AB	6.82 (0.46) AB	0.99 (0.02) CD	309.26 (9.35) A	133.56 (14.34) A	47.73 (2.36) AB	21.51 (0.86) AB	65.14 (4.65) ABC	45.47 (2.05) AB	313.00 (10.41) AB
PCT721	438.95 (4.51) ABC	7.04 (0.47) AB	1.01 (0.02) BCD	289.12 (8.06) AB	134.39 (12.08) A	43.79 (2.10) ABCD	20.37 (0.75) ABC	62.67 (4.37) ABC	41.21 (1.97) ABCD	287.08 (11.50) ABCDE
PCT712	434.76 (5.50) ABC	7.36 (0.35) AB	1.04 (0.02) ABCD	287.59 (7.84) AB	129.83 (12.98) A	45.21 (2.00) ABCD	19.40 (0.76) BCD	59.23 (2.89) ABC	39.12 (0.98) CDEF	277.23 (5.64) BCDEF
PCT631	435.75 (2.74) ABC	7.25 (0.47) AB	1.03 (0.02) ABCD	268.97 (6.83) B	135.21 (9.89) A	39.86 (1.85) CD	19.23 (0.63) BCD	60.36 (4.10) ABC	37.21 (1.92) CDEF	262.17 (12.27) DEFG
PCT622	431.57 (3.74) ABC	7.58 (0.35) AB	1.06 (0.01) ABC	267.45 (6.49) B	130.66 (10.71) A	41.28 (1.75) BCD	18.26 (0.64) CDE	57.16 (2.73) ABC	35.35 (1.03) DEF	253.14 (6.82) EFG
PCT613	427.39 (4.75) BC	7.90 (0.23) AB	1.09 (0.02) AB	265.93 (6.44) B	126.11 (11.62) A	42.70 (1.65) ABCD	17.29 (0.65) DE	54.15 (1.64) BC	33.67 (0.31) EF	244.76 (2.17) FG

Note: Numbers following letters in treatment abbreviations represent mass ratios of different tree species' leaf litter.

DOC Content Dynamics

Single-Species Litter Initial DOC content was highest in *T. sinensis* litter, intermediate in *C. camphora*, and significantly lowest in *P. massoniana* (Figure 1 [Figure 1: see original paper]). After 6 months of decomposition, DOC content decreased significantly in *T. sinensis*, showed no significant change in *C. camphora*, but increased significantly in *P. massoniana*, demonstrating carbon enrichment. During 6–12 months, DOC content decreased significantly in all three species, then increased during 12–24 months, showing enrichment phenomena. Overall, DOC content decreased during the first year (except in *P. massoniana*), but increased during the second year across all species.

One-Conifer-One-Broadleaf Mixed Litter DOC content in PC and PT mixtures showed significant differences among combinations (Figure 2 [Figure 2: see original paper]). Initial DOC content (at 0 months) increased with broadleaf proportion in both PT and PC mixtures. In PT mixtures, PT82 was consistently highest throughout the two-year decomposition period; PT73 was significantly higher than PT64 only at 12 months, with no significant differences at other times (Figure 2A). In PC mixtures, PC82 was consistently highest; PC73 was significantly lower than PC64 at 12 months but higher at 24 months, with no significant differences at other times (Figure 2B). Over time, PT82 showed increase-decrease-increase-decrease patterns, PT73 showed increase-decrease, and PT64 showed decrease-increase-decrease, with all PT mixtures peaking at 6 months (Figure 2A). All PC mixtures showed increase-decrease patterns from 0–12 months, stabilizing during 18–24 months, also peaking at 6 months (Figure 2B). Most mixed treatments exhibited carbon enrichment during early decomposition (0–6 months), after which DOC content gradually decreased, with higher broadleaf proportions resulting in lower later-stage DOC content.

One-Conifer-Two-Broadleaf Mixed Litter DOC content in PCT mixtures is shown in Figure 3 [Figure 3: see original paper]. Initial content increased with broadleaf proportion, especially *T. sinensis* proportion, following the order: PCT631 > PCT622 > PCT613 > PCT712 > PCT721 > PCT811. Specific patterns across decomposition periods showed: PCT712 at 6 months, PCT811 at 12 months, PCT631 at 18 months, and PCT721 at 24 months had highest DOC content; while PCT631 at 6 months, PCT631/622/613 at 12 months, PCT622 at 18 months, and PCT613 at 24 months had lowest content. Generally, broadleaf proportions $ $40% (except at 18 months) resulted in lower DOC content. Over time, PCT811, PCT721, and PCT622 showed increase-decrease-increase patterns, while PCT712, PCT631, and PCT613 showed increase-decrease-increase-decrease patterns, all peaking at 6 months. All PCT mixtures exhibited significant carbon enrichment during early decomposition (0–6 months), decreased during 6–12 months, then increased again during 12–18 or 18–24 months, showing minor secondary carbon enrichment.

Mixing Effects on DOC Release

Non-additive effects on DOC release rates were observed during all four sampling periods (Figure 4 [Figure 4: see original paper]). After 6 months, 8.33% (1/12) of mixed litters showed synergistic effects (observed - expected > 0, $P < 0.05$) and 58.33% (7/12) showed antagonistic effects (observed - expected < 0, $P < 0.05$) (Figure 4A). After 12 months, 41.67% (5/12) showed synergistic effects and 8.33% (1/12) showed antagonistic effects (Figure 4B). At 18 months, all mixed treatments had observed - expected values > 0, with 91.67% (11/12) exhibiting synergistic effects (Figure 4C). At 24 months, 66.67% (8/12) showed synergistic effects (Figure 4D). Overall, antagonistic effects dominated early decomposition (0–6 months), synergistic effects strengthened during 6–18 months, but non-additive effects (mainly synergistic) weakened in final stages (18–24 months).

Synergistic effect frequency varied by species combination: PT (75%, 9/12) > PCT (45.83%, 11/24) > PC (41.67%, 5/12). Among all treatments, PT64 exhibited synergistic effects throughout decomposition, while PT73, PCT622, and PCT613 showed synergistic effects during most periods (3/4). In contrast, PC mixtures (PC82, PC73, PC64), PT82, PCT811, PCT721, and PCT631 showed no synergistic effects during the first year (6 and 12 months). Mixing effects varied with species combination, mixing ratio, and decomposition time, with broadleaf proportion \$ \$30% containing *T. sinensis* (PT64, PT73, PCT622, and PCT613) showing stronger synergistic effects.

Figure 4 Relative mixing effect of 12 mixed leaf litter treatments on DOC release rate during four decomposition periods. (A) 6 months; (B) 12 months; (C) 18 months; (D) 24 months. Numbers above the x-axis represent the proportion of mixed treatments showing synergistic effects; numbers below represent antagonistic effects. * indicates significant difference ($P < 0.05$), ** indicates extremely significant difference ($P < 0.01$) between observed and expected values.

PLS Regression Analysis of Initial Chemistry and DOC Release Mixing Effects

Variable importance in projection (VIP) values indicated differential explanatory power of initial litter quality on DOC release mixing effects (Figure 5 [Figure 5: see original paper]). The relative importance of initial chemical parameters in explaining DOC release rate was: lignin/N > lignin/P > condensed tannin content > P content > N content > C/N > C/P > lignin content > C content > cellulose content > N/P > total phenol content. DOC release rate showed significant positive correlations with N and P contents, and significant negative correlations with lignin content, condensed tannin content, C/N, C/P, lignin/N, and lignin/P.

Figure 5 PLS regression analysis for the initial concentrations of litter mixtures in explaining variation of the DOC mixed effect (observed - expected). VIP values > 1 indicate significant contributions of predictive factors. Black circles indicate positive correlations; white circles indicate negative correlations.

Discussion

Litter represents a critical component of forest ecosystem nutrient cycling and carbon dynamics. Despite comprising a small proportion of total forest biomass, litter regulates soil fertility through nutrient return, maintaining stand productivity and biodiversity. Nutrient turnover in litter occurs faster than in living trees through metabolic processes. Mixed decomposition studies commonly use non-additive and additive effects to assess mass and substance changes. This study found antagonistic effects dominated early decomposition (0–6 months), synergistic effects strengthened during 6–18 months, but weakened in final stages (18–24 months).

During early decomposition (0–6 months), litter structure remained relatively intact with high concentrations of recalcitrant compounds like lignin and tannins. Litter must first be fragmented by soil fauna before fungal and bacterial colonization [Deng CJ et al., 2022], limiting leaching and microbial decomposition. The carbon enrichment observed at 6 months may reflect autumn-winter conditions (August 2016 to February 2017) when litterfall was abundant but rainfall was low, reducing leaching, while low winter temperatures decreased microbial activity and DOC biodegradation. When element release rates fall below dry mass loss rates, element concentrations increase, showing enrichment [Wang XH et al., 2004]. During 6–18 months, synergistic effects strengthened as litter underwent humification and nutrient transfer occurred from high-quality to low-quality litter, increasing microbial abundance and activity [Deng CJ et al., 2022] and promoting decomposition and DOC release. After initial decomposition, litter fragmentation accelerated leaching of soluble carbohydrates. Weakened synergistic effects in late decomposition may result from reduced DOC concentrations and increased relative abundance of lignin-derived recalcitrant compounds, yielding leachates with more aromatic compounds and fewer bioavailable nutrients [Del et al., 2017], creating negative complementary effects [Kalbitz et al., 2006; Butenschoen et al., 2014].

Mixing effects varied with species combination and ratio. Broadleaf proportion \$ 30 \$3/4), promoting DOC release. This aligns with previous research [Wardle et al., 1997; Gartner & Cardon, 2004] showing that nutrient-rich broadleaf litter (high N, P) facilitates decomposition of nutrient-poor conifer litter, with stronger effects when nutrient differences are greater. When species proportions are unequal, each species' contribution to decomposition varies [Hoorens et al., 2003; Hättenschwiler, 2005], and uneven mixing ratios can improve microenvironmental conditions [Vestgarden, 2001]. Mixed litter increases compound diversity, meeting requirements of different decomposers and accelerating organic carbon decomposition [Pérez-harguindeguy et al., 2000]. DOC biodegradation nutrient limitation patterns resemble litter decomposition processes [Del et al., 2017], where N and P as key factors regulate decomposition rates through microbial nutrient availability [Vestgarden, 2001; Xiang & Bauhus, 2007]. *T. sinensis* had significantly higher N and P contents than *P. massoniana* and *C. camphora*, and mixing increased readily decomposable nutrient content and improved litter quality [Wardle et al., 1997]. *T. sinensis* litter is softer and more easily humified by rainfall leaching [Li X et al., 2016], rapidly improving substrate quality for soil fauna and microbes, ultimately promoting decomposition and nutrient release. Thus, mixtures with higher *T. sinensis* or broadleaf proportions showed synergistic effects during the first decomposition year.

PLS regression confirmed that mixing effects (observed - expected) correlated closely with initial litter quality. Significant positive relationships with N and P contents and negative relationships with lignin, condensed tannins, C/N, C/P, lignin/N, and lignin/P align with previous research [Su ZX et al., 2022; Zhou TY et al., 2022], indicating DOC release is primarily controlled by initial litter properties. Mixing effects depend on nutrient content, stoichiometric ratios, and recalcitrant compound concentrations. Mixtures with broadleaf proportion \$ \$30% containing *T. sinensis* (PT64, PT73, PCT622, and PCT613) had relatively high initial N and P contents, accelerating microbial nutrient transfer to compensate for stoichiometric imbalances between litter and consumers [Wang YT and Lu JB, 2017], thereby promoting decomposition and DOC release from low-quality litter. Soil community structure and feeding preferences also affect decomposition, as soft, nutrient-rich litter (e.g., *T. sinensis*) is more easily colonized and consumed [Baña et al., 2014]. Mixing with soluble nutrient-rich broadleaf litter increases microbial diversity, altering bacterial community structure and metabolism and enhancing microbial activity [Cassart et al., 2020], promoting DOC biodegradability. Our previous research [Zhang Y et al., 2023] also found higher soil fauna densities in broadleaf-rich mixtures. Therefore, mixtures with broadleaf proportion \$ \$30% containing *T. sinensis* have relatively high N and P contents and larger leaf surface areas, facilitating soil biological colonization and decomposition.

In conclusion, mixing *P. massoniana* with two native broadleaf species promoted DOC release, with *P. massoniana*-*T. sinensis* mixtures at \$ \$30% broadleaf proportion particularly enhancing synergistic DOC release. Initial litter quality factors including N content, P content, lignin content, condensed tannin content, lignin/N, and lignin/P were key determinants of DOC release in this region. When establishing mixed *P. massoniana* plantations, *T. sinensis* should be considered as a companion species, with a broadleaf litter proportion of 30% to promote forest carbon cycling. These results provide theoretical guidance for transforming pure *P. massoniana* plantations into mixed stands.

References

DENG CJ, YUAN F, PU TD, et al., 2022. Influence of soil fauna on litter decomposition in central Guizhou karst forest[J]. For Res, 35(3): 72-81.

DING YD, XU JQ, ZHENG J, et al., 2021.Quantity and optical characteristics of dissolved organic matter derived from decomposing leaf litter on the ground and in the air in typical subtropical plantations[J]. Chin J Ecol, 40(6): 1599-1608.

HU JW, YANG GJ, LIU ZH, et al., 2021. Growth and stand structure differences of *Toona sinensis* plantation from different slope positions[J]. J NW For Univ, 36(5): 82-87.

JIANG X, CHEN H, HU TX, et al., 2015. Inhibition of decomposing leaf litter of *Cinnamomum camphora* on growth of *Pharbitis nil* and the alleviation effect of nitrogen application[J]. Chin J Appl and Environ Biol, 21(5): 926-932.

LI JM, ZHANG YT, LI X, et al., 2017. Impact of precipitation Intensity on the decomposition of floor litter and the fine roots of *Picea schrenkiana*[J]. Bull Bot Res, 37(3): 360-369.

LIN KM, ZHANG ZQ, YE FM, et al., 2010. Dynamic analysis of decomposition characteristics and content change of nutrient elements of leaf litter of *Cunninghamia lanceolata*, *Phoebe boumei* and *Schima superba* under *C. lanceolata* artificial forest[J]. J Plant Resour Environ, 19(2): 34-39.

LI X, ZHANG J, YANG WQ, et al., 2016. Effect of forest gap on carbon release of *Toona Ciliata* leaf litter[J]. J Nat Resour, 31(7): 1114-1126.

LI YN, ZHOU XM, ZHANG NL, et al., 2016. The research of mixed litter effects on litter decomposition in terrestrial ecosystems[J]. Acta Ecol Sin, 36(16): 4977-4987.

LI ZA, ZHOU B, DING YZ, et al., 2004. Key factors of forest litter decomposition and research progress[J]. Chin J Ecol, 23(6): 77-83.

LV SY, 2001. Several basic viewpoints on construction of mixed forests[J]. Yunnan For Sci Technol, 1(1): 26-28.

SU ZX, SU BQ, SHANGGUAN ZP, 2022. Advances in effects of plant litter decomposition on the stability of soil organic carbon[J]. Res Soil Water Conserv, 29(2): 406–413.

WANG XH, HUANG JJ, YAN ER, 2004. Leaf litter decomposition of common trees in Tiantong[J]. Chin J Plant Ecol, 28: 457–467.

WANG YT, LU JB, 2017. A review on litter decomposition and its impact factor in terrestrial ecosystems[J]. Bull Sci Technol, 33(10): 2–3.

WAN XB, WANG QG, YAN GY, et al., 2019. Response of ecological stoichiometric characteristics and photosynthetic characteristics of plant leaves to long-term N deposition in natural secondary forest[J]. Bull Bot Res, 39(3): 407-420.

WU MJ, YOU YJ, ZHANG XH, et al., 2019. Effects of infected pure *Pinus massoniana* different disturbance patterns on stand structure of plantation[J]. Chin J Appl Ecol, 30(1): 58-66.

XIAO LY, 2015. Researches on the leaf litter decomposition dynamic of *Eucalyptus urophylla*×*Eucalyptus grandis* mixed with other species leaf litters [D]. Chongqing: Southwest University.

XIAO N, MO XQ, TAN XM, et al., 2022. Effects of multi-layer of *Pinus massoniana* and mixed-age forest management plantations on carbon components and transformation of soil aggregates[J]. Guihaia, 42(4): 595-607.

XIE YS, MENG JH, ZENG J, et al., 2023. Analysis on the effect of close-to-nature transformation of *Pinus massoniana* pure forest plantation[J]. For Res, 36(2):31-38.

YANG XF, YE JS, 2001. Preliminary study on directed cultivation methods for big diameter timber of *Cunninghamia lanceolata*[J]. Jiangxi For Sci Technol, (2): 32-34.

ZHANG XX, LIU H, WANG B Y, et al., 2019. Characteristics of the mixed decomposition of fresh litter of *Piceaasperata* and broadleaved species[J]. Ecol Environ, 28(2): 235-244.

ZHANG Y, LI X, SONG SM, et al., 2023. Characteristics of soil fauna community structure during mixed decomposition of needle and broad leaf litter[J]. J For Environ, 43(1): 92–102.

ZHANG Y, YUAN YL, LI X, et al., 2022. Variation characteristics of activities of carbon cycle-related enzymes of mixed leaf litters of *Pinus massoniana* and three broad-leaved tree species during decomposition period[J]. J Plant Resour Environ, 31(1): 29-41.

ZHOU TY, XIAO Y, HUANG QY, et al., 2022. Forest litter decomposition: research progress and prospect[J]. Chin Agric Sci Bull, 38(33): 44–51.

BAÑA Z, AYO B, MARRASÉ C, et al., 2014. Changes in bacterial metabolism as a response to dissolved organic matter modification during protozoan grazing in coastal cantabrian and mediterranean waters[J]. Environ Microbiol, 16(2): 498–511.

BRADFORD MA, BERG B, MAYNARD DS, et al., 2016. Understanding the dominant controls on litter decomposition[J]. J Ecol, 104(1): 229-238.

BUTENSCHOEN O; KRASHEVSKA V, MARAUN M, et al., 2014. Litter mixture effects on decomposition in tropical montane rainforests vary strongly with time and turn negative at later stages of decay[J]. Soil Biol Biochem, 77: 121–128.

CASSART B, BASIA AA, JONARD M, et al., 2020. Average leaf litter quality drives the decomposition of single-species, mixed-species and transplanted leaf litters for two contrasting tropical forest types in the Congo Basin (DRC)[J]. Ann For Sci, 77(2): 1-20.

DEL GIUDICE R, LINDO Z, 2017. Short-term leaching dynamics of three peatland plant species reveals how shifts in plant communities may affect decomposition processes[J]. Geoderma, 285: 110–116.

DON A, KALBITZ K, 2005. Amounts and degradability of dissolved organic carbon from foliar litter at different decomposition stages[J]. Soil Biol Biochem, 37: 2171-2179.

GARCÍA-PALACIOS P, SHAW EA, WALL D, et al., 2016. Contrasting mass-ratio vs. niche complementarity effects on litter C and N loss during decomposition along a regional climatic gradient[J]. J Ecol, 105(4): 968-978.

GARTNER TB, CARDON ZG, 2004. Decomposition dynamics in mixed-species leaf litter[J]. Oikos, 104(2): 230–246.

HÄTTENSCHWILER S, 2005. Effects of tree species diversity on litter quality and decomposition[J]. Ecol Stud, 176: 149–164.

HOORENS B, AERTS R, STROETENGA M, 2003. Does initial litter chemistry explain litter mixture effects on decomposition[J]. Oecologia, 137(4): 578–586.

JOLY FX, FROMIN N, KIIKKILÄ O, et al., 2016. Diversity of leaf litter leachates from temperate forest trees and its consequences for soil microbial activity[J]. Biogeochemistry, 129: 1-14.

KALBITZ K, KAISER K, BARGHOLZ J, et al., 2006. Lignin degradation controls the production of dissolved organic matter in decomposing foliar litter[J]. Eur J Soil Sci, 57(4): 504–516.

MASTNÝ J, KAŠTOVSKÁ E, BÁRTA J, et al., 2018. Quality of DOC produced during litter decomposition of peatland plant dominants[J]. Soil Biol Biochem, 121: 221-230.

MASUDA C, KANNO H, MASAKA K, et al., 2022. Hardwood mixtures facilitate leaf litter decomposition and soil nitrogen mineralization in conifer plantations[J]. For Ecol Manage, 507: 120006.

PÉREZ-HARGUINDEGUY N, DÍAZ S, CORNELISSEN JHC, et al., 2000. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina[J]. Plant Soil, 218(1): 21-30.

ROSENFIELD MV, KELLE JK, CLAUSEN C, et al., 2020. Leaf traits can be used to predict rates of litter decomposition[J]. Oikos, 129: 1589-1596.

SALAMANCA EF, KANEKO N, KATAGIRI S, 1998. Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods[J]. Ecol Eng, 60: 53-73.

SCHOFIELD JA, HAGERMAN AE, HAROLD A, 1998. Loss of tannins and other phenolics from willow leaf litter[J]. J Chem Ecol, 24(8): 1409-1421.

VANDERBILT KL, WHITE CS, HOPKINS O, et al., 2008. Aboveground decomposition in arid environments: results of a long-term study in central new mexico[J]. J Arid Environ, 72(5): 696-706.

VESTGARDEN LS, 2001. Carbon and nitrogen turnover in the early stage of scots pine (*Pinus sylvestris* L.) needle litter decomposition: effects of internal and external nitrogen[J]. Soil Biol Biochem, 33(4/5): 465–474.

WARDLE DA, BONNER KI, NICHOLSON KS, 1997. Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function[J]. Oikos, 79(2): 247–258.

WU PP, DING YD, LI SL, et al., 2021. Carbon, nitrogen and phosphorus stoichiometry controls interspecific patterns of subtropical plantations of China[J]. iForest-Biogeosci For, 14: 80-85.

XIANG W, BAUHUS J, 2007. Does the addition of litter from N-fixing *Acacia mearnsii* accelerate leaf decomposition of *Eucalyptus globulus*?[J]. Aust J Bot, 55(5): 576–583.

ZHENG JQ, XU ZH, WANG YZ, et al., 2014. Non-additive effects of mixing different sources of dissolved organic matter on its biodegradation[J]. Soil Biol Biochem, 78: 160-169.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv — Machine translation. Verify with original.