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Research Article

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Hydrothermal thresholds and microbial carbon use efficiency regulate seasonal litter decomposition dynamics in an arid ecosystem

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Abstract:

Aims

Litter decomposition is a key process regulating carbon (C) cycling and nutrient turnover in arid and semi-arid regions. Seasonal shifts in microbial physiological trade-offs and enzyme stoichiometric strategies are critical regulatory mechanisms for predicting litter decomposition in drylands, yet the synergistic effects of precipitation and phosphorus (P) deposition on these processes remain unclear.

Methods

We conducted a 720-day in situ litter decomposition experiment. Two precipitation levels (ambient precipitation; +50% precipitation) and four P addition gradients (0, 1, 2.5, 5 g P m⁻² yr⁻¹) were established. Litter mass remaining, soil physicochemical properties, microbial biomass, microbial nutrient utilization efficiency, and extracellular enzyme activities were measured. Based on these data, we systematically examined how hydro-thermal thresholds regulate microbial physiological trade-offs to drive seasonal litter decomposition dynamics.

Results

Litter mass loss ranged from 36.9% to 52.9% and was tightly coupled to brief hydrothermal windows in the growing season. P addition accelerated decomposition only during the summer hydrothermal window by enhancing microbial C use efficiency (CUE) and hydrolase activity. Winter low temperatures triggered physiological drought and disrupted nutrient regulatory pathways. Consequently, microbial metabolism shifted from a hydrolase-dominated growth strategy to an oxidase-dominated nutrient-mining strategy. Mixed-species litter did not show stable synergistic effects compared with single-species litter. Instead, it exhibited pronounced seasonal reversal. Substrate stoichiometric complementarity maintained microbial metabolism in summer. In winter, high lignin content imposed metabolic bottlenecks, leading to lower decomposition rates than single-species litter types. Structural equation modeling (SEM) revealed divergent regulatory pathways. Summer decomposition was directly driven by extracellular enzyme activity. Winter

decomposition depended on microbial biomass to sustain conservative metabolism.

Conclusions

Incorporating seasonal dynamics of microbial CUE and its trade-offs with enzymatic stoichiometry into C cycle models for arid regions can improve the prediction accuracy of litter-derived C sequestration potential under global change.

Keywords: Hydrothermal thresholds· Litter decomposition· Microbial carbon use efficiency· Microbial extracellular enzymes· Seasonal dynamics

Introduction

Litter decomposition is a central biogeochemical process governing soil organic carbon (SOC) accumulation and nutrient cycling in arid and semi-arid regions, directly regulating the persistence and fertility of soil C pools (Parton et al. 2007; Bai et al. 2025; Li et al. 2025). This decomposition process is highly dependent on brief yet intense hydro-thermal pulses, intensive ultraviolet radiation, and seasonal reconfiguration of microbial physiological functions (Austin & Ballaré 2024; Yang et al. 2024a). Precipitation pulses during the growing season can rapidly activate dormant microbial communities, triggering bursts of biogeochemical transformations. In contrast, low temperatures and drought stress during the non-growing season force microbes into dormancy or maintain extremely low metabolic activity (Schimel et al. 2007; Day et al. 2022). Crucially, low temperatures exert multiple constraints on microbial processes. They not only directly suppress extracellular enzyme kinetics by reducing reaction rates, but also impair cell membrane fluidity, disrupt transmembrane ion transport, and interfere with osmoregulatory mechanisms. These combined effects induce a soil microenvironmental stress analogous to water scarcity, which is termed "physiological drought". This stress significantly weakens the coupling between soil nutrient availability and its biogeochemical effects. Nevertheless, its quantitative regulatory impacts remain poorly quantified (Jefferies et al. 2010; Immiger et al. 2024). Additionally, photochemical oxidation driven by intensive ultraviolet radiation serves as a distinctive pre-weathering process. It modifies the chemical structure of recalcitrant organic matter, thereby affecting subsequent microbial colonization and enzymatic degradation efficiency (Gallo et al. 2009; Liu et al. 2025). Under global change, shifting precipitation patterns and increasing atmospheric phosphorus (P) deposition further strengthen nonlinear interactions among these multi-scale processes (Liu et al. 2024a). Consequently, traditional decomposition models based on mean annual climate averages fail to accurately capture C turnover dynamics. These dynamics are constrained by hydro-thermal thresholds and driven by microbial physiological responses.

Conventional litter decomposition models mostly rely on mean annual climatic variables. Such models cannot adequately capture C turnover dynamics constrained by hydrothermal thresholds and governed by microbial physiological responses (Mougi et al. 2023; Li et al. 2025). According to the resource conservation hypothesis (Allison & Vitousek 2005), microbial communities adjust extracellular enzyme stoichiometric allocation to cope with mismatches between substrate resources and cellular elemental demands (Gong et al. 2023). Exogenous P addition is widely used to alleviate microbial P limitation and accelerate litter decomposition (Shi et al. 2021; Zhang et al. 2022; Chen et al. 2023). However, this stimulation shows clear seasonal asymmetry. Under warm and moist conditions, elevated C use efficiency (CUE) can promote microbial metabolism. Under cold or drought-induced osmotic stress, the stimulatory effect may diminish or even disappear (Manzoni et al. 2012; Li et al. 2024; Liu et al. 2024b). Empirical studies in arid regions confirm that nutrient additions promote decomposition only when moisture and temperature thresholds are

simultaneously met (Yang et al. 2020; Gong et al. 2022). Yet current C cycling frameworks lack mechanistic understanding of how coupled precipitation–heat dynamics regulate microbial physiological trade-offs and reshape extracellular enzyme investment strategies in arid ecosystems.

Mixed-species litter decomposition often exhibits non-additive effects that deviate from the additive patterns observed in single-species litter. Traditional explanations primarily emphasize physical contact and litter leachate interactions (Hättenschwiler et al. 2005; Gartner & Cardon 2008). In resource-limited arid landscapes, substrate stoichiometric complementarity is frequently masked by strong seasonal environmental filtering (Liu et al. 2025). Accumulating evidence shows that synergistic effects common in temperate ecosystems often weaken to neutrality or shift to antagonism under chronic resource limitation (Zhou et al. 2020; Bai et al. 2025). This context dependence indicates that mixed-species litter decomposition trajectories are not solely governed by initial stoichiometric ratios. Instead, they depend on the capacity of microbial communities to transition from hydrolytic to oxidative enzyme activities (Hättenschwiler et al. 2005; Sinsabaugh et al. 2008; Yang et al. 2020). Most existing studies treat substrate quality, environmental filtering, and microbial responses as independent drivers. Integrated frameworks linking the coupled mechanism of “initial stoichiometry–enzyme succession–seasonal transition” remain scarce. In particular, quantitative assessments of how winter low temperatures interact with photodegradation to impose metabolic bottlenecks on decomposition are still lacking.

To address these research gaps, we conducted a 720-day field experiment with a two-factor controlled design in the oasis–desert ecotone along the southern margin of the Tarim Basin. The experiment used single and mixed-species litter of two dominant shrubs: *Alhagi camelorum* Fisch. and *Karelinia caspica*. We tested three scientific hypotheses: (i) The promoting effect of P addition on litter decomposition is strictly constrained by hydrothermal thresholds. P addition accelerates decomposition only during the summer hydrothermal window by enhancing microbial CUE. Winter low temperatures induce physiological drought and block this stimulatory pathway; (ii) The non-additive effects of mixed-species litter exhibit seasonal reversal. Stoichiometric complementarity drives synergistic decomposition in summer. In winter, high lignin content creates metabolic bottlenecks, leading to antagonistic effects; (iii) Initial litter stoichiometry determines the seasonal trajectory of decomposition by regulating microbial functional shifts from hydrolytic to oxidative enzyme activities. Our findings clarify the microbial mechanisms controlling litter C turnover in arid landscapes. They provide field-based evidence for incorporating seasonal microbial physiological parameters into regional soil C models.

Materials and methods

Study site

The field experiment was conducted at the Cele Desert Grassland Ecosystem National Field Scientific Observation and Research Station (80°43'45"E, 37°00'57"N),

Xinjiang, China (Fig. S1). This site is located in the oasis–desert ecotone along the southern margin of the Tarim Basin. It represents a typical warm-temperate continental extremely arid landscape. The annual mean precipitation is only 42.6 mm, with recent maximums reaching about 100 mm. Annual evaporation exceeds 2600 mm. The average annual temperature is 12.8°C, with an extreme maximum of 45.7°C and an extreme minimum of −22.3°C. The soil in this region is classified as a mobile aeolian sandy soil. It is characterized by low organic matter content and available P levels below 5 mg/kg. Vegetation is dominated by desert shrubs, including *Alhagi camelorum* Fisch., *Tamarix ramosissima*, *Karelinia caspia*, and *Populus euphratica*. These species form a typical desert vegetation community in arid landscapes (Gao et al. 2025).

Experimental design

The experiment commenced on 7 December 2023. A randomized block design was adopted with 16 treatments and three replicates. A total of 48 plots (4 × 4 m each) were established. Plots were spaced 1 m apart to minimize interference from lateral precipitation and nutrient leaching. Two precipitation treatments were applied: natural precipitation (CK) and a 50% increase above the local maximum annual rainfall (approximately 150 mm yr^{−1}, denoted as W). Four P addition gradients were established: 0 g P m^{−2} yr^{−1} (CK), 1 g P m^{−2} yr^{−1} (LP), 2.5 g P m^{−2} yr^{−1} (MP), and 5 g P m^{−2} yr^{−1} (HP). These precipitation and P levels were based on recent local records of maximum precipitation and average annual P deposition (Du et al. 2020; Gao et al. 2025; Liu et al. 2025). They aim to simulate typical coupled changes in precipitation and P availability across arid landscapes (Fig. S2). The P source was analytical-grade NaH₂PO₄·2H₂O (≥99.9%), prepared as stock solutions of 0, 2.0, 5.0, and 10.0 g P L^{−1}. The 16 specific treatment combinations were: control (CK), precipitation only (W), low P (LP), medium P (MP), high P (HP), and interactive combinations of precipitation with different P levels (WLP, WMP, WHP). Detailed methodologies for precipitation manipulation and P addition are provided in Appendix S1.

Sample collection and processing

We selected freshly fallen leaves of two dominant shrub species—*Alhagi camelorum* Fisch. and *Karelinia caspia*—as litter materials. These two species differ significantly in their initial chemical compositions (Fan et al. 2021). Litter mixtures were prepared at a 1:1 mass ratio. Freshly fallen leaves were collected in early December 2023. Impurities such as soil particles and living plant tissues were removed. The litter was thoroughly mixed and air-dried. On 4 December 2023, subsamples were oven-dried at 75°C for 48 h. The dried litter was then placed into 10 cm × 10 cm nylon decomposition bags with a 1 mm mesh size. Each bag contained approximately 10 g of litter and was weighed to 0.01 g using an electronic balance. Three subsamples were taken to determine initial concentrations of C, N, P, cellulose, and lignin (Table 1). A total of 240 decomposition bags were prepared, based on a factorial design of 2 litter types × 8 treatments × 3 replicates × 5 sampling sites. On 7 December 2023, the bags were randomly placed on the soil surface within each subplot. They were fully

exposed to sunlight and precipitation, simulating natural litter distribution in desert environments. Each bag was secured at its four corners with 8 cm stainless steel pins driven into the sand. Throughout the 720-day decomposition period, plots were inspected biweekly. Each month, newly emerged annual herbaceous plants within a 10 cm radius around each litter bag were manually removed without herbicide application. If strong winds displaced any bags, they were promptly repositioned and the disturbance was recorded. Given the low rodent density at the site, no protective fencing was installed. No obvious signs of animal disturbance were observed during the experiment. Litter bags were collected at 90, 180, 360, 540, and 720 days after placement. A total of 48 bags were retrieved at each sampling event.

Determination of litter and soil properties

Litter samples were transported to the laboratory within 6 h of collection in an ice box ($< 4^{\circ}\text{C}$). Surface sand particles were gently removed using a soft brush. Samples were then oven-dried at 75°C to constant weight. Mass retention rate was calculated based on the final dry weight. For analyses of C, N, P, and lignin, selected samples were freeze-dried to constant weight. They were ground using a ball mill and sieved through a 0.15 mm mesh. The powdered samples were sealed in polyethylene ziplock bags and stored in the dark at -20°C for no more than 45 days prior to analysis (Liu et al. 2025). Total C (TC) and total N (TN) contents were determined using a CHNS elemental analyzer (Elementar, Hanau, Germany) (Shi et al. 2021). Total P (TP) was measured by the molybdenum–antimony colorimetric method. Lignin content was analyzed using a fiber analyzer (FOSS, Hilleroed, Denmark), following a combined modified Klason method and acid detergent lignin (ADL) method (Van Soest 1967; Hou et al. 2021). Detailed procedures are provided in Appendix S2.

At day 540 (summer) and day 720 (winter) of decomposition, 48 litter bags were retrieved. Samples were transported to the laboratory within 6 h in an ice box ($< 4^{\circ}\text{C}$). During sampling, personnel wore gloves and used sterile brushes to gently remove soil adhering to the litter surface. The soil was collected into labeled 1.5 mL centrifuge tubes, with three replicates per treatment. Fresh soil samples for extracellular enzyme activity analysis were immediately frozen at -20°C to avoid repeated freeze–thaw cycles and were analyzed within 7 days of collection. Soil samples for physicochemical property analysis were air-dried indoors. They were crushed with a plastic rod, sieved through a 0.15 mm mesh, sealed in polyethylene ziplock bags, and stored in the dark at room temperature. Soil pH was measured using a calibrated pH meter. SOC, TN, and TP were determined using a CHNS elemental analyzer (Luo et al. 2020). Available P (AP) was measured by the molybdenum blue colorimetric method. Nitrate-N (NO_3^- -N) and ammonium-N (NH_4^+ -N) were analyzed using a flow analyzer. Microbial biomass C (MBC), N (MBN), and P (MBP) were determined using the chloroform fumigation– K_2SO_4 extraction method (Anderson & Ingram 1993). Extracts were filtered through a $0.45\ \mu\text{m}$ membrane and analyzed separately: C and N by an elemental analyzer, and P by the molybdenum blue method. Correction coefficients were applied ($\text{EC} = 0.45$, $\text{EN} = 0.54$, $\text{EP} = 0.40$).

Assay of soil enzymatic activities and microbial communities

Soil extracellular enzyme activities were measured using fluorometric assays for hydrolytic enzymes and colorimetric assays for oxidase enzymes (Shi et al. 2018; Yang et al. 2024b). Five enzymes were assayed, representing four functional categories: C-acquiring enzyme: β -1,4-glucosidase (BG); N-acquiring enzyme: leucine aminopeptidase (LAP); P-acquiring enzyme: alkaline phosphatase (ALP); Lignin-degrading oxidases: peroxidase (POD) and polyphenol oxidase (PPO). The detailed procedure was as follows. One gram of fresh soil was weighed into a 50 mL centrifuge tube. It was mixed with 125 mL of 50 mM sodium acetate buffer (pH 5.0) and shaken to prepare a soil suspension. Then, 200 μ L of the soil suspension was pipetted into a 96-well microplate. This was combined with 50 μ L of either a specific fluorescent substrate (for BG, LAP, and ALP) or a chromogenic substrate (for POD and PPO). Hydrolytic enzyme plates were incubated at 20°C in the dark for 4 h. Oxidase enzyme plates were incubated for 5 h. After incubation, fluorescence intensity (excitation/emission: 365/450 nm) or absorbance (470 nm) was measured using a Synergy H1 multi-mode microplate reader (BioTek, USA). Enzyme activities were calculated based on standard curves.

Data analysis

Litter decomposition rate and remaining mass

Retrieved litter samples were oven-dried at 75 °C to constant weight. Residual sand and soil fragments were removed by passing the litter through a 1 mm mesh sieve. Dry mass was measured to the nearest 0.01 g. Litter mass remaining (MR , %) was then calculated. Decomposition rate constants (k) were estimated using the single-exponential decay model. The equations for litter mass remaining (Gong et al. 2023), the formula for calculating k (Olson, 1963), and the formula for average litter retention time are as follows:

$$MR = (X_t / X_0) \times 100\% \quad (1)$$

$$X_t = X_0 e^{-kt} \quad (2)$$

$$MRT = 1/k \quad (3)$$

where X_0 (g) is the dry mass of litter at the beginning of the experiment, X_t (g) is the residual litter mass at decomposition time t , e is the base of the natural logarithm, and k is the litter decomposition rate.

Dynamic indicators of elements

We evaluated changes in nutrient retention during decomposition using the nutrient accumulation index (R). Values below 100% indicated net nutrient release from the litter. Values above 100% indicated nutrient accumulation (McGroddy et al. 2004):

$$R = M_t \times C_t / (M_0 \times C_0) \times 100\% \quad (4)$$

In the calculations, C_0 (mg.g⁻¹) and M_0 (g) represented the initial nutrient concentration and initial litter mass, while C_t (mg.g⁻¹) and M_t (g) corresponded to the nutrient concentration and litter mass at sampling time t .

Microbial C, N, and P use efficiency were calculated based on enzyme stoichiometry

(Sinsabaugh et al. 2013):

$$CUE_{C:N} = CUE_{max} \times (S_{C:N} / (S_{C:N} + K_{C:N})) \quad (5)$$

$$S_{C:N} = (1 / EEA_{C:N}) \times (B_{C:N} / L_{C:N}) \quad (6)$$

$$NUE = NUE_{max} \times (1 + K_{N:C}/S_{N:C} + K_{N:P}/S_{N:P}) \quad (7)$$

$$PUE = PUE_{max} \times (1 + K_{P:C}/S_{P:C} + K_{P:N}/S_{P:N}) \quad (8)$$

CUE_{max} : Theoretical maximum C use efficiency, typically set at 0.6; $K_{C:N}$: Half-saturation constant representing the ratio of enzyme activity when microbial nutrient uptake reaches half its maximum potential, generally set at 0.5; $B_{C:N}$: Ratio of MBC to MBN; $L_{C:N}$: Soil SOC to TN ratio; $EEA_{C:N}$: Activity ratio of ln(BG) to ln(LAP). NUE_{max} : Theoretical maximum nitrogen use efficiency, typically set at 1; $K_{N:P}$: Half-saturation constant representing the ratio of enzyme activity when microbial nutrient uptake reaches half its maximum potential, generally set at 0.5; $S_{N:C}$, $S_{N:P}$: Enzyme activity ratios, representing ln(BG/LAP) and ln(BG/ALP), respectively. PUE_{max} : Theoretical maximum P use efficiency, typically set at 1; $K_{P:N}$: Half-saturation constant representing the ratio of enzyme activity when microbial nutrient uptake reaches half its maximum potential, generally set at 0.5; $S_{P:C}$, $S_{P:N}$: Enzyme activity ratios, representing ln(BG/ALP) and ln(ALP/LAP), respectively.

Statistical analysis

Single-factor analysis of variance (ANOVA) and Tukey's HSD test were conducted using the "multcomp" package in R. These tests evaluated the effects of different treatments on litter decomposition rates, soil physicochemical properties, microbial biomass MBC, MBN, and MBP, extracellular enzyme activities, their stoichiometric ratios, and nutrient use efficiencies. Linear mixed-effects models (LMMs, using the "lme4" package) were applied to assess the main and interactive effects of precipitation, P addition, and time on each variable. In these models, plot was treated as a random effect, while all other factors were treated as fixed effects (Wang et al. 2020). Based on this framework, we further used LMMs to separately examine the interactions among precipitation, P addition, and litter type during summer and winter. Spearman correlation analysis was employed to explore relationships among litter characteristics, soil properties, microbial biomass, and enzyme activities. Canonical redundancy analysis (RDA) was used to assess the combined effects of litter composition and soil physicochemical properties on soil enzyme activities under different precipitation and P addition conditions. Significance was evaluated via 999 permutations. Finally, structural equation modeling (SEM) was constructed using the piecewiseSEM package in R. This model quantified the direct and indirect effects of various factors on litter mass loss in summer and winter. Model fit was assessed using multiple criteria: $P > 0.05$, lower AIC values, and lower Fisher's C values, all indicating better goodness-of-fit (Tian et al. 2021).

Results

The dynamics of litter mass remaining and decomposition rate

The 720-day decomposition experiment showed continuous litter mass loss across all treatments. Overall mass loss ranged from 36.87% to 52.90% (Fig. 1). Both increased precipitation and P addition significantly promoted litter decomposition ($p < 0.05$). However, their interactive effect was significant only during summer (540 days) (Table 3). Specifically, at 540 days, mass loss in the increased precipitation treatment was significantly higher than in the control. By 720 days (winter), single-species litter showed significantly greater mass loss than mixed-species litter (Fig. 1). Based on the Olson exponential decay model, increased precipitation significantly increased the decomposition rate constant (k) and shortened the MRT ($p < 0.05$; Table 2). For single-species litter, k increased monotonically with increasing P concentration. However, mixed-species litter showed no significant response. Model estimates indicated that single-species litter decomposed fastest under the WHP treatment, whereas mixed-species litter reached its maximum decomposition rate under the WMP treatment.

Across both seasons, increased precipitation and P addition significantly increased the loss of cellulose and N from litter. They also elevated C/N and lignin/N ratios, while reducing C/P, N/P, and lignin/P ratios ($p < 0.05$; Fig. 2). However, the positive effects of increased precipitation on cellulose loss, C/N ratio, and lignin/P ratio were not significant in winter ($p > 0.05$; Table 3). Variance analysis revealed that the three-way interaction among precipitation, P addition, and litter type significantly influenced most nutrient loss and stoichiometric ratios only during summer ($p < 0.05$). By winter, single-species litter exhibited higher losses of C and cellulose, along with greater lignin remaining. These were accompanied by higher C/P, N/P, and lignin/P ratios, but lower C/N and lignin/N ratios (Fig. 2).

The dynamics of soil physicochemical properties

Precipitation, P addition, and litter type significantly affected most soil physicochemical properties (Table S1). Soil pH responded to P addition with clear seasonal reversal. Under ambient precipitation in summer, pH increased (Fig. 3). Under increased precipitation, pH decreased. In winter, pH generally declined regardless of moisture conditions ($p < 0.05$). The effect of P addition on SOC depended on litter type. Under single-species litter treatments, SOC significantly decreased. Under mixed-species litter, SOC accumulated in summer and showed differentiation in winter ($p < 0.05$). TN dynamics were more complex and jointly regulated by season and moisture conditions ($p < 0.05$). In contrast, TP and AP responded consistently to P addition. Both increased with higher P concentrations, reaching peak values under high P in single-species litter treatments ($p < 0.05$). Increases in inorganic N (NO_3^- , NH_4^+) were mainly observed in single-species litter treatments. They were largely suppressed in mixed-species litter treatments ($p < 0.05$). Notably, precipitation significantly influenced NO_3^- and NH_4^+ contents only in winter for both litter types. In single-species litter treatments, they increased significantly ($p < 0.05$). In mixed-species litter treatments, they decreased ($p > 0.05$). Stoichiometric ratios indicated that P addition reduced the SOC/TN ratio under increased

precipitation in summer for both litter types. In winter, it increased this ratio in most single-species litter treatments but decreased it in mixed-species litter ($p < 0.05$). Both SOC/TP and TN/TP ratios were significantly reduced by P addition ($p < 0.05$).

The dynamics of microbial biomass C, N, P, stoichiometric ratios and utilization efficiency

Microbial biomass and stoichiometry were jointly regulated by environmental factors (Table S2). The effect of P addition on biomass exhibited significant seasonal asymmetry. It promoted MBC and MBC/MBP accumulation in summer but mostly inhibited them in winter (Fig. 4a, c). Under mixed-species litter, promotion occurred only under increased precipitation in summer ($p < 0.05$). MBN showed similar patterns. P addition generally reduced MBP ($p < 0.05$). CUE displayed a clear seasonal reversal. In single-species litter treatments, P addition enhanced CUE in summer (Fig. 4b, d). This effect was maintained only under increased precipitation in winter. In mixed-species litter treatments, P addition promoted CUE in both seasons. In contrast, NUE and PUE showed opposite trends to CUE ($p < 0.05$).

The dynamics of extracellular enzymes and stoichiometric ratios

Increased precipitation significantly affected five enzymes and their stoichiometric ratios. P addition significantly influenced only enzyme activities (Table S3). BG activity was significantly enhanced by both P addition and increased precipitation in both seasons. LAP activity was promoted in summer but inhibited under ambient precipitation in winter. ALP activity increased under ambient precipitation in summer but was generally suppressed in winter. PPO activity increased in both seasons, while POD activity was inhibited. This suggests that P addition altered lignin degradation pathways ($p < 0.05$; Fig. 5). Enzyme stoichiometric ratios further revealed microbial resource allocation strategies. P addition increased the $\ln(\text{BG})/\ln(\text{LAP})$ ratio under ambient precipitation in single-species litter treatments but decreased this ratio in mixed-species litter during summer. The $\ln(\text{BG})/\ln(\text{ALP})$ ratio decreased in summer and increased under increased precipitation in winter. Notably, the $\ln(\text{POD})/\ln(\text{LAP})$ ratio showed a slight promotion in mixed-species litter under ambient precipitation in winter, though not significantly ($p > 0.05$).

Factors influencing litter decomposition under precipitation and P addition

Spearman correlation analysis revealed significant seasonal variations in the coupling between litter mass loss and microbial physiological traits ($p < 0.05$; Fig. 6a). In summer, single-species litter mass loss was significantly positively correlated with C, N, and P losses, pH, TP, MBC/MBN, hydrolytic enzymes (BG, LAP), and CUE. It was negatively correlated with C/P, SOC/TP, TN/TP, oxidative enzymes (PPO, POD), and high enzyme investment ratios. For mixed-species litter, mass loss showed similar patterns. It was also positively correlated with ALP and negatively correlated with

SOC, SOC/TP, and oxidative enzymes. In winter, single-species litter mass loss was significantly positively associated with PPO, high enzyme investment ratios $\ln(\text{BG}/\text{ALP})$, and nutrient use efficiencies (NUE, PUE). It showed significant negative correlations with TN/TP, MBN, MBN/MBP, and ALP. In contrast, mixed-species litter mass loss remained positively correlated with $\ln(\text{BG}/\text{LAP})$ and NUE, and negatively correlated with SOC and SOC/TN ($P < 0.05$). RDA further highlighted seasonal differences in environmental drivers (Fig. 6b). In summer, the first two RDA axes explained 65.97% and 82.79% of the variation for single- and mixed-species litter, respectively ($p < 0.05$). These patterns were primarily driven by pH, nutrient stoichiometry, and enzyme activities. In winter, the RDA for single-species litter explained 82.79% of the variation, but the permutation test was not significant ($p > 0.05$). Similarly, the RDA for mixed-species litter explained 95.37% of the variation, yet the permutation test was also non-significant ($p = 0.215$). Treatment groups overlapped substantially along the ordination axes.

Structural equation modeling (SEM) explained 79% and 64% of the variation in litter mass loss during summer and winter, respectively (Fig. 7). In summer, precipitation, P addition, and litter type all had significant direct positive effects on mass loss. Precipitation indirectly promoted decomposition by enhancing enzyme activity. P addition mainly facilitated decomposition indirectly by improving soil physicochemical properties (e.g., pH, AP) (Fig. 7a). In winter, the direct effect of P addition was no longer significant. It exerted only a weak indirect positive influence via improved soil conditions. Although the direct effect of precipitation diminished, it still indirectly supported decomposition by maintaining microbial biomass (Fig. 7b). Across both seasons, enzyme activity showed significant positive effects on CUE. However, its direct effect on mass loss was not significant in winter.

Discussion

Seasonal asymmetry in the effects of hydrothermal thresholds and P additions

As hypothesized, increased precipitation significantly enhanced decomposition rate constants (k) and shortened mean residence time. However, the synergistic effect with P addition was only evident during the summer hydrothermal window (Fig. 1; Table 2). This pattern of “summer activation and winter dormancy” supports the low-temperature inhibition hypothesis (Parton et al. 2007). From the perspective of microbial resource economics theory, the root of this seasonal asymmetry lies in trade-offs in microbial C allocation strategies. In summer, P addition significantly promoted microbial biomass accumulation (MBC, MBN) and improved CUE (Fig. 4a, c). This indicates that P alleviated microbial P limitation, enabling greater C allocation to growth (Vitousek 1994). SEM further confirmed that P addition indirectly promoted decomposition in summer by improving soil physicochemical properties (pH, AP) (Fig. 7a). Notably, decomposition did not cease entirely during winter due to low temperatures. Instead, it showed a markedly reduced response to P addition, likely related to cold-induced physiological drought (Jefferies et al. 2010). Under such

conditions, cell membrane fluidity decreases substantially, transmembrane ion transport is impaired, and exogenous P input exacerbates osmotic imbalances between microbial cells and the external environment. These changes suppress microbial biomass and even reduce LAP activity (Allison & Vitousek 2004). These findings align closely with Manzoni et al.'s (2012) concept of "microbial physiological thresholds." When soil precipitation potential falls below a critical threshold, the energetic cost of osmoregulation rises sharply, CUE declines, and external nutrients are less effectively converted into biomass. In this study, winter cold induced phase transitions in cell membranes and intracellular ice formation, creating physiological stress analogous to drought. This forced microbes into maintenance metabolism (Schimel et al. 2007; Jefferies et al., 2010). Under these conditions, exogenous P cannot be efficiently assimilated, and nutrient additions have no significant effect on decomposition.

This physiological decoupling explains why single-species litter exhibited greater mass loss than mixed litter during winter. It also challenges the empirical assumption that nutrient addition inevitably promotes litter decomposition. More importantly, our seasonally asymmetric results suggest that short-term experiments are prone to overestimating the positive effect of P deposition on litter decomposition in arid ecosystems. Long-term, multi-season observations are therefore essential to reveal the true drivers of litter decomposition.

Dynamic non-additivity of mixed-species litter: from "growth synergy" to "metabolic bottleneck"

This study found that mixed-species litter decomposition did not exhibit the synergistic effects commonly observed in temperate ecosystems (Bai et al. 2025). Instead, it showed a significant seasonal reversal (Table 2; Fig. 1b). These results support the idea that mixed effects are driven by stoichiometric control of enzyme succession, rather than simple physical contact mechanisms (Gartner & Cardon 2008). Two mechanisms may explain the synergistic effect in summer. First, during the summer hydrothermal window, mixed-species litter exhibits broader initial C:N:P ratios and greater resource complementarity. This reduces fluctuations in the $\ln(\text{BG})/\ln(\text{LAP})$ ratio, thereby stabilizing enzyme investment and maintaining high CUE and decomposition rates (Sinsabaugh et al. 2008; Xiao et al. 2021). Second, increased precipitation availability amplifies this complementary effect by enhancing nutrient diffusion and accelerating enzymatic reaction rates (Li et al. 2022). Correlation analysis revealed that mixed-species litter mass loss was positively correlated with ALP activity and negatively correlated with the SOC/TP ratio (Fig. 6a). This further confirms the role of stoichiometric complementarity in sustaining metabolic efficiency. However, this advantage disappeared in winter and shifted to an antagonistic effect. Data showed that single-species litter had higher C/P, N/P, and lignin/P ratios in winter, whereas mixed-species litter exhibited higher C/N ratios (Fig. 2). These stoichiometric differences may drive distinct microbial survival strategies. Single - species litter maintains basic metabolic turnover through rapid release of

labile C. In contrast, mixed-species litter contains higher lignin content. Under low-temperature stress, this may create a "metabolic bottleneck" that partially restricts decomposition (Vitousek 1994; Hättenschwiler et al. 2005). Based on this, we propose the "seasonal metabolic bottleneck hypothesis": the non-additive effects of mixed-species litter are not fixed traits. Instead, they undergo functional transformation as microbial strategies shift from "growth" to "survival." This hypothesis aligns with studies on metabolic thresholds under resource limitation in arid regions (Bai et al. 2025).

Notably, even under ambient precipitation in winter, mixed-species litter still fine-tuned the investment ratio between oxidative and hydrolytic enzymes $\ln(\text{POD})/\ln(\text{LAP})$. It also maintained positive correlations with $\ln(\text{BG}/\text{LAP})$ and NUE (Fig. 5b, 6b). This suggests that mixed substrates do not fully shift into conservative metabolism. Instead, stoichiometric complementarity delays the transition of microbes toward complete oxidative pathways (Hättenschwiler et al. 2005), demonstrating the buffering role of substrate heterogeneity in seasonal shifts of functional strategies. However, the promoting effect of P addition on MBC/MBP in mixed-species litter was only significant under increased precipitation in summer (Fig. 5c). This indicates that the buffering capacity of substrate stoichiometry on metabolic efficiency strongly depends on the physiological activation thresholds set by precipitation availability (Joly et al. 2017). These findings challenge the conventional view of stable mixed effects. They highlight that in extremely arid landscapes, resource complementarity is often masked by strong environmental filtering, leading to seasonal reversals in non-additive effects (Bai et al. 2025; Zhou et al. 2020).

Moreover, the higher lignin content in mixed-species litter may be associated with stronger microbial nitrogen retention (Fig. 3), consistent with the nitrogen demand for oxidase synthesis (Xiao et al. 2021; Gong et al. 2023). However, the metabolic contributions of specific microbial taxa still require further investigation. Overall, mixed-species litter plays a dual role under extreme conditions. It promotes decomposition through stoichiometric complementarity in summer and buffers environmental stress via substrate heterogeneity in winter. This illustrates the seasonal regulation of decomposition by the "stoichiometric buffering" mechanism. It should be noted that our interpretation of "stoichiometric complementarity" and "metabolic bottleneck" is primarily based on indirect evidence from enzyme stoichiometry and CUE. It lacks independent validation from metrics such as substrate utilization efficiency or metabolite profiles. Therefore, these mechanisms should be regarded as working hypotheses. Future studies could further test them using substrate-induced respiration experiments, metabolomics analysis, or isotope labeling techniques.

Seasonal decoupling of photodegradation–microbial coupling and enzymatic strategy shifts

Correlation analysis and SEM consistently demonstrated that litter decomposition in summer was dominated by hydrolytic enzymes (BG and LAP) (Fig. 6a, 7). Microbes adopted an "acquisition–growth" strategy, prioritizing the utilization of labile

substrates and nutrient retention. This strategy was characterized by high CUE and elevated hydrolytic enzyme activity (Gong et al. 2023). In contrast, during winter, single-species litter shifted toward an oxidase-dominated “nutrient mining” mode represented by PPO. This mode exhibited low CUE and high oxidase activity (Sinsabaugh et al. 2008). This pattern aligns with recent findings in arid ecosystems, where C:N:P acquisition enzyme investment dynamically adjusts in response to temperature and moisture pulses (Yang et al. 2020; Zheng et al. 2023). More importantly, this enzymatic shift may be linked to the photo-degradation–microbial coupling process characteristic of arid regions. Strong solar radiation in summer reduces lignin polymerization via photolytic cleavage, creating favorable conditions for oxidase synthesis following precipitation pulses (Day et al. 2022; Austin & Ballaré, 2024). Conversely, low winter temperatures simultaneously suppress both photochemical reactions and enzyme activity, leading to overall decomposition suppression (Liu et al. 2025).

RDA further quantified this seasonal transition (Fig. 6b). Environmental factors strongly drove microbial functional expression in summer. In winter, however, microbial processes became desensitized to environmental gradients. Microbes no longer adjusted enzyme investments according to resource stoichiometry, instead entering a conservative survival state (Manzoni et al. 2012). An interesting observation was the significant seasonal reversal in CUE (Fig. 4b, d). P addition enhanced summer CUE in single-species litter, but this effect was sustained only under increased precipitation in winter. In mixed-species litter, however, P addition promoted CUE in both seasons. These results reveal strong context dependence in microbial responses to nutrient additions. The direction and magnitude of these responses were jointly regulated by season, moisture conditions, and litter type. Further observations showed that CUE exhibited opposite trends to NUE and PUE (Fig. 4b, d). This reflects internal trade-offs in microbial resource allocation: as C acquisition efficiency increases, microbes tend to reduce investment in nitrogen and P acquisition, and vice versa. This trade-off mechanism aligns closely with ecological enzyme stoichiometry theory (Sinsabaugh et al. 2013). It indicates that microbes dynamically adjust their enzyme portfolios to optimize resource acquisition efficiency. From a thermodynamic perspective, microbes prioritize maximizing growth efficiency (high CUE) when resources are abundant. Under resource limitation or environmental stress, they switch to maintaining cellular functions (low CUE). This represents a core component of microbial adaptation strategies (Dijkstra et al. 2011; Lipson 2015).

It should be noted that “desensitization” does not equate to metabolic stasis. Rather, it reflects reduced sensitivity of microbes to environmental fluctuations, with priority given to sustaining basic cellular functions (Schimel et al. 2007; Manzoni et al. 2012). Our study also revealed that enzyme activity significantly and positively affected CUE in both summer and winter. However, in winter, the direct effect of enzyme activity on mass loss was insignificant (Fig. 7). This suggests that enzymatic breakdown products in winter are primarily assimilated and stored by microbes rather than released through respiration. This functionally confirms that microbes adopt a

“conservative survival strategy” during winter (Bolscher et al. 2024). This characteristic may help explain why C turnover persists slowly but continuously in arid regions during the non-growing season (Jefferies et al. 2010; Dijkstra et al. 2011). It also suggests that simply setting winter decomposition rates to zero or a constant in dryland C cycling models could underestimate their actual contribution (Parton et al. 2007). Overall, our findings support a cascading regulatory mechanism: initial litter C:N:P stoichiometry → microbial enzyme investment strategy → final fate of litter decomposition. They provide crucial field evidence for incorporating microbial physiological parameters into soil C models for arid regions.

Ecological insights and limitations

This study demonstrates that hydrothermal thresholds govern the seasonal dynamics of litter decomposition in extremely arid landscapes by regulating microbial resource allocation strategies. The seasonal reversal of non-additive effects in mixed-species litter indicates that C stability during non-growing seasons is primarily controlled by microbial physiological conservatism rather than short-term climatic fluctuations. Future regional biogeochemical models should integrate the seasonal dynamics of CUE, enzyme investment trade-offs, and hydrothermal threshold effects to improve predictions of soil C turnover in arid regions. Nevertheless, this study has several limitations. (1) The 1 mm mesh size of decomposition bags excluded macrofauna, which may underestimate the contribution of physical fragmentation to winter decomposition. (2) P addition simulated atmospheric deposition but did not distinguish bioavailability differences among various mineral P forms. Isotopic tracing could help clarify this aspect in future studies. (3) The lack of quantitative evidence at the lignin molecular level (e.g., FTIR/NMR) limits a deeper understanding of photo-degradation–microbial coupling mechanisms. (4) The "seasonal metabolic bottleneck" hypothesis is based on data from a single site with two dominant species. It requires validation across multiple sites and species combinations to assess its generality. (5) This study did not quantify the effects of photo-degradation pretreatment. Nylon mesh bags partially blocked UV-B radiation. Simultaneous measurements of DOM fluxes generated via photochemical breakdown and their microbial utilization efficiency were not conducted. This may underestimate the role of photo-degradation in initiating microbial decomposition during hydrothermal windows.

Conclusion

In the extremely arid area, litter decomposition is governed by microbial resource allocation strategies regulated by hydrothermal thresholds. P addition promotes decomposition only during the summer hydrothermal window. This promotion operates by increasing microbial CUE and enhancing investment in hydrolytic enzymes. This period represents a critical window for external nutrient regulation of litter C turnover. Winter low temperatures induce physiological drought and sever the direct nutrient-driven stimulation of decomposition. Consequently, microbes shift from a hydrolytic enzyme-dominated “growth mode” to an oxidase-dominated

“nutrient mining mode.” Mixed-species litter decomposition exhibits a significant seasonal reversal, transitioning from synergistic effects in summer to antagonistic effects in winter. The metabolic bottleneck imposed by high lignin content is the primary driver of winter antagonism. In contrast, chemical stoichiometric complementarity among substrates buffers environmental stress. In winter, microbes become desensitized to environmental gradients, directing more enzymatic products toward assimilation and storage rather than respiratory consumption. Ultimately, litter decomposition depends on the dynamic match between initial substrate stoichiometry and microbial physiological plasticity. These findings highlight that incorporating seasonal CUE dynamics and extracellular enzyme investment trade-offs into biogeochemical models can provide a physiological basis for parameterizing soil C cycling models in arid landscapes.

Authors’ contribution Meiqi Li: Writing – review and editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Yalan Liu: Supervision, Investigation, Formal analysis. Peng Wang: Supervision. Xiangyi Li: Writing – review and editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the findings of this study will be made available by the corresponding author upon reasonable request.

References

- Allison SD, Vitousek PM (2004) Rapid nutrient cycling in leaf litter of invasive plants in Hawai’i. *Oecologia* 146:612–619. <https://doi.org/10.1007/s00442-004-1679-z>
- Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol Biochem* 37:937–944. <https://doi.org/10.1016/j.soilbio.2004.09.014>
- Anderson JA, Ingram JSI (1993) Tropical soil biology and fertility: a handbook of methods. CAB International, Wallingford, Oxon, UK.

- Austin AT, Ballaré CL (2024) Photodegradation in terrestrial ecosystems. *New Phytol* 244:769–785. <https://doi.org/10.1111/nph.20105>
- Bai L, Song C, Li M et al (2025) Global patterns and drivers of decomposition of mixed-species litter. *GEODERMA* 451:117015. <https://doi.org/10.1016/j.geoderma.2025.117015>
- Bolscher T, Vogel C, Olagoke FK, Meurer KHE, Herrmann AM, Colombi T, Brunn M, Domeignoz-Horta LA (2024) Beyond growth: the significance of non-growth anabolism for microbial carbon-use efficiency in the light of soil carbon stabilisation. *Soil Biol Biochem* 193:109400. <https://doi.org/10.1016/j.soilbio.2024.109400>
- Chen C, Xiao WY (2023) The global positive effect of phosphorus addition on soil microbial biomass. *Soil Biol Biochem* 176:108882. <https://doi.org/10.1016/j.soilbio.2022.108882>
- Day TA, Urbine JM, Bliss AS (2022) Supplemental precipitation accelerates decay but only in photodegraded litter and implications that sunlight promotes leaching loss. *BIOGEOCHEMISTRY* 158:113–129. <https://doi.org/10.1007/s10533-022-00888-x>
- Dijkstra P, Thomas SC, Heinrich PL, Koch GW, Schwartz E (2011) Effect of temperature on metabolic activity of intact microbial communities: evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency. *Soil Biol Biochem* 43:2023–2031. <https://doi.org/10.1016/j.soilbio.2011.05.018>
- Du E, Terrer C, Pellegrini AFA et al (2020) Global patterns of terrestrial nitrogen and phosphorus limitation. *Nat Geosci* 13:221–226. <https://doi.org/10.1038/s41561-019-0530-4>
- Fan LJ, Li CD, Li XY et al (2021) Effects of sand burial on litter decomposition rate and salt content dynamics in an extremely arid region. *Chin J Plant Ecol* 45:144–153. <https://doi.org/10.17521/cjpe.2020.0273>
- Gallo ME, Porras-Alfaro A, Odenbach KJ, Sinsabaugh RL (2009) Photoacceleration of plant litter decomposition in an arid environment. *Soil Biol Biochem* 41:1433–1441. <https://doi.org/10.1016/j.soilbio.2009.03.011>
- Gao Y, Tariq A, Zeng FJ, Li XY, Sardans J, Al-Bakre DA, Peñuelas J (2025) Metagenomics reveals divergent functional profiles of soil C and nitrogen cycles in an experimental drought and P-poor desert ecosystem. *Appl Soil Ecol* 207:105946. <https://doi.org/10.1016/j.apsoil.2025.105946>
- Gartner TB, Cardon ZG (2008) Leaf litter mixtures and their effects on decomposition: a meta-analysis. *ECOLOGY* 85:2077–2086. <https://doi.org/10.1890/03-0662>
- Gong JR, Zhang ZH, Zhu CC et al (2022) The response of litter decomposition to phosphorus addition in typical temperate grassland in Inner Mongolia. *J Arid Environ* 197:104677. <https://doi.org/10.1016/j.jaridenv.2022.104677>
- Gong JR, Dong XD, Li XB et al (2023) Phosphorus fertilization affects litter quality and enzyme activity in a semiarid grassland. *Plant Soil* 492:91–108. <https://doi.org/10.1007/s11104-023-06053-9>
- Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in

- terrestrial ecosystems. *Annu Rev Ecol Evol Syst* 36:191–218.
<https://doi.org/10.1146/annurev.ecolsys.36.091704.085500>
- Hou SL, Hättenschwiler S, Yang JJ et al (2021) Increasing rates of long-term nitrogen deposition consistently increased litter decomposition in a semi-arid grassland. *New Phytol* 229:296–307. <https://doi.org/10.1111/nph.16911>
- Immiger S, Meier DV, Schintlmeister A et al (2024) Survival and rapid resuscitation permit limited productivity in desert microbial communities. *Nat Commun* 15:8392. <https://doi.org/10.1038/s41467-024-52734-3>
- Jefferies RL, Walker NA, Edwards KA, Lundberg P, Sullivan PF (2010) Is the decline of soil microbial biomass in late winter coupled to changes in the physical state of cold soils? *Soil Biol Biochem* 42:129–135.
<https://doi.org/10.1016/j.soilbio.2009.10.008>
- Joly FX, Kurupas KL, Throop HL (2017) Pulse frequency and soil-litter mixing alter the control of cumulative precipitation over litter decomposition. *ECOLOGY* 98:2255–2260. <https://doi.org/10.1002/ecy.1921>
- Li ZL, Peng Q, Dong YS et al (2022) The influence of increased precipitation and nitrogen deposition on the litter decomposition and soil microbial community structure in a semiarid grassland. *Sci Total Environ* 844:157115.
<https://doi.org/10.1016/j.scitotenv.2022.157115>
- Li JW, Wu JZ, Yu JY et al (2024) Soil enzyme activity and stoichiometry in response to precipitation changes in terrestrial ecosystems. *Soil Biol Biochem* 191:109321.
<https://doi.org/10.1016/j.soilbio.2024.109321>
- Li Z, Wang FW, Wen Y et al (2025) Temporal dynamics of climate sensitivity of litter decomposition in a semi-arid grassland. *GEODERMA* 451:117015.
<https://doi.org/10.1016/j.geoderma.2025.117015>
- Lipson DA (2015) The complex relationship between microbial growth rate and yield and its implications for ecosystem processes. *Front Microbiol* 6:615.
<https://doi.org/10.3389/fmicb.2015.00615>
- Liu YL, Zhang AL, Li XY et al (2024a) Litter decomposition rate response to multiple global change factors: a meta-analysis. *Soil Biol Biochem* 197:109533.
<https://doi.org/10.1016/j.soilbio.2024.109533>
- Liu Y, Wang G, Xu Z et al (2024b) Precipitation pulses regulate phosphorus limitation and microbial C use efficiency in arid grassland soils. *Glob Chang Biol* 30:e17068. <https://doi.org/10.1111/gcb.17068>
- Liu YL, Li L, Zhang ZH et al (2025) Ultraviolet radiation effects on biodegradation depend on precipitation amount, litter quality, and time in an arid land. *Appl Soil Ecol* 215:106451. <https://doi.org/10.1016/j.apsoil.2025.106451>
- Luo R, Kuzyakov Y, Liu D et al (2020) Nutrient addition reduces carbon sequestration in a Tibetan grassland soil: disentangling microbial and physical controls. *Soil Biol Biochem* 144:107801. <https://doi.org/10.1016/j.soilbio.2020.107801>
- Manzoni S, Taylor PG, Richter A et al (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol* 196:79–91.
<https://doi.org/10.1111/j.1469-8137.2012.04225.x>
- McGroddy ME, Silver WL, de Oliveira RC (2004) The effect of P availability on

decomposition dynamics in a seasonal lowland Amazonian forest. *ECOSYSTEMS* 7:172–179. <https://doi.org/10.1007/s10021-003-0208-y>

Mougi A, Kondoh M, Ushimaru A (2023) Eco-evolutionary dynamics of pulsed resources in arid ecosystems. *Ecol Lett* 26:1025–1034. <https://doi.org/10.1111/ele.14235>

Olson JS (1963) Energy storage and the balance of producers and decomposers in ecological systems. *ECOLOGY* 44:322–331. <https://doi.org/10.2307/1932179>

Parton W, Silver WL, Burke IC et al (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. *Glob Biogeochem Cycles* 21:GB4016. <https://doi.org/10.1029/2006GB002878>

Schimel JP, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and the structure and function of soil microbial communities. *Soil Sci Soc Am J* 71:1123–1133. <https://doi.org/10.2136/sssaj2006.0309>

Shi A, Penfold C, Marsden PJ (2018) High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *J Vis Exp* 139:e58161. <https://doi.org/10.3791/58161>

Shi JY, Gong JR, Baoyin TT et al (2021) Short-term phosphorus addition increases soil respiration by promoting gross ecosystem production and litter decomposition in a typical temperate grassland in northern China. *CATENA* 197:104952. <https://doi.org/10.1016/j.catena.2020.104952>

Sinsabaugh RL, Hill BH, Shah JJF (2008) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *NATURE* 462:795–798. <https://doi.org/10.1038/nature08632>

Sinsabaugh RL, Manzoni S, Moorhead DL, Richter A (2013) Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecol Lett* 16:930–939. <https://doi.org/10.1111/ele.12113>

Tian P, Liu S, Zhao X et al (2021) Past climate conditions predict the influence of nitrogen enrichment on the temperature sensitivity of soil respiration. *Commun Earth Environ* 2:78. <https://doi.org/10.1038/s43247-021-00259-8>

Van Soest PJ (1967) Development of a comprehensive system of feed analyses and its application to forages. *J Anim Sci* 26:119–128. <https://doi.org/10.2527/jas1967.261119x>

Vitousek PM (1994) *Nutrient Cycling and Nutrient Limitation in Tropical Forest Ecosystems*. Princeton University Press, Princeton, NJ, USA.

Wang QW, Pieristè M, Liu C et al (2020) The contribution of photodegradation to litter decomposition in a temperate forest gap and understory. *New Phytol* 229:2625–2636. <https://doi.org/10.1111/nph.17011>

Xiao W, Chen C (2021) Functional and phylogenetic diversity promote litter decomposition across terrestrial ecosystems. *Ecol Lett* 23:1488–1499. <https://doi.org/10.1111/ele.13561>

Yang K, Zhu J, Zhang W et al (2020) Ecoenzymatic stoichiometry reveals microbial nutrient limitation and resource allocation in response to precipitation change in a desert steppe. *GEODERMA* 375:114851. <https://doi.org/10.1016/j.geoderma.2020.114851>

- Yang X, Jiang N, Sun D et al (2024a) Dry-wet cycles induce the decoupling of carbon and nitrogen mineralization at high temperatures in semi-arid grassland soil. *Soil Biol Biochem* 188:109227. <https://doi.org/10.1016/j.soilbio.2023.109227>
- Yang L, Canarini A, Zhang W et al (2024b) Microbial life-history strategies mediate microbial carbon pump efficacy in response to N management depending on stoichiometry of microbial demand. *Glob Chang Biol* 30:e17311. <https://doi.org/10.1111/gcb.17311>
- Yuan Y, Li Y, Mou ZJ et al (2021) Phosphorus addition decreases microbial residual contribution to soil organic carbon pool in a tropical coastal forest. *Glob Chang Biol* 27:454–466. <https://doi.org/10.1111/gcb.15468>
- Zhang JF, Zhou JG, Lambers H et al (2022) Nitrogen and phosphorus addition exerted different influences on litter and soil carbon release in a tropical forest. *Sci Total Environ* 832:155049. <https://doi.org/10.1016/j.scitotenv.2022.155049>
- Zheng M, Chen Y, Liu L et al (2023) Seasonal shifts in extracellular enzyme stoichiometry reflect microbial nutrient acquisition strategies in alpine and arid soils. *BIOGEOCHEMISTRY* 164:331–347. <https://doi.org/10.1007/s10533-023-01054-2>
- Zhou SX, Butenschoen O, Barantal S et al (2020) Decomposition of leaf litter mixtures across biomes: the role of litter identity, diversity and soil fauna. *J Ecol* 108:2374–2385. <https://doi.org/10.1111/1365-2745.13447>

Table 1 Initial chemical properties of two types of litter

Factors	Single-litter	Mix-litter
C (g/kg)	419.61 ± 4.91a	368.94 ± 5.51b
N (g/kg)	14.85 ± 3.29a	11.34 ± 3.47b
P (g/kg)	0.89 ± 1.15a	0.69 ± 1.01b
Lignin (%)	10.67 ± 0.45b	12.91 ± 0.51a
Cellulose (%)	2.73 ± 0.27a	2.69 ± 0.24b
Hemicellulose (%)	11.16 ± 0.41a	9.84 ± 0.82b
C/N	28.26 ± 0.39b	32.52 ± 0.67a
C/P	467.38 ± 0.28b	532.48 ± 0.37a
N/P	16.54 ± 0.87a	16.37 ± 0.91a
C/N/P	31.75 ± 0.49b	47.15 ± 0.16a
Lignin/N	7.18 ± 0.58b	11.38 ± 0.49a
Lignin/P	118.81 ± 0.76b	186.26 ± 0.37a

Note: Different lowercase letters indicate significant differences between single and mixed-species litter ($p < 0.05$).

Table 2 Decomposition rate constant (k), and the time required for model-predicted mass loss to reach 50% ($T_{50\%}$) and 95% ($T_{95\%}$)

Species	Treatments	k ($\text{g}^{-1} \text{a}^{-1}$)	R^2	P	$T_{50\%}$	$T_{95\%}$
Single-litter	CK	0.483±0.003c	0.953	< 0.001	1.436	6.217
	LP	0.554±0.023c	0.856	< 0.001	1.250	5.412
	MP	0.617±0.004bc	0.895	< 0.001	1.124	4.865
	HP	0.677±0.011b	0.845	< 0.001	1.023	4.429
	W	0.643±0.014b	0.898	< 0.001	1.077	4.663
	WLP	0.716±0.004b	0.958	< 0.001	0.968	4.190
	WMP	0.794±0.006a	0.902	< 0.001	0.873	3.778
	WHP	0.801±0.011a	0.828	< 0.001	0.863	3.736
Mixed litter	CK	0.516±0.008c	0.953	< 0.001	1.308	5.660
	LP	0.530±0.019c	0.856	< 0.001	1.343	5.816
	MP	0.694±0.008b	0.895	< 0.001	0.998	4.322
	HP	0.628±0.016bc	0.845	< 0.001	1.104	4.777
	W	0.627±0.004bc	0.898	< 0.001	1.105	4.782
	WLP	0.667±0.013b	0.958	< 0.001	1.009	4.369
	WMP	0.718±0.021a	0.902	< 0.001	0.967	4.180
	WHP	0.698±0.009a	0.828	< 0.001	1.003	4.345

Note: Different lowercase letters within a column indicate significant differences among treatments ($p < 0.05$). Values are presented as mean ± standard error ($n = 3$). Treatment codes are provided in Fig. 1.

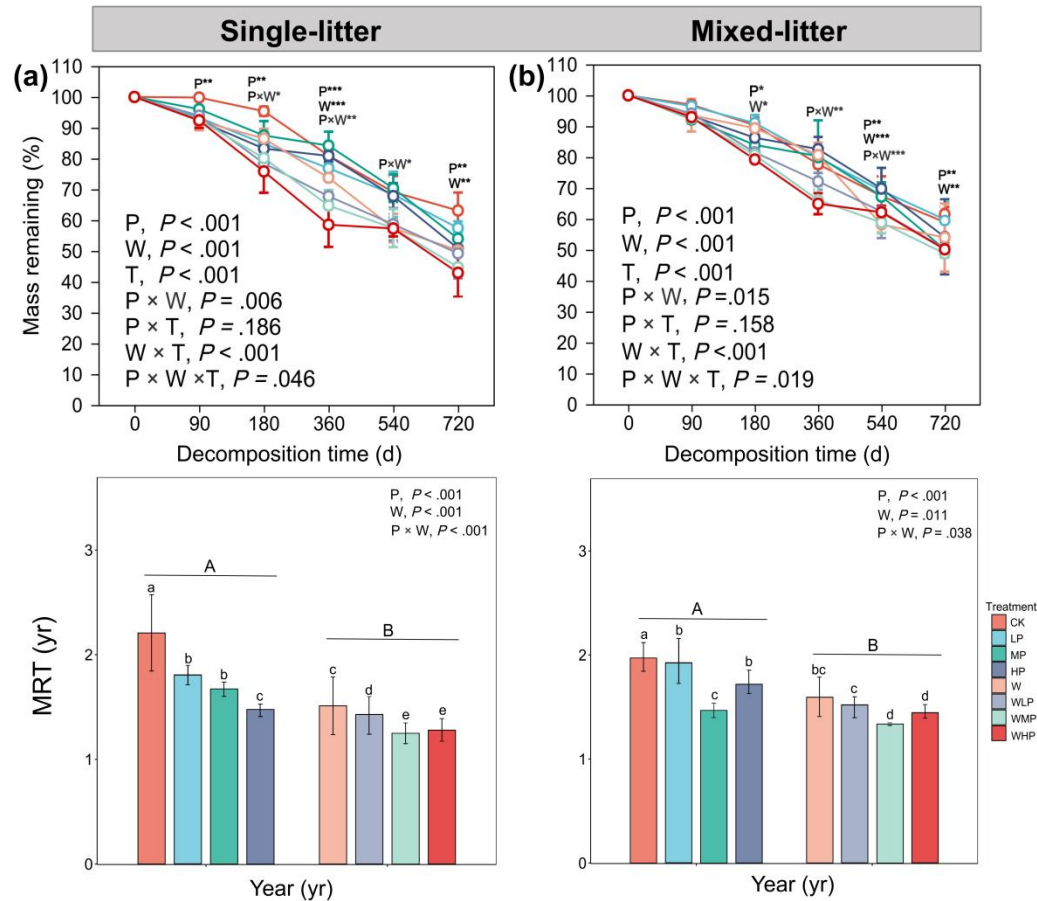


Fig. 1 Mass remaining dynamics and mean residence time of single (a) and mixed (b) litter. P: P addition; W: precipitation increase; T: decomposition duration; $P \times W$: two-way interaction between P addition and precipitation increase; $P \times T$: two-way interaction between P addition and decomposition duration; $W \times T$: two-way interaction between precipitation increase and decomposition duration; $P \times W \times T$: three-way interaction among P addition, precipitation increase and decomposition duration. CK: ambient precipitation and P deposition; W: 150% of ambient precipitation; LP: $1 \text{ g P m}^{-2} \text{ yr}^{-1}$; MP: $2.5 \text{ g P m}^{-2} \text{ yr}^{-1}$; HP: $5 \text{ g P m}^{-2} \text{ yr}^{-1}$; WLP: combined W and LP treatments; WMP: combined W and MP treatments; WHP: combined W and HP treatments. Different lowercase letters indicate significant differences among treatments ($p < 0.05$, Tukey's HSD test). Error bars represent standard error ($n = 3$). Significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

Table 3 Linear mixed-effects models revealed the effects of phosphorus addition (P), precipitation increase (W), and litter type (S) on litter decomposition dynamics during summer and winter

Time	Treatments		P	W	S	W*P	P*S	W*S	W*P*S
	<i>df</i>		3	1	2	3	6	2	6
Summer	Mass loss	<i>F</i>	.314	53.827	.716	.195	.641	1.493	.030
		<i>P</i>	.002	< .001	.025	< .001	.094	.231	.093
	C loss	<i>F</i>	7.263	82.207	3.689	2.365	1.546	1.546	1.347
		<i>P</i>	.001	< .001	.064	.089	.222	.232	.276
	N loss	<i>F</i>	28.375	52.682	6.384	5.927	1.179	2.336	1.224
		<i>P</i>	< .001	< .001	.017	.002	.333	.136	.317
	P loss	<i>F</i>	27.397	23.740	105.339	2.446	5.925	.220	7.180
		<i>P</i>	< .001	< .001	< .001	.082	.002	.642	.001
	L loss	<i>F</i>	8.667	50.975	487.901	6.713	7.190	36.732	5.880
		<i>P</i>	< .001	< .001	< .001	.001	.001	< .001	.003
	Cellulose loss	<i>F</i>	2.462	263.446	29.720	14.186	9.709	4.136	13.720
		<i>P</i>	.080	< .001	< .001	< .001	< .001	.005	< .001
	C/N	<i>F</i>	19.458	19.363	39.589	11.635	1.536	.151	2.606
		<i>P</i>	< .001	< .001	< .001	< .001	.224	.700	.069
	C/P	<i>F</i>	68.588	256.835	33.454	1.849	6.809	6.074	7.471
		<i>P</i>	< .001	< .001	< .001	.048	.001	.019	.001
	N/P	<i>F</i>	140.007	213.762	103.869	7.033	6.762	15.155	4.268
		<i>P</i>	< .001	< .001	< .001	.001	.001	< .001	.012
	Lignin/N	<i>F</i>	57.050	35.978	.001	10.900	9.900	12.126	5.797
		<i>P</i>	< .001	< .001	.980	< .001	.003	.001	.003
	Lignin/P	<i>F</i>	22.069	57.477	121.565	15.198	2.180	.596	12.029
		<i>P</i>	< .001	< .001	< .001	< .001	.110	.446	< .001
Winter	Mass loss	<i>F</i>	4.912	20.247	1.123	.372	.623	2.097	.467
		<i>P</i>	.006	< .001	.297	.774	.605	.157	.707
	C loss	<i>F</i>	2.766	30.958	1.004	1.004	.372	.372	1.452
		<i>P</i>	.038	< .001	.324	.876	.774	.832	.246
	N loss	<i>F</i>	11.802	49.969	1.362	.696	.899	.022	2.269
		<i>P</i>	< .001	< .001	.252	.561	.452	.883	.099
	P loss	<i>F</i>	42.601	.594	136.481	.146	2.149	.620	.457
		<i>P</i>	.066	.447	< .001	.932	.114	.437	.714
	L loss	<i>F</i>	9.886	59.265	440.071	.909	8.396	2.517	.485
		<i>P</i>	< .001	< .001	< .001	.447	< .001	.122	.695
	Cellulose loss	<i>F</i>	5.916	1.661	.243	.710	10.092	2.523	.875
		<i>P</i>	.002	.207	.207	.553	< .001	.122	.464
	C/N	<i>F</i>	25.891	10.530	60.277	1.972	2.739	.336	12.439
		<i>P</i>	< .001	.053	< .001	.138	.060	.566	< .001
	C/P	<i>F</i>	21.244	74.267	52.303	.354	6.151	.189	7.911
		<i>P</i>	< .001	< .001	< .001	.787	.002	.667	.577
	N/P	<i>F</i>	66.001	133.188	133.188	133.188	12.236	2.476	9.900
		<i>P</i>	< .001	< .001	< .001	.265	< .001	.125	.627
	Lignin/N	<i>F</i>	27.871	125.638	8.171	3.892	1.564	2.062	2.121
		<i>P</i>	< .001	< .001	.007	.018	.217	.161	.117
	Lignin/P	<i>F</i>	11.928	1.494	122.874	.561	9.585	.001	6.282
		<i>P</i>	< .001	.230	< .001	.645	< .001	.978	.002

Note: Values shown in bold denote statistically significant effects at $p < 0.05$.

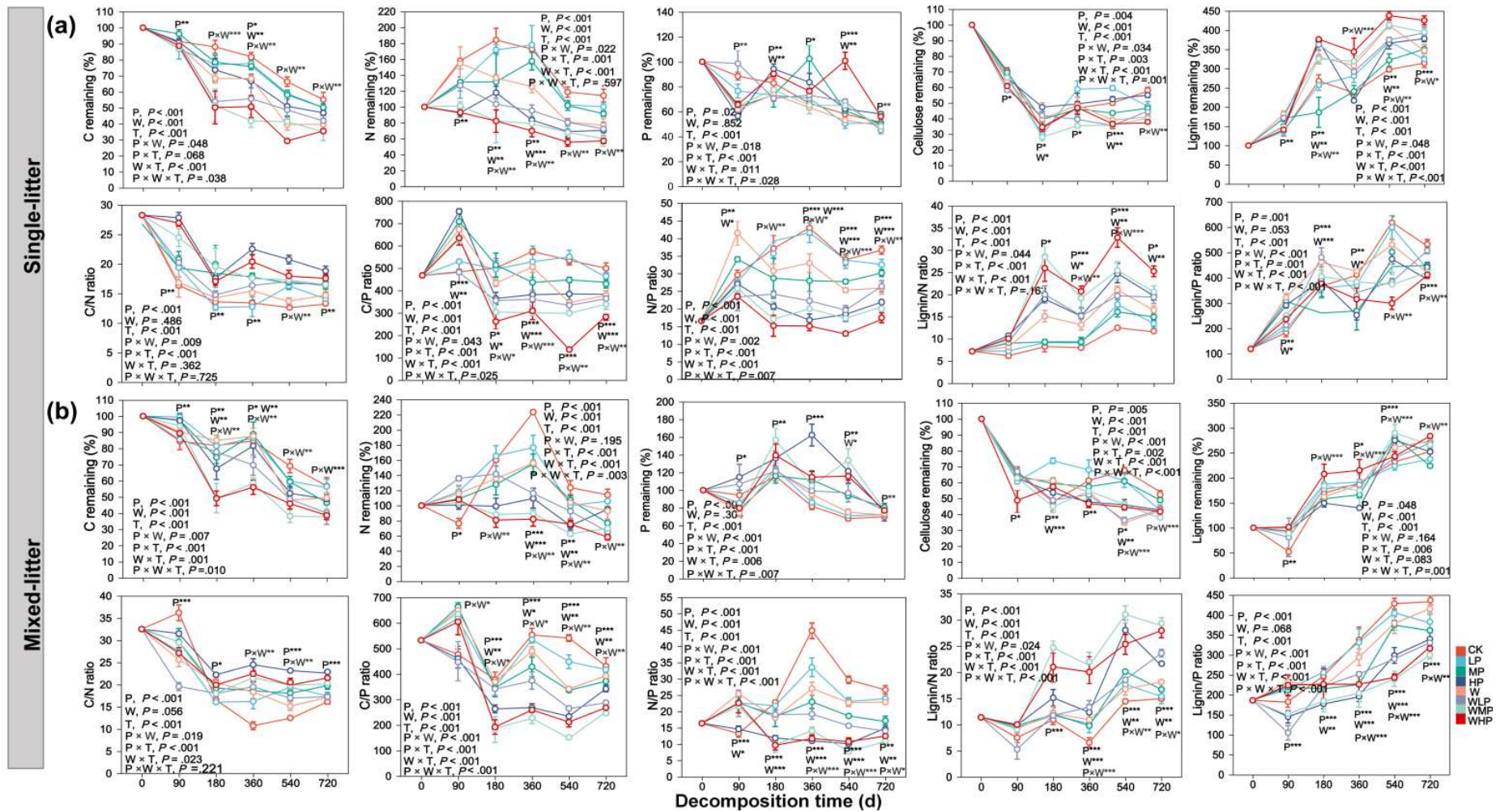


Fig. 2 Dynamics of nutrient remaining and stoichiometric ratios of single (a) and mixed (b) litter. Error bars represent standard error ($n = 3$). Significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. Treatment codes are provided in Fig. 1

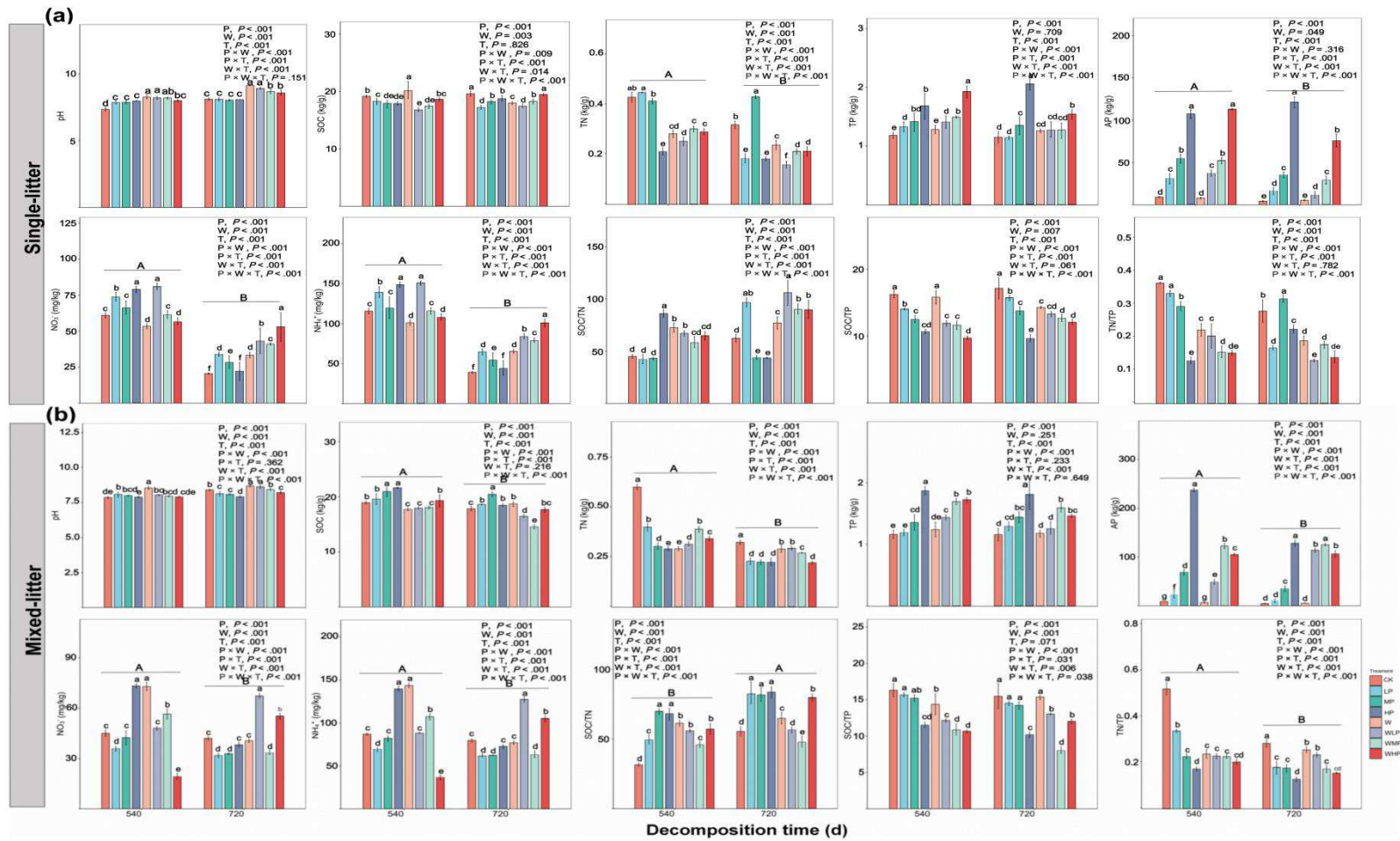


Fig. 3 Dynamics of soil physicochemical properties and stoichiometric ratios of single (a) and mixed (b) litter. Different lowercase letters indicate significant differences among treatments at the same sampling time ($p < 0.05$, Tukey's HSD test). Error bars represent standard error ($n = 3$). Significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. Treatment codes are provided in Fig. 1.

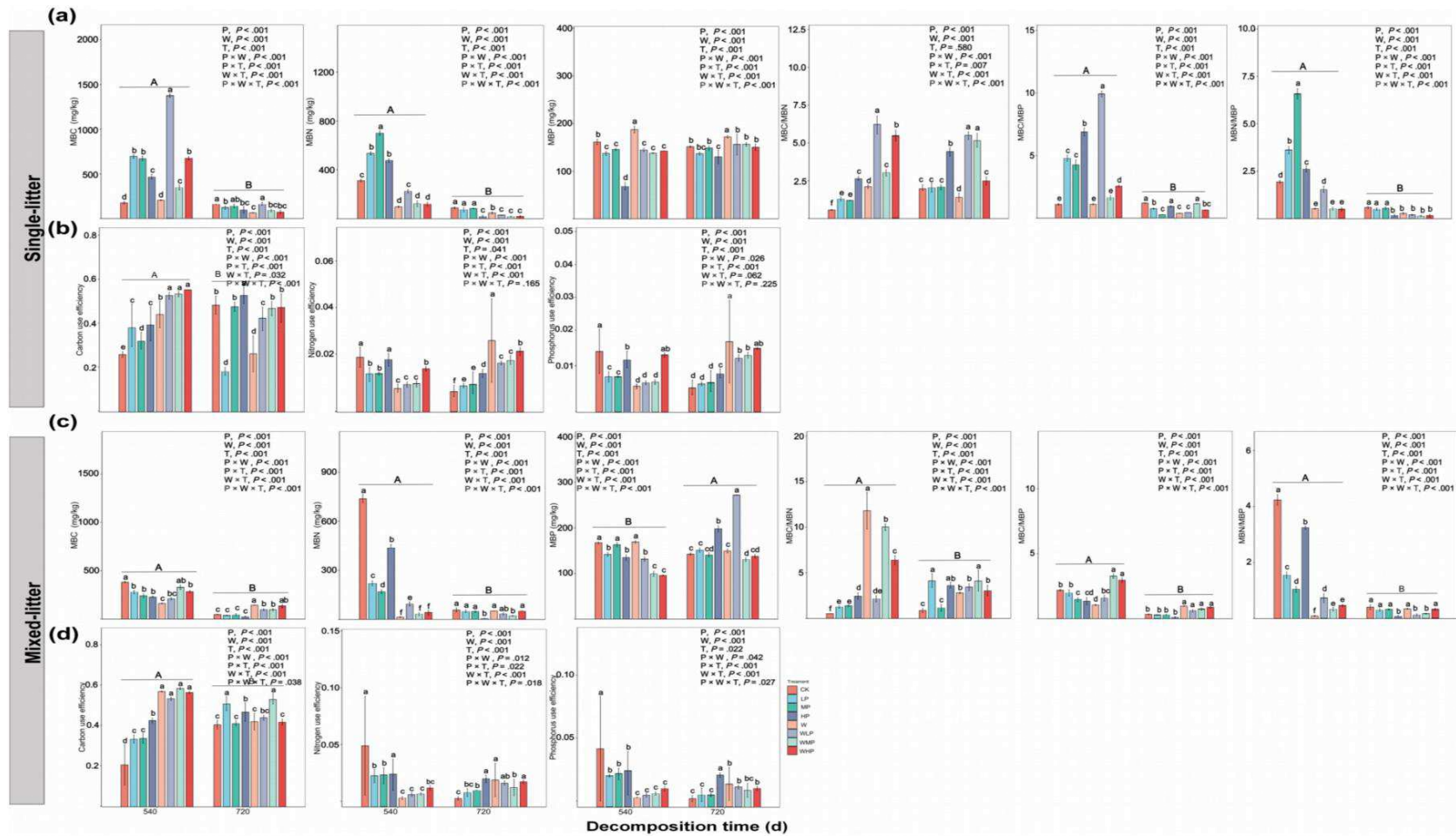


Fig. 4 Dynamics of soil microbial biomass C, N, and P, their stoichiometric ratios, and nutrient use efficiencies of single (a) and mixed (b) litter. Different lowercase letters indicate significant differences among treatments at the same sampling time ($p < 0.05$, Tukey's HSD test). Error bars represent standard error ($n = 3$). Significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. Treatment codes are provided in Fig. 1.

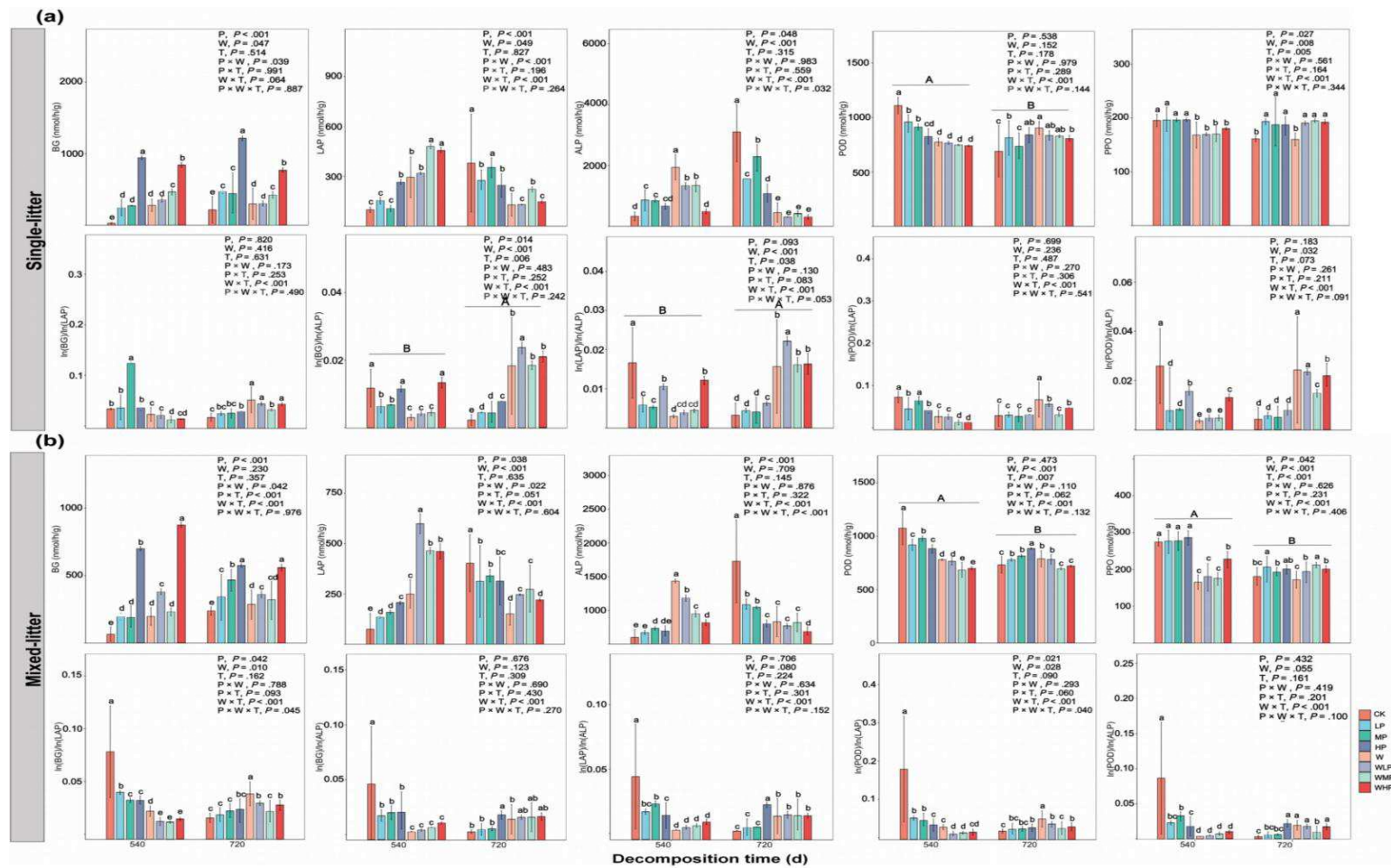


Fig. 5 Dynamics of soil extracellular enzyme activities and stoichiometric ratios of single (a) and mixed (b) litter. Different lowercase letters indicate significant differences among treatments at the same sampling time ($p < 0.05$, Tukey's HSD test). Error bars represent standard error ($n = 3$). Significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. Treatment codes are provided in Fig. 1.

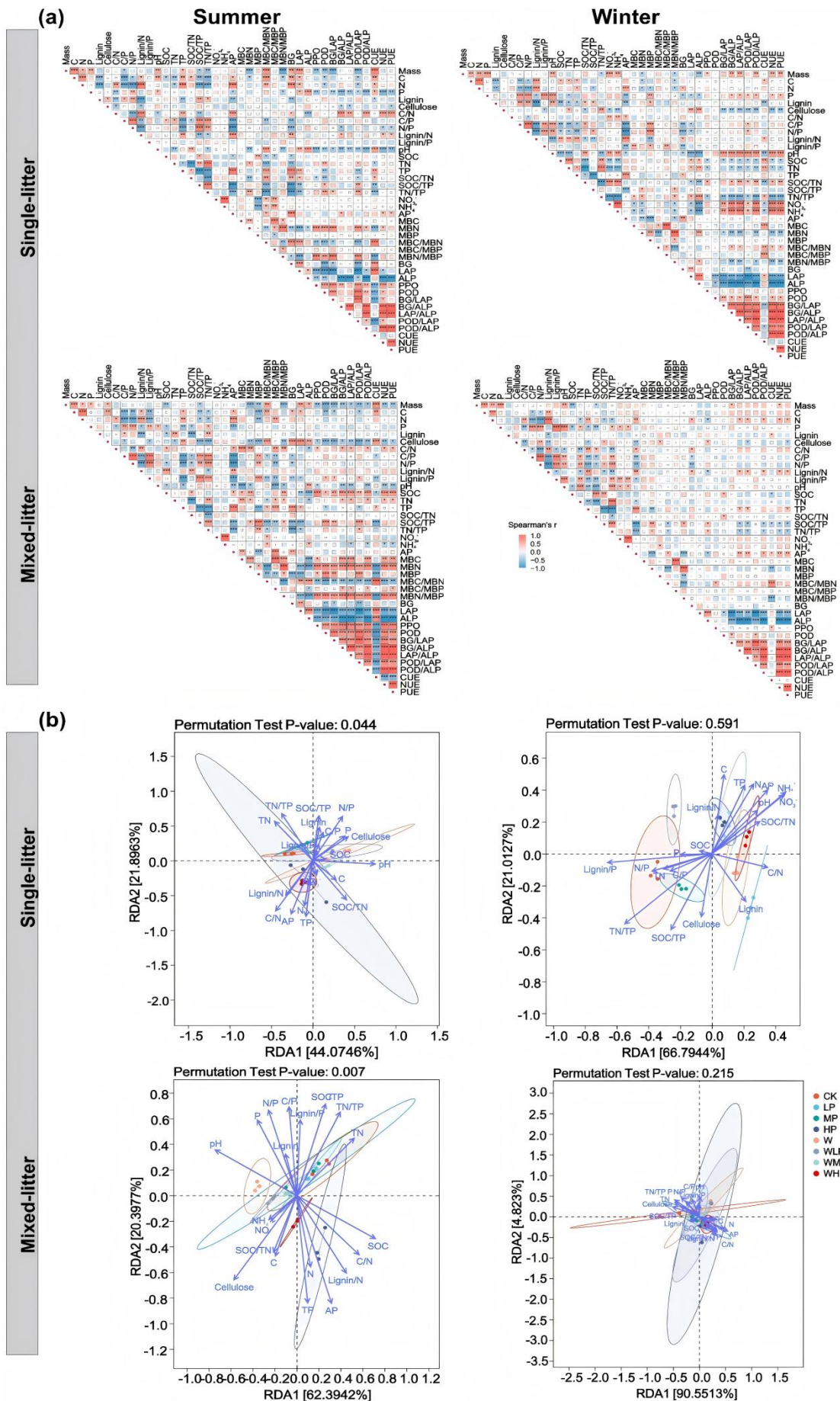


Fig. 6 (a) Correlations between litter mass loss of the two litter types during summer and winter and litter nutrients, soil physicochemical properties, microbial biomass C, N and P, extracellular enzyme activities, and their stoichiometric ratios. **(b)** Redundancy analysis (RDA) showing the effects of litter components and soil physicochemical properties on soil enzyme activities and their stoichiometry under precipitation and P addition. Mass: Mass loss; C: C loss; N: N loss; P: P loss; Lignin: Lignin loss; Cellulose: Cellulose loss; BG/LAP: $\ln(\text{BG})/\ln(\text{LAP})$ ratio; BG/ALP: $\ln(\text{BG})/\ln(\text{ALP})$ ratio; LAP/ALP: $\ln(\text{LAP})/\ln(\text{ALP})$ ratio; POD/LAP: $\ln(\text{POD})/\ln(\text{LAP})$ ratio; POD/ALP: $\ln(\text{POD})/\ln(\text{ALP})$. Significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. Treatment codes are provided in Fig. 1.

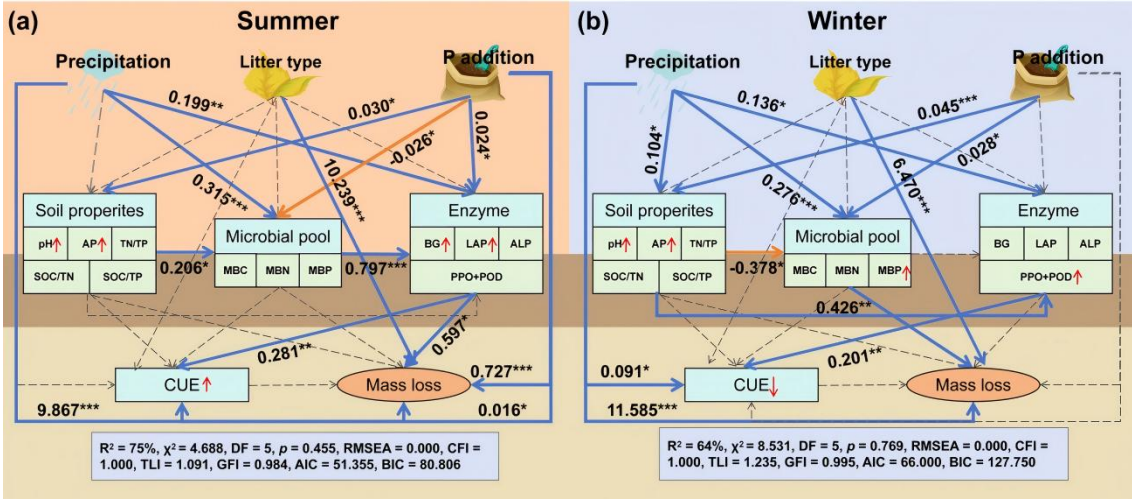


Fig. 7 Structural equation models (SEMs) showing the major pathways through which precipitation, P addition, and litter type influence litter mass loss in summer **(a)** and winter **(b)**. Latent variables are represented by ellipses, and observed variables by rectangles. Values adjacent to arrows are standardized path coefficients. Blue and orange arrows denote positive and negative effects, respectively, while dashed arrows indicate non-significant relationships. R^2 values below each response variable represent the proportion of variance explained by the model. Significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

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