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RESEARCH ARTICLE

The litter C/N ratio governs the dual role of ectomycorrhizal fungi in decomposition and plant nutrition absorption

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Abstract

1. Ectomycorrhizal fungi (EcMF) modulate litter decomposition and facilitate nutrient acquisition through the soil–mycorrhiza–plant pathway. Although litter chemistry has been considered an important factor affecting litter decomposition, how it regulates EcMF-induced decomposition and subsequent plant growth remains unclear.
2. Using a microcosm experiment with five litter types, we assessed decomposition rates, carbon (C) and nitrogen (N) loss, microbial biomass, enzyme activities and plant growth under ectomycorrhizal and non-ectomycorrhizal seedlings.
3. Results indicated that effects of EcMF on litter decomposition were litter-specific and time-dependent. After 120 days, EcMF enhanced decomposition of low C/N litters (*Carex breviculmis*, *Lindera glauca* and *Liquidambar formosana*) by 13%–28%, but suppressed that of high C/N litters (*Quercus acutissima* and *Pinus massoniana*) by 19%–20%. Initially (30 days), EcMF inhibited low C/N litter decomposition, later shifting to promotion, whereas high C/N litter decomposition was consistently suppressed. Correlation analysis revealed that the hyphal effects on decomposition correlated negatively with litter stage C/N ratio. EcMF significantly increased plant N concentration and biomass under low C/N litters (biomass increased by 20%–30%), but only marginally enhanced biomass under high C/N litters (14%–16%) without improving N concentration. Partial least squares path modelling revealed that EcMF promoted C and N release from low C/N litter, thereby increasing N availability and plant growth. Even under high C/N conditions, EcMF directly supported plant growth despite suppressed decomposition.
4. These findings underscore the dual roles of EcMF, which are regulated by litter C/N ratio, in mediating decomposition and plant nutrient acquisition, highlighting the importance of litter–microbe interactions in forest nutrient cycling and productivity.

KEYWORDS
C/N ratio, ectomycorrhizal fungi, hyphal effect, litter decomposition, plant growth, plant N concentration

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1 | INTRODUCTION

Litter decomposition is the core process of carbon (C) and nutrient cycling in terrestrial ecosystems, regulating soil organic matter formation, nutrient release and global C balance (Aber & Melillo, 1980; Prescott, 2010). It is estimated that forest ecosystems produce over 60 Pg of litter annually, with approximately 50% of this input ultimately contributing to soil C pools through decomposition (Pan et al., 2011). As a critical pathway in nutrient cycling, litter returns essential elements such as C and nitrogen (N) to the soil in bioavailable forms, thereby continuously supporting plant growth and sustaining ecosystem productivity (Wu et al., 2021). Understanding the mechanisms that drive litter decomposition is therefore essential for predicting how ecosystem functions will respond to environmental changes.

Litter decomposition has traditionally been viewed as being dominated by free-living saprophytic microorganisms (Kjoller & Struwe, 1992). However, emerging evidence has found the critical role of plant-symbiotic ectomycorrhizal fungi (EcMF) in modulating this process. EcMF form mutualisms with trees, exchanging photosynthetically derived C for host nutrients like N and phosphorus via extraradical mycelial networks (Smith & Wan, 2019). They regulate decomposition through two contrasting mechanisms. On one hand, EcMF can suppress decomposition via the 'Gadgil effect' (Fernandez & Kennedy, 2016; Gadgil & Gadgil, 1971), wherein they compete with saprophytic fungi for nutrients and release enzymes to extract N from organic matter—a process known as 'mining' (Bödeker et al., 2016; Fernandez et al., 2019). This mining increases substrate C/N ratios, inducing N limitation that impairs saprotrophic decomposition efficiency (Lindahl & Tunlid, 2015; Smith & Wan, 2019). On the other hand, EcMF can enhance decomposition via priming effects, potentially by alleviating microbial C limitation through the release of labile C or necromass (Johansson et al., 2009; Lang et al., 2021; Wilkinson et al., 2011). Furthermore, EcMF also drive C decomposition during N mining (Argiroff et al., 2022; Lindahl et al., 2021), since releasing N from organic matter necessitates the enzymatic hydrolysis of C skeletons (Jones et al., 1998; Packard et al., 2025). Thus, this dual role of inhibition and facilitation suggests that EcMF act as dynamic regulators of breakdown.

The variation in ectomycorrhizal fungal effects on litter decomposition may be influenced by substrate chemical composition (Choreño-Parra & Treseder, 2024; Fernandez et al., 2019). Litter chemistry differs among tree species, and both local and global studies show that components such as C and N are strongly linked to decomposition rates (Liao et al., 2022). Since competition for N underpins the Gadgil effect, low-N litter with a high C/N ratio is likely more susceptible to such inhibition. This is supported by the frequent observation of EcMF-mediated suppression in conifer-dominated systems (Fernandez et al., 2019; Fernandez & Kennedy, 2016), where high C/N litter intensifies N competition and reduces decomposition efficiency (Smith & Wan, 2019). In subtropical mixed forests, EcMF networks mediate decomposition of diverse litter sources, yet how litter C/N stoichiometry regulates the role of EcMF in this process remains poorly understood.

Moreover, EcMF play a crucial role not only in regulating soil decomposition but also serve as key bridge driving nutrient cycling within the soil–mycorrhiza–plant system (Liu et al., 2017; Wu, 2011). Through their extensive hyphal networks, EcMF transport nutrients (e.g. N and P) released during decomposition to host plants, significantly enhancing nutrient uptake (Liu et al., 2017; Trap et al., 2016; Wu, 2011). This is achieved by extending the root absorption network (Ditengou et al., 2015), secreting extracellular hydrolases (Jones et al., 1998) and interacting synergistically with other microorganisms to accelerate mineralization (Mei et al., 2023). The mutualistic relationship between EcMF and plants is based on a 'nutrient trade' meaning that the growth-promoting effects of EcMF strongly depend on soil nutrient conditions. It is widely recognized that EcMF-mediated benefits become more pronounced under N- or P-limited conditions. Similarly, when colonizing poorly decomposable litter, EcMF may take up amino acids and amino sugars directly (Chen et al., 2024; Hazard et al., 2017), or degrade proteins by secreting extracellular enzymes (Bending & Read, 1996; Read & Perez-Moreno, 2003), reducing N acquisition by other microorganisms (Trap et al., 2016). This 'N-channelling' mechanism likely facilitates the direct transfer of litter-derived N to host plants, contributing to enhanced forest productivity (Näsholm et al., 2009; Wu, 2011). Thus, litter C and N concentration under EcMF mediation may influence litter-derived nutrients of plant via ectomycorrhizal pathways.

In recent years, studies on EcMF-mediated litter decomposition have often employed methods such as trenching or girdling to reduce EcMF abundance before assessing decomposition rates (Fernandez et al., 2019; Fernandez & Kennedy, 2016). However, these approaches cannot fully eliminate EcMF interference. In contrast, we generated ectomycorrhizal and non-ectomycorrhizal seedlings to explicitly control EcMF presence. Furthermore, litters of five common subtropical plant species were used to better represent natural conditions and broaden the range of C/N ratios examined. Through a litterbag microcosm decomposition experiment with different litter types, we assessed the decomposition rates, C and N loss patterns, microbial biomass and enzyme activity of litter in ectomycorrhizal and non-ectomycorrhizal seedlings, analysed changes in N concentration and growth of host plants, aiming to reveal how litter C/N ratio affects ectomycorrhizal fungal-mediated decomposition and host plant growth. We tested two hypotheses: first, EcMF suppress decomposition more strongly in high C/N litter but enhance it under low C/N conditions; and second, EcMF accelerate nutrient absorption and host plant growth, particularly in high C/N ratio litter that resists decomposition.

2 | MATERIALS AND METHODS

2.1 | Biological materials and soil

Seeds of *Pinus massoniana*, a common EcMF host plant, were obtained from the Institute of Botany, Chinese Academy of Sciences in Beijing. The seeds were surface-sterilized with 3% (v/v) sodium hypochlorite and 70% (v/v) ethanol for 3 min, then cultured in sterile vermiculite.

One-month-old seedlings were transplanted as host plants into the ectomycorrhizal construction system described below.

Forest soils (yellow-brown soil, pH = 5.5, silty clay loam) were collected from the Zijin mountain, Nanjing, in southern China (32°4′N, 118°51′E), sieved (2 mm), and mixed with autoclaved vermiculite (vermiculite: soil = 3:1, v/v) to serve as the inoculation substrate (C concentration: 0.51%; N concentration: 0.06%). All materials were autoclaved to eliminate indigenous microorganisms.

Litter from five common species—*Carex breviculmis*, *Lindera glauca*, *Liquidambar formosana*, *Quercus acutissima* and *P. massoniana*—was collected from the same site for decomposition experiments; their initial chemical properties are listed in Table 1.

The *Suillus* genus is a common EcMF group in southern Chinese forests (Zhang et al., 2017). We collected *Suillus clintoniauns* (PX715512) fruiting bodies from Zhejiang Province (29°34′N, 118°54′E) and isolated the saprophytic fungus *Irpex lacteus* (PQ803832.1) from the fruiting bodies, confirming their natural coexistence. *Irpex laceratus* has the ability to degrade cellulose and lignin (Qin et al., 2023). Due to difficulties in isolating a pure EcMF strain, we used the closely related *Suillus grevillei* (PZ246099; obtained from the China Forestry Culture Collection Center and preserved at Nanjing Normal University; 95% ITS similarity) as our experimental EcMF.

2.2 | Construction of ectomycorrhizal plants

A total of 600 *P. massoniana* seedlings were planted: 300 into 50 pots inoculated with 5 g (dry weight) of *S. grevillei*, and 300 into 50 control pots with autoclaved inoculum (Mei et al., 2023). All plants were grown in a greenhouse (16-h photoperiod, 28°C, 60% relative humidity) at Nanjing University.

After 6 months, seedlings showing ectomycorrhizal colonization via root binary branching (Figure S1) were designated the +EcMF group; those from autoclaved-inoculated pots served as the −EcMF control (CK). Five seedlings per treatment were harvested initially, showing no significant phenotypic differences (Table S1). Ectomycorrhizal colonization was assessed by measuring ergosterol concentration in fine roots (diameter ≤2 mm) from five randomly selected plants per treatment. Ergosterol, a fungal membrane sterol, served as a biomarker for EcMF biomass (Ekblad et al., 1998). The methods are described in the Supporting Information. The mean ergosterol concentration was 1.69 $\mu g\,g^{-1}$ dry root in CK and 15.33 $\mu g\,g^{-1}$ dry root in the EcMF treatment (Table S1).

TABLE 1 Initial chemical characteristics of litters.

Litter types	Total C (%)	Total N (%)	C/N
C. breviculmis	41.63 ± 0.69	1.19 ± 0.05	35.01 ± 0.52
L. glauca	49.21 ± 1.53	1.12 ± 0.03	43.9 ± 1.12
L. formosana	45.01 ± 0.74	0.88 ± 0.03	51.09 ± 0.94
Q. acutissima	51.99 ± 0.89	0.83 ± 0.04	62.71 ± 0.93
P. massoniana	52.01 ± 1.27	0.62 ± 0.04	83.85 ± 1.14

Note: Values are means and standard deviations ($n = 3$).

2.3 | Decomposition experiment design

The microcosm decomposition experiment was conducted in pots (150 mm diameter × 140 mm height), each containing six *P. massoniana* seedlings, including EcMF-colonized (EcMF) and non-colonized (CK) treatments. Litter samples were air-dried for 2 weeks, then cut into 2 cm segments. For each bag (2 × 7 cm, 30 μm nylon mesh), 1 g of material was sealed, gamma-irradiated (total dose 50 kGy in two rounds with a 1-week interval) (Lensi et al., 1991) and inoculated with saprophytic fungus *I. lacerates* (5-mm diameter fungal plug).

Five replicate bags per litter type per treatment were buried 1 cm deep (Figure 1, Table 2). Sampling was performed at 30-day intervals. The experimental design included two seedling treatments × five litter types × five sampling time points × five replicates, resulting in 250 litter bags. To evaluate the interactive effects of EcMF and litter type on plant growth, control pots without litter bags were set up for both the EcMF and CK treatments, with five replicates each.

All microcosms were randomly arranged in a greenhouse under controlled conditions (16-h photoperiod, 28°C, 60% relative humidity) and watered weekly with deionized water. The experiment lasted 120 days (December 2024–April 2025). Litter samples were collected monthly, and in the final month, host plants and soil were also harvested for subsequent measurements.

2.4 | Mass remaining, C and N remaining of litter

Litters were harvested after 30, 60, 90 and 120 days of decomposition. Retrieved litter bags were weighed to determine total remaining fresh mass. A subsample (approximately 0.3 g) was oven-dried at 65°C for 48 h to determine moisture content, enabling calculation of total remaining dry mass. The decomposition rate was expressed using the exponential decay rate coefficient, k, in the model $M_t/M_0 = e^{-kt}$, where M_0 represents initial dry mass and M_t denotes dry mass at time t, and t is the sampling time (month) (Olson, 1963).

Dried litter was ground to <1 mm and analysed for C and N concentration via dry combustion (Vario MICRO cube, Elementar, Germany). Litter C remaining (%) was calculated as: $100 - 100 \times [(M_i \times C_i) - (M_p \times C_p)]/(M_i \times C_i)$, where M_i and M_p are the initial and post-incubation litter masses, respectively, and C_i and C_p are the initial and posterior C concentrations, respectively. N remaining (%) was calculated analogously.

2.5 | Analysis of microbial biomass and enzyme activity in litters

Microbial biomass was estimated using the substrate-induced respiration (SIR) method (Anderson & Domsch, 1978). Briefly, 0.2 g of litter was placed in a 100 mL vial, amended with 2 mL of glucose solution (100 mg glucose per g dry sample), and incubated at 25°C for 1 h.

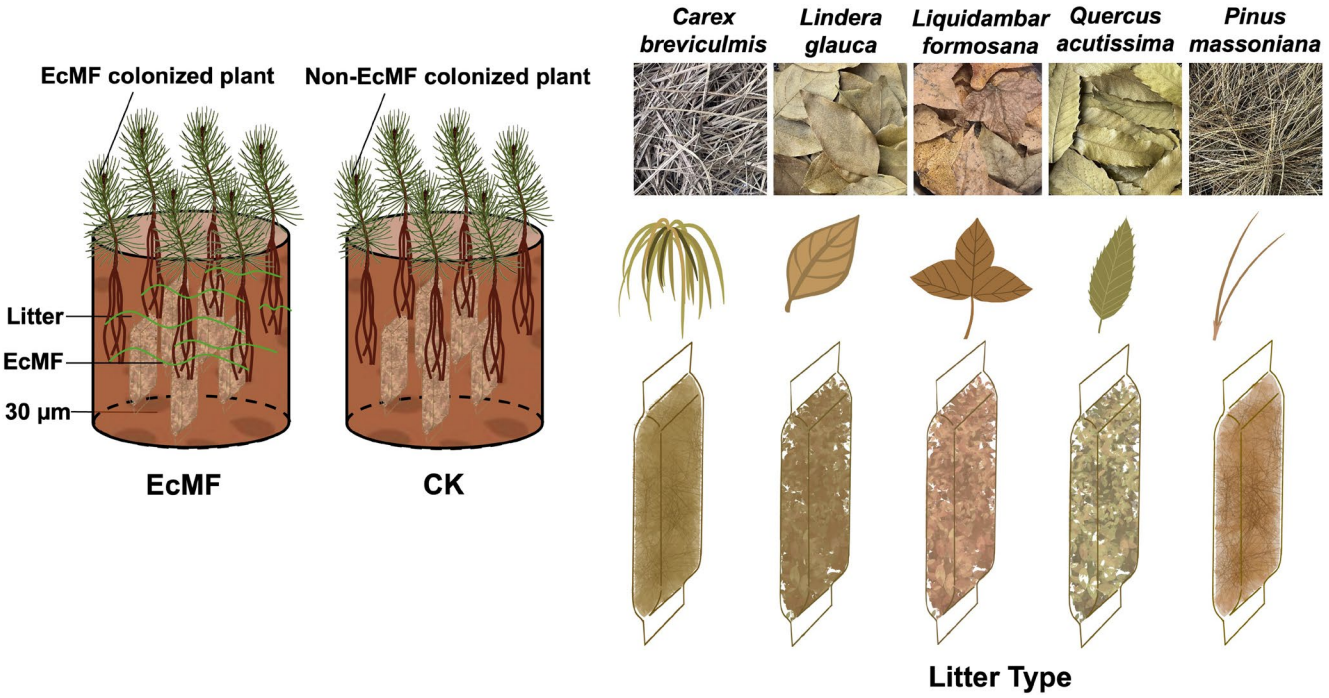


FIGURE 1 Design of experimental microcosm pots. The ectomycorrhizal seedlings and non-ectomycorrhizal seedlings were planted in the microcosm pots. The litter was placed in nylon mesh bags (pore size 30 μm) and buried below the soil. The types of litter included: *C. breviculmi*, *L. glauca*, *L. formosana*, *Q. acutissima* and *P. massoniana*.

TABLE 2 Replication statement for the litter decomposition experiment.

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Individuals	Litters	5 of each treatment (five litter types; two plant mycorrhizal colonization statuses)
Plants	Plants	5 of each treatment (no litter and five litter types; two plant mycorrhizal colonization statuses)

Note: Five litter types included litter from *C. breviculmis*, *L. glauca*, *L. formosana*, *Q. acutissima* and *P. massoniana*. The two plant mycorrhizal colonization statuses were EcMF-colonized and non-EcMF-colonized plants.

CO_2 production was measured with an infrared gas analyser (Bailey et al., 2002).

To quantify the biomass of EcMF *S. grevillei* and the saprophytic fungus *I. lacteus* in litter bags, quantitative PCR (qPCR) was employed. The methods are described in the Supporting information, and the primers are listed in Table S2. Results are expressed as $\log_{10}$-transformed gene copy numbers.

The activities of β-glucosidase (BG), cellobiohydrolase (CBH), polyphenol oxidase (PPO), peroxidase (POD), β-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) were determined following Sinsabaugh et al. (2008). For each assay, 0.2 g of litter was homogenized in 2 mL of acetic acid buffer (pH = 5). Then, 100 μL of homogenate was combined with 150 μL of buffer (control) or substrate (test) in a microplate. For POD, 10 μL of 0.3% H_2O_2 was added. After incubation (duration per enzyme given in Table S3), 100 μL of the reaction mixture was transferred to a new plate, and 5 μL of 1.0 M NaOH was added to stop the reaction and develop colour. Absorbance was read at 405 nm (BG, CBH, LAP, NAG) or 450 nm (POD, PPO). Enzyme-specific substrates, concentrations and EC numbers are listed in Table S3. Activity is expressed as μmol substrate hydrolysed per hour per g dry litter ($\mu mol\, h^{-1}\, g^{-1}$).

2.6 | Plant biomass and N concentration

Plants were harvested after 120 days. Roots were separated from soil, rinsed with distilled water and dried at 65°C to constant weight to determine biomass. Dried plant material was ground to <1 mm and analysed for total C and N concentration using dry combustion with an elemental analyser (Vario MICRO cube, Elementar, Germany).

2.7 | Statistical analysis

All statistical analyses were performed in SPSS (version 22.0). Data were tested for normality and homogeneity of variances. Litter decomposition rates, mass remaining, litter N and litter C/N between EcMF and CK treatments were compared using independent-samples *t*-tests. The hyphal effect on a given variable

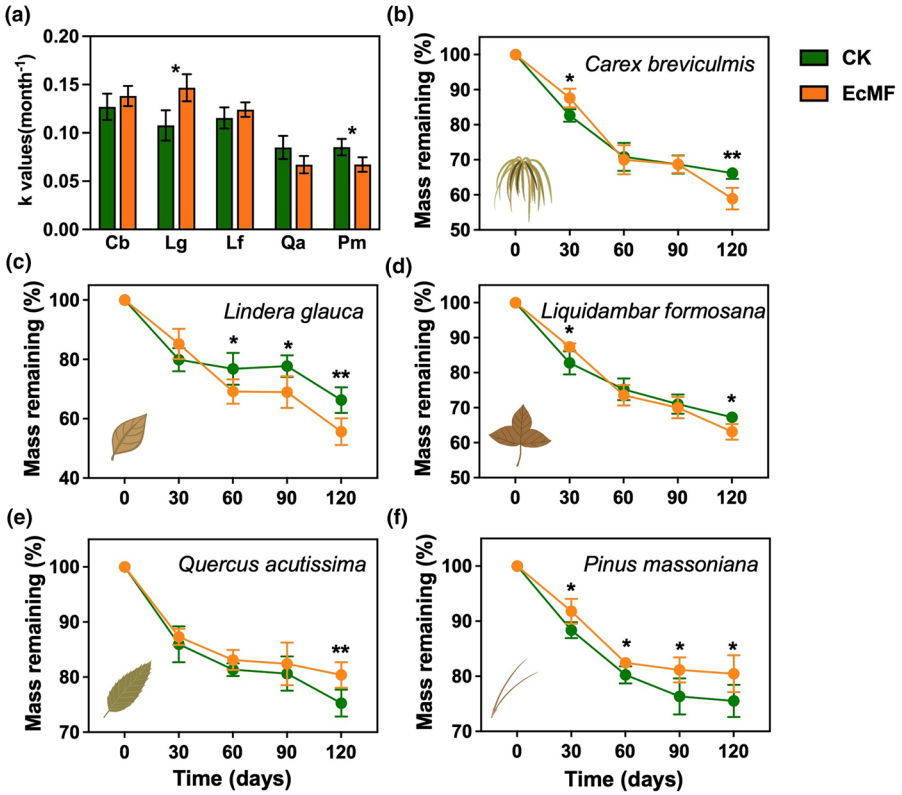


FIGURE 2 Decomposition rates k (a) and mass remaining (b–f) of the five litter types during the 120-day decomposition process under treatments with EcMF (EcMF) and without EcMF (CK). Values are means and standard deviations ($n = 5$). Asterisks indicate significant differences between the EcMF treatment and the CK treatment for the same litter type (Student's *t*-test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

was calculated as: Value (EcMF)/Value (CK), where a value >1 indicates a positive effect and <1 a negative effect. Extracellular enzyme activities (EEA) were grouped as: C-acquiring (CEEA; average of CBH and BG effects), oxidative (OEEA; average of PPO and POD effects) and N-acquiring (NEEA; average of LAP and NAG effects). Linear regression (R 'stats' package) examined relationships between the hyphal effect and stage-specific litter C/N ratios and N concentration, using averaged values per sampling stage. One-way ANOVA with Tukey's HSD test evaluated differences in plant indicators among inoculation treatments and litter types. To visualize the role of EcMF across C/N ratios and decomposition stages, partial least squares path modelling (PLS-PM) was implemented using the 'plspm' package in R; potential pathways were identified via the 'innerplot' function. Figures were produced in Origin Pro 9.0.

3 | RESULTS

3.1 | Effect of EcMF on litter decomposition

The impact of EcMF on litter decomposition varied with litter type and decomposition stage over the 4-month experiment. EcMF increased the value of the exponential decay rate k for *C. breviculmis*, *L. glauca* and *L. formosana*, while decreasing it for *Q. acutissima* and *P. massoniana*, with significant differences observed for *L. glauca* and *P. massoniana* ($p < 0.05$) (Figure 2a). At day 30, EcMF significantly inhibited decomposition of *C. breviculmis* and *L. formosana* ($p < 0.05$). However, by day 120, EcMF significantly promoted decomposition of *C. breviculmis*, *L. glauca* and *L. formosana*, increasing mass loss by 23%, 28% and 13%, respectively ($p < 0.05$; Figure 2b–d). In contrast, *Q. acutissima* and *P. massoniana* were consistently suppressed by EcMF throughout the experiment, with significant reductions in mass loss of 19% and 20%, respectively, at day 120 ($p < 0.05$; Figure 2e,f). Based on the consistent response patterns—stimulation in litters with lower C/N ratios (*C. breviculmis* = 35.01, *L. glauca* = 43.9, *L. formosana* = 51.09) and suppression in those with higher C/N ratios (*Q. acutissima* = 62.71, *P. massoniana* = 83.85)—we categorized the former as low C/N litter and the latter as high C/N litter.

C-remaining patterns (Figure S2a) aligned with mass loss: for low C/N litter, EcMF initially increased C remaining ($p < 0.05$) but lowered it by the endpoint ($p < 0.05$); for high C/N litter, C remaining was consistently higher under EcMF, with significant differences at day 120 ($p < 0.05$). N remaining increased during decomposition, especially in high C/N litter (Figure S2b). EcMF treatment generally resulted in lower residual N, significantly so in low C/N litter ($p < 0.05$), indicating facilitated N decomposition (Figure S2b). No significant difference in N remaining was observed for high C/N litter.

3.2 | Hyphal effect on microbial characteristics

The hyphal effect (value in EcMF treatment/value in control) indicates inhibition when <1 and stimulation when >1. Based on microbial biomass estimated by substrate-induced respiration, for low

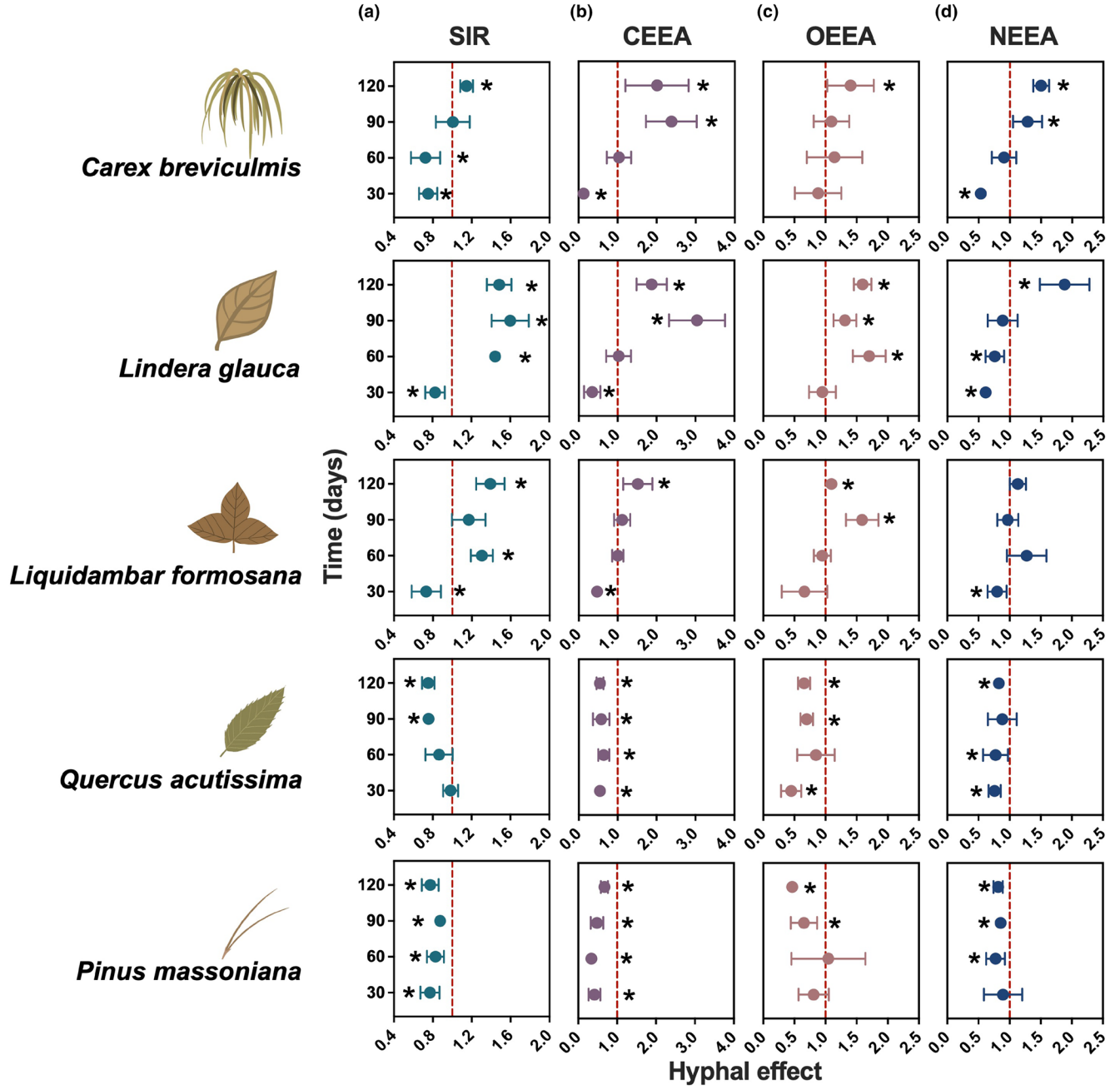


FIGURE 3 Hyphal effects substrate-induced respiration (SIR) (a), C-acquiring extracellular enzyme activities (CEEA) (b), N-acquiring extracellular enzyme activities (NEEA) (c), and oxidative extracellular enzyme activities (OEEA) (d) of the five litter types during the 120-day decomposition process. Values are means and standard deviations ($n = 5$). Asterisks indicate significant effects (Student's *t*-test: $p < 0.05$).

C/N litter, the hyphal effect was <1 initially ($p < 0.05$), then increased to >1 later ($p < 0.05$; Figure 3a). For high C/N litter, values were predominantly <1 across decomposition, indicating consistent suppression of microbial biomass by EcMF. Additionally, fungal colonization in litter bags was quantified during initial and late decomposition. No colonization of EcMF *S. grevillei* was detected in controls treatments, whereas stable colonization was maintained in EcMF treatments (Figure S3a). Notably, EcMF altered the colonization of saprophytic fungus *I. lacerates* ($p < 0.05$; Figure S3b), which was consistent with the microbial respiration.

In low C/N litter, C-acquiring (CEEA) and N-acquiring (NEEA) extracellular enzyme activities were initially suppressed ($p < 0.05$) but enhanced later ($p < 0.05$) by EcMF (Figure 3b,c). Oxidative enzyme activity (OEEA) showed no significant response initially but was stimulated later ($p < 0.05$; Figure 3d). For high C/N litter, all three enzyme categories were consistently suppressed, especially later in decomposition ($p < 0.05$; Figure 3b–d).

In summary, trends in microbial biomass and enzyme activities aligned with decomposition dynamics, and the EcMF effects depended on litter C/N ratio and decomposition stage.

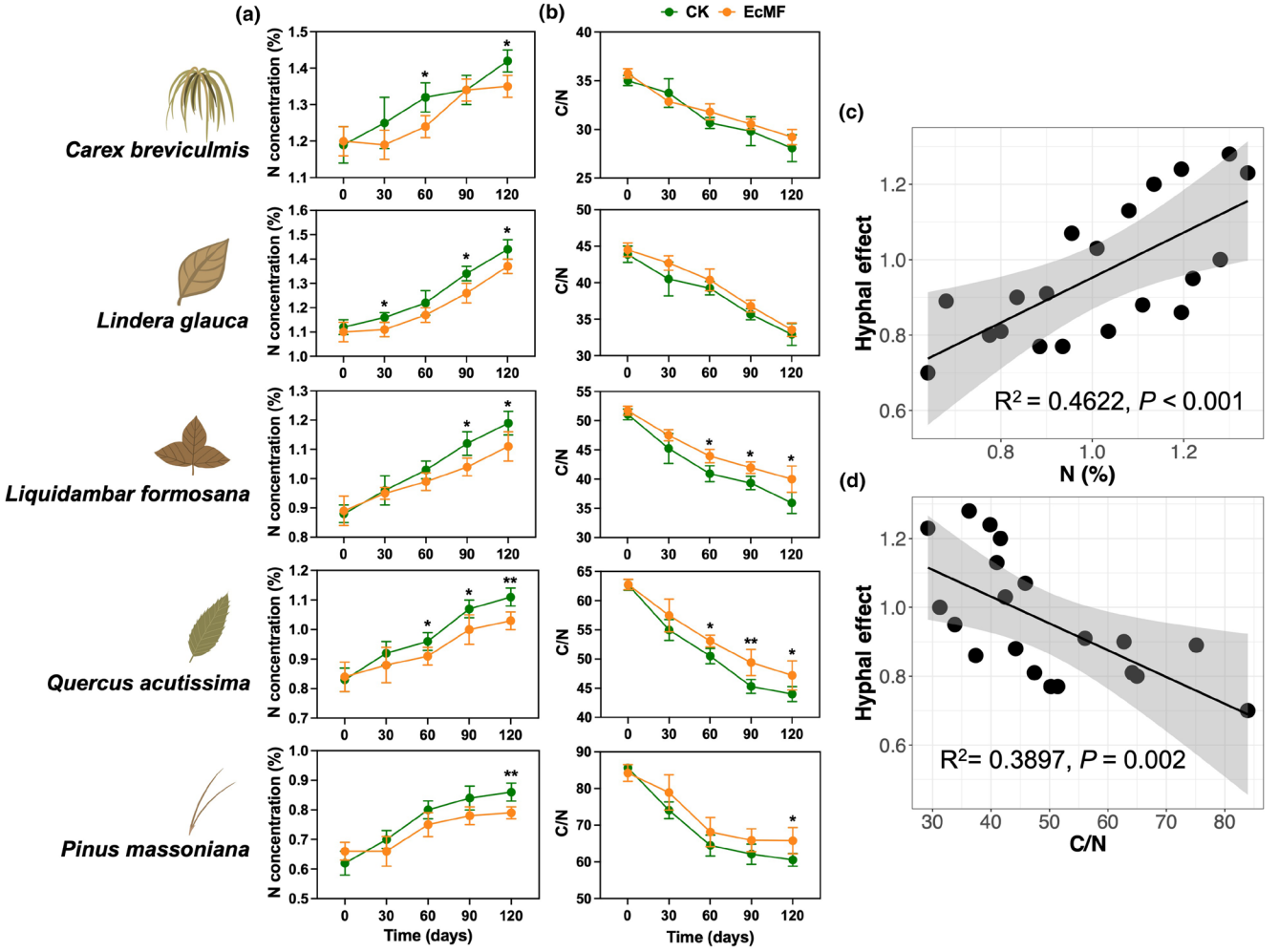


FIGURE 4 The N concentration (a) and C/N ratio (b) of the five litter types during the 120-day decomposition process under treatments with EcMF (EcMF) and without EcMF (CK). Values are means and standard deviations ($n = 5$). Asterisks indicate significant differences between the EcMF treatment and the CK treatment for the same litter type (Student's *t*-test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). The correlation of the stage N concentration (c) and the stage C/N ratio (d) with the hyphal effect. The black lines are the regression trend lines, and the grey area around each trend line is the confidence interval within 95%.

3.3 | Factors related to the hyphal effect on litter decomposition

To explore why the hyphal effect shifted from negative to positive during low C/N litter decomposition, we hypothesized a link to the dynamics of litter N concentration and C/N ratio. Analysis showed that N concentration gradually increased (Figure 4a) while the C/N ratio decreased over time in all five litter types (Figure 4b). After 4 months, N concentration was significantly lower in the EcMF treatment than in the control for all litter types ($p < 0.05$; Figure 4a), whereas the C/N ratio tended to be higher under EcMF, with a significant difference in *L. formosana*, *Q. acutissima* and *P. massoniana* ($p < 0.05$; Figure 4b).

Correlation analysis revealed a significant positive relationship between stage-specific N concentration and the hyphal effect on mass loss ($p < 0.05$; Figure 4c), and a significant negative correlation with the stage-specific C/N ratio ($p < 0.05$; Figure 4d). A regression model further described the relationship between the stage C/N ratio and the hyphal effect (HE): $C/N = 101.02 - 54.17 \times HE$ ($R^2 = 0.39$, $p < 0.01$). The model indicates that the hyphal effect shifts from stimulating decomposition at lower C/N ratios to inhibiting it at higher ratios, with a predicted transition around a C/N ratio of 47 (though the wide confidence interval limits the precision of this estimate).

3.4 | Plant biomass and N concentration

Plant biomass and N concentration were measured after 120 days of decomposition (Figure 5a,b). Plant N concentration did not differ between the EcMF treatment and the control (CK) in the absence of litter (Figure 5a). Under low C/N litter (*C. breviculmis*, *L. glauca* and *L. formosana*), N concentration was significantly higher with EcMF than with CK ($p < 0.05$). No such difference was observed for high C/N litter. Among CK treatments, plant N concentration did not vary significantly across litter types; under EcMF, however, N concentration was significantly higher with low C/N litter than with no litter

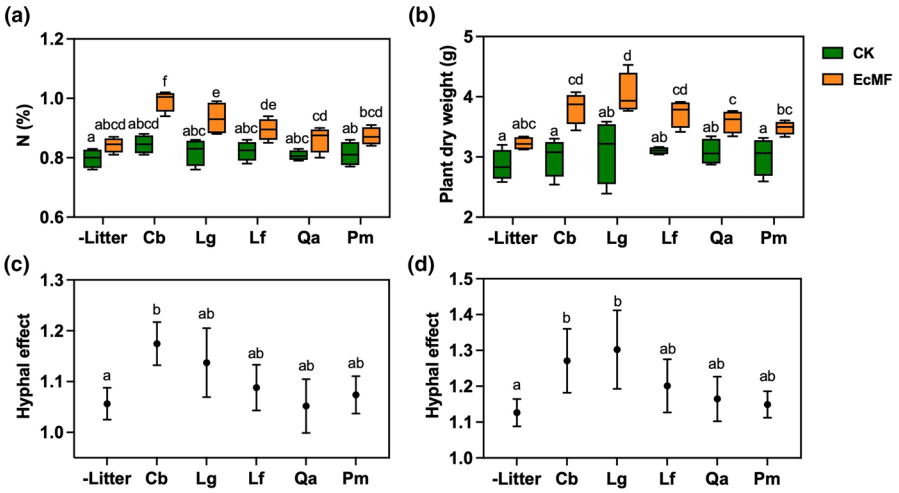


FIGURE 5 Plant growth under five litter types and no litter after the decomposition process under treatments with EcMF (EcMF) and without EcMF (CK). N concentration (a) and dry weight (b) of plants under each treatment. Hyphal effect on the N concentration (c) and dry weight (d). Values are means and standard deviations ($n = 5$). Letters indicate significant differences among treatments according to one-way ANOVA and Tukey's HSD test ($p < 0.05$).

or high C/N litter ($p < 0.05$). Specifically, the highest N concentration was observed in EcMF-treated plants grown with *C. breviculmis* litter (the lowest C/N ratio), followed by *L. glauca* and *L. formosana* litter (Figure 5a).

Plant dry weight did not differ between EcMF and CK in the absence of litter. In the presence of litter, however, plant biomass was consistently greater in the EcMF treatment across all litter types ($p < 0.05$; Figure 5b). Compared with CK, EcMF increased plant biomass by 27%, 30% and 20% for *C. breviculmis*, *L. glauca* and *L. formosana*, respectively, but only by 16% and 14% for *Q. acutissima* and *P. massoniana*.

The hyphal effect on plant N concentration was greatest for *C. breviculmis* litter (Figure 5c), while the effect on dry weight was most pronounced for *C. breviculmis* and *L. glauca* (Figure 5d). Overall, low C/N litter more strongly enhanced both N concentration and biomass under EcMF mediation. Subsequently, we conducted correlation analyses between litter properties (C/N ratio and N concentration) and the hyphal effects on plant growth (Figure S4). The results showed that the hyphal effect on plant N concentration was positively related to litter N concentration (Figure S4b), and the hyphal effect on plant dry weight was negatively correlated with litter C/N ratio (Figure S4c). These results indicate that litter with low C/N ratio and high N concentration elicits stronger EcMF-mediated promotion of plant growth.

3.5 | Impact of EcMF on litter mass loss and plant biomass

Partial Least Squares Path Modelling (PLS-PM) was used to elucidate the potential pathways of EcMF on the litter mass loss and plant biomass. The pathways affecting litter mass loss varied with litter type and decomposition stage (Figure S5). In the early stage of decomposition, EcMF directly reduced microbial activity and C loss in both high and low C/N ratio litters, and these factors contributed directly to mass loss. In the later decomposition stage, EcMF promoted the decomposition of low C/N ratio litter (total effect: +0.77) but inhibited the decomposition of high C/N ratio litter (total effect: −0.79; Figure S6).

For plant responses (Figure 6), with low C/N litter, EcMF directly promoted greater C and N loss, which increased plant N concentration and ultimately enhanced plant biomass. EcMF also enhanced microbial activity, directly promoting plant biomass. The total effects of EcMF, plant N and microbial activity on plant biomass were 0.80, 0.45 and 0.41, respectively (Figure S7). With high C/N litter, plant biomass was directly and positively influenced by EcMF (total effect: 0.77), but no significant effects of plant N or litter loss on plant biomass were observed (Figure S7). In summary, EcMF-mediated plant growth via distinct mechanisms depending on litter type: for low C/N litter, promotion occurred through enhanced C and N release and subsequent nutrient uptake; for high C/N litter, this pathway was not supported by the model.

4 | DISCUSSION

EcMF exhibit a dual regulatory effect on litter decomposition; their effect direction and size depend strongly on the litter C/N ratio. Furthermore, EcMF play a crucial bridging role between plant growth and litter nutrient absorption. In this study, we conducted a microcosm decomposition experiment using five types of litter with varied C/N ratios to quantitatively assess the effects of EcMF on both the decomposition process and plant growth. The results indicate that, first, the effect of EcMF on litter decomposition varied depending on litter type and decomposition stage. This variation was specifically associated with the initial litter C/N ratio, and the hyphal effect declined with increasing litter C/N ratio, which is consistent with hypothesis 1. Second, EcMF enhanced plant growth when litter was added as part of the substrate, and this hyphal effect depended on C/N ratio litter: it was more pronounced under low C/N conditions. This can be attributed to the ability of EcMF to facilitate the release of C and N from low C/N litter and promote plant N uptake, thereby increasing plant biomass. These findings provide partial support for hypothesis 2. In conclusion, our study demonstrates that the

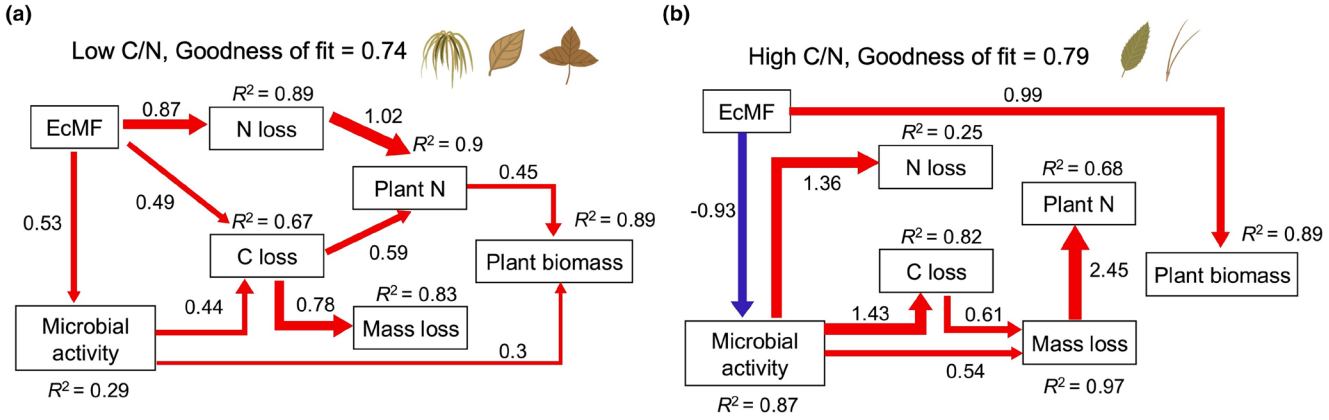


FIGURE 6 Partial least squares path modelling (PLS-PM) showing the direct and indirect effects of EcMF after 120 days of decomposition, microbial activity, C loss, N loss, mass loss, plant N and plant biomass. Low C/N include: *C. breviculmis*, *L. glauca*, and *L. formosana* (a). High C/N include: *Q. acutissima* and *P. massoniana* (b). The width of the arrows is proportional to the strength of the path coefficients. Blue and red arrows indicate significant negative and significant positive effects ($p < 0.05$), respectively. Numbers on the arrows indicate significant standardized path coefficients. R^2 values indicate the explained variance. Microbial activity include: SIR, BG, CBH, NAG, LAP, PPO, POD.

litter C/N ratio modulates the decomposition effects of EcMF and further influences plant N uptake and growth (Figure 7).

4.1 | The C/N ratio of litter regulates hyphal effects on litter decomposition

Our findings demonstrate that EcMF can both suppress and enhance litter decomposition, contingent on substrate chemistry. EcMF suppressed the decomposition of high C/N litter, consistent with the 'Gadgil effect' (Fernandez & Kennedy, 2016; Gadgil & Gadgil, 1971), whereas they enhanced the decomposition of low C/N litter, reflecting a 'priming effect' (Brzostek et al., 2015; Johansson et al., 2009). Although these results are derived from laboratory-based decomposition experiments, they align with the variable effects of EcMF reported in the studies across broader spatial scales (Choreño-Parra & Treseder, 2024; Fernandez & Kennedy, 2016). However, previous trenching studies found that root inputs promote pine litter decomposition, which differs from our results (Zhu & Ehrenfeld, 1996). This discrepancy may be explained by their extremely nutrient-poor soils, where root C universally alleviates microbial limitation and consistently stimulates decomposition. It should also be noted that trenching removes both roots and their associated EcMF, making it impossible to isolate the specific role of EcMF—a methodological distinction that may partly explain the discrepancies between studies (Fernandez & Kennedy, 2016). More recently, DeLancey et al. (2025) reported that litter type did not modulate EcMF-driven decomposition, likely because they used only conifer litter, whereas our cross-species litter types encompassed greater chemical diversity. Their observation of low EcMF abundance in the litter layer further implies that trenching effects may be indirectly modulated by roots or mineral soil EcMF through N supply (Lin et al., 2019). In contrast, our inoculation approach effectively controlled for EcMF presence in litterbags, better isolating the fungal effect itself.

The stage of decomposition also modulated EcMF effects. For low C/N litter, EcMF initially suppressed but later promoted decomposition, a pattern consistent with reports of early competition followed by later facilitation (Mielke et al., 2025). As decomposition proceeds, litter N concentration typically rises and the C/N ratio declines (Averill et al., 2014). We found a negative correlation between the stage-specific C/N ratio and the hyphal effect, indicating that the substrate C/N ratio governs the occurrence of Gadgil or priming effects by modulating nutrient competition (Averill et al., 2014; Choreño-Parra & Treseder, 2024). Our model predicted suppression when litter C/N exceeded approximately 47, corroborating meta-analytical evidence that the Gadgil effect is strongest in substrates with C/N > 40 (Choreño-Parra & Treseder, 2024). In high C/N litter, microorganisms immobilize N from the environment, resulting in litter N mass exceeding initial values (Figure S2b). Although EcMF inoculation reduced litter N concentration (Figure 4a), PLS-PM revealed no direct effect of EcMF on litter N loss (Figure 6b). This is likely due to the combined effects of strong microbial N immobilization and low overall mass loss. The coupling between N stoichiometry and EcMF hyphal effects on decomposition, along with the observed declines in microbial biomass (Figure 3a, Figure S3b) and enzyme activities (Figure 3b–d) in high C/N litter, suggests that EcMF primarily suppress decomposition via N competition, thereby inhibiting saprotrophic fungal activity. Other potential mechanisms—such as chemical inhibition, mycoparasitism and competition for water (Fernandez & Kennedy, 2016)—warrant further investigation with more targeted experiments.

Conversely, low C/N litter with rich nutrients and reduced competition allows EcMF to stimulate decomposition. Potential mechanisms include the production of oxidative enzymes that depolymerize organic matter, making it more accessible to saprotrophs (Argiroff et al., 2022; Lindahl et al., 2021; Lindahl & Tunlid, 2015), and the release of labile C via exudates or necromass (Fernandez & Kennedy, 2018; Wilkinson et al., 2011). In our study, EcMF increased

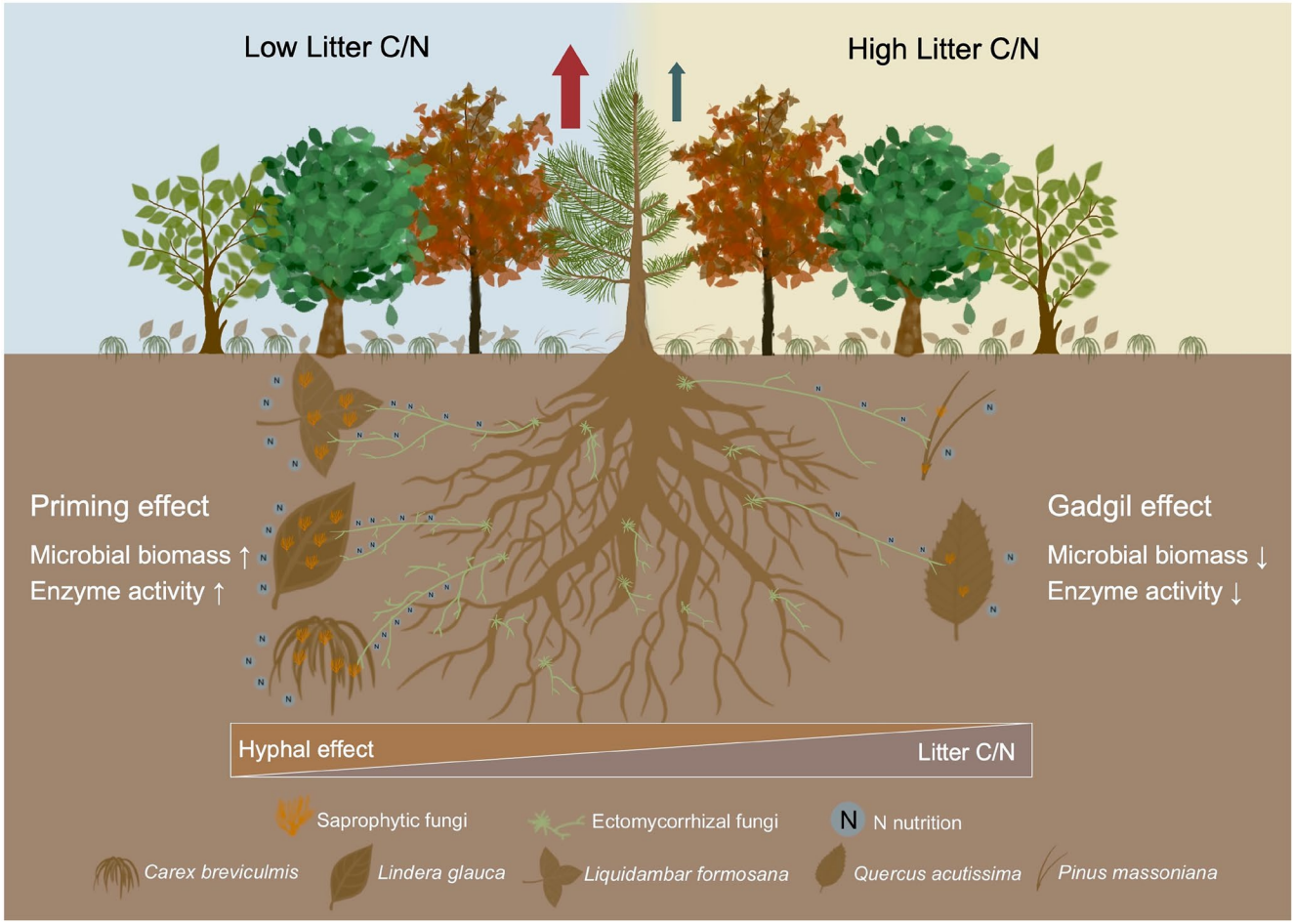


FIGURE 7 Schematic representation of the regulatory role of litter C/N ratio on the function of ectomycorrhizal fungi in litter decomposition and plant N acquisition.

microbial biomass (Figure 3a, Figure S3b) and enzyme activities in later stages for low C/N litter (Figure 3b–d), accelerating C loss and confirming a priming effect.

4.2 | The effect of EcMF on plant nutrient absorption varies with C/N ratio of litters

EcMF promote plant growth by enhancing nutrient availability and expanding the root absorption area (Ditengou et al., 2015; Jones et al., 1998). In the absence of EcMF inoculation, litter addition did not significantly alter plant N concentration or biomass (Figure 5a,b), likely because plant roots were excluded from litter bags and nutrients were immobilized by soil microorganisms. In contrast, EcMF hyphal bridges connected the decomposition zone with roots, enabling direct nutrient transfer from litter to plants (Becquer et al., 2014; Cao et al., 2022; Mei et al., 2023). This supports the role of EcMF as a critical link between soil nutrient cycling and plant growth, particularly in mobilizing nutrients from organic substrates (Argiroff et al., 2022; Liu et al., 2017). Beyond litter-derived nutrients, EcMF also enhance mineralization of soil organic N and phosphorus through extracellular enzyme secretion and microbial synergism, with released nutrients efficiently transported to plants via the mycorrhizal network (Baskaran et al., 2017; Mei et al., 2023).

The growth-promoting effect of EcMF depends on soil nutrient conditions (Smith & Smith, 2011). Nutrient-poor environments typically amplify EcMF benefits, as plants allocate more photosynthate to mycorrhizae to acquire limiting N and P (Chen et al., 2017; Liu et al., 2017), and nutrient stress can stimulate EcMF colonization and hyphal extension (Franklin et al., 2014; Li et al., 2020). Although high C/N litter is considered more nutrient-poor, we found the hyphal effect on plant growth was stronger under low C/N litter. This is explained by differential decomposition dynamics: for low C/N litter, EcMF promoted plant growth by accelerating C and N release (increasing plant N concentration; Figure 5a) and stimulating microbial activity (Figure 6a), both of which enhanced nutrient flux to plants (Cao et al., 2022; Cheeke et al., 2017). For high C/N litter, EcMF also increased plant biomass, but to a lesser extent (Figure 5b), likely because slow decomposition combined with fungal inhibition limited nutrient transfer. Notably, EcMF did not significantly enhance plant N under high C/N litter conditions. This is likely because N released from high C/N litter only met or slightly exceeded fungal growth demand, leaving little surplus N for translocation to host plants. Moreover, under high C/N conditions, plant biomass was

directly promoted by EcMF rather than via decomposition pathways (Figure 6b), suggesting alternative mechanisms such as expanded root foraging capacity or improved bioavailability of soil nutrients (Ditengou et al., 2015; Jones et al., 1998). In summary, the growth-promoting effect of EcMF on host plants is regulated by litter C/N ratio. Similarly, Aucina et al. (2007) linked higher seedling survival with low C/N oak litter to greater nutrient release, compared to pine litter. In contrast, Trap et al. (2016) observed no significant EcMF effect when litter originated from the same tree species, likely due to limited chemical divergence between litter from young and old beech. By using five litter types across a range of C/N ratios, our study systematically clarifies how litter chemistry modulates the hyphal effect, providing broader and more precise conclusions.

Our study demonstrates that litter chemistry strongly mediates the effects of EcMF on both decomposition and plant growth. However, these findings are based on experiments using a single EcMF species, *Suillus grevillei*. Due to substantial functional diversity among ectomycorrhizal lineages—especially in decomposition-related enzymes, as seen in more oxidative genera like *Cortinarius* and *Hebeloma*—the observed interactions may differ across fungi with varied metabolic traits (Lindahl et al., 2021; Pellitier & Zak, 2018). Similarly, potential limitations arise from the use of only one saprotrophic fungus. In natural ecosystems, decomposition is mediated by diverse saprotrophic assemblages with divergent nutrient demands and physiological capabilities, and reliance on a single isolate cannot fully represent the complexity of real-world ecological interactions. Finally, C and N exchange dynamics between seedlings and their associated EcMF may differ substantially from those involving mature trees (Pardo et al., 2013). Accordingly, the litter chemistry-dependent mechanisms identified in this study warrant further testing across a broader taxonomic range in future research.

5 | CONCLUSION

This study demonstrates that EcMF significantly regulates litter decomposition, with the direction and magnitude of their effect being strongly and negatively correlated with litter C/N ratio. Specifically, EcMF generally enhanced the decomposition of low C/N litter but suppressed that of high C/N litter. This regulation also displayed distinct stage-dependency: for low C/N litter, EcMF initially inhibited decomposition before shifting to promotion, whereas suppression of high C/N litter persisted throughout the process. Furthermore, when litter was present as a substrate, EcMF significantly promoted plant growth, an effect that diminished as litter C/N increased. Mechanistically, EcMF facilitated the release of C and N from low C/N litter, enhancing plant N recycling and biomass accumulation. Under high C/N conditions, although EcMF suppressed litter decomposition, it still provided promotion to plant growth. The results deepen our understanding of the functional mechanisms of EcMF in forest ecosystem nutrient cycling and provide an important theoretical basis for future research on the impact of litter–microbe interactions on forest productivity.

AUTHOR CONTRIBUTIONS

Yan Mei: Writing-Original draft preparation, Formal analysis, Data Curation, Writing-Review and Editing, Visualization. Qingyu Lan: Data Curation, Investigation, Sample collection, Methodology. Xin Liang: Writing-Review and Editing. Qianwei Li, Guangyan Li, Qi Chen, Meng Lin: Reviewing. Yong Jia, Xingjun Tian: Conceptualization and conceived concept of field experiment, Funding acquisition, Writing-Review and Editing. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

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 STATEMENT

Data were available from Dryad Digital Repository: https://doi.org/10.5061/dryad.3tx95x6vp (Mei et al., 2026).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. The plant height, dry weight and the fungal biomass (concentration of ergosterol) in fine roots under EcMF inoculation treatment and non-EcMF inoculation treatments, at the beginning of decomposition experiment.

Table S2. Specific primers used for the quantitative PCR analysis.

Table S3. Enzyme commission (EC) number, specific substrates, substrate concentrations and incubation time.

Figure S1. The characteristics of ectomycorrhizal seedlings. Photos of non-ectomycorrhizal seedlings (a) and ectomycorrhizal seedlings (b). The white circles indicate the root binary branches structure of the ectomycorrhizal seedlings. Photos of the root structures of non-ectomycorrhizal seedlings (c) and the root binary branches structure ectomycorrhizal seedlings (d).

Figure S2. C mass remaining (a) and N mass remaining (b) of the five litter types during the 120-day decomposition process under treatments with EcMF and without EcMF (CK). Values are means and standard deviations ($n = 4$).

Figure S3. Colonization of EcMF *S. grevillea* (a) and saprophytic fungus *I. lacerates* (b) in five litter types at 30 and 120 days of decomposition under EcMF-inoculated (EcMF) and non-inoculated (CK) treatments. Values are presented as means ± standard deviations ($n = 3$).

Figure S4. The correlation of the initial C/N ratio (a) and the initial N concentration (b) with the hyphal effect on plant N concentration. The correlation of the initial C/N ratio (c) and the initial N concentration (d) with the hyphal effect on plant dry weight.

Figure S5. Partial least squares path modelling (PLS-PM) showing the direct and indirect effects of EcMF, microbial activity, C loss, N loss and mass loss. Low C/N include: *Carex breviculmis*, *Lindera glauca* and *Liquidambar formosana*. High C/N include: *Quercus acutissima* and *Pinus massoniana*. Initial stage indicates the decomposition period of 30 days and later stage indicates the decomposition period of 120 days. The width of the arrows is proportional to the strength of the path coefficients. Blue and red arrows indicate significant negative and significant positive effects ($p < 0.05$), respectively. The dashed-line box indicates no significant correlation. Numbers on the arrows indicate significant standardized path coefficients. R^2 values indicate the explained variance. Microbial activity include SIR, BG, CBH, NAG, LAP, PPO, POD.

Figure S6. The total effects of EcMF, microbial activity, litter C loss and litter N loss on litter mass loss from Partial least squares path modelling (PLS-PM).

Figure S7. The total effects of EcMF, microbial activity, litter C loss, litter N loss, litter mass loss and plant N concentration on plant biomass from Partial least squares path modelling (PLS-PM). Low C/N litter as the substrate (a), high C/N litter as the substrate (b).

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