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RESEARCH ARTICLE

Litter nutrient release and allelopathy jointly contribute to the diversity–invasibility relationship

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Abstract

1. Elton's biotic resistance hypothesis suggests that diverse communities are more resistant to biological invasions. While below-ground mechanisms play a crucial role in regulating the diversity–invasibility relationship, the role of litter as a key regulator of below-ground processes remains underexplored.
2. We used 15 common native plant species from temperate forests in China to create litter mixtures with five different levels of species richness (1, 3, 6, 9, 15), and the invasive plant *Phytolacca americana* as a model invader. We investigated how these litter mixtures affected the growth of the invader and explored the roles of nutrient release and allelopathy in this process.
3. Our results demonstrate that increased litter species richness decreased the relative abundance of dominant saprotrophic fungi, resulting in reduced nutrient release and suppressed the growth of *P. americana*. Additionally, the allelopathic inhibitory effects of litter on the invader intensified as litter species richness increased.
4. These findings elucidate the roles and mechanisms of litter species richness in resisting invasion, demonstrating that the combined effects of nutrient dynamics and allelopathy are major drivers of the diversity–invasibility relationship. We also highlight the potential of incorporating native plant litter into ecosystem management plans to enhance community resistance to invasions, as well as ecosystem stability and sustainability.

KEYWORDS
allelochemicals, community invasibility, litter, microbes, nutrients, plant invasion, species richness

1 | INTRODUCTION

Biological invasions pose a growing threat to global biodiversity and ecosystem functions (Courchamp et al., 2017; Diagne et al., 2021). This threat has prompted extensive research into factors affecting ecosystem resistance to invasion (Seebens et al., 2017; Van Kleunen et al., 2015). Elton's biotic resistance hypothesis posits that more diverse communities inherently possess greater resilience against invasions (Elton, 1958). This negative diversity–invasibility relationship has been strongly supported, especially in studies highlighting

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resource competition (Levine et al., 2004; Li et al., 2021). Recent studies have highlighted the critical role of below-ground processes, particularly soil microbial communities, in driving negative diversity–invasibility relationships (Liao et al., 2015; Zhang et al., 2020). However, the effect of litter, a major driver of below-ground dynamics, on the diversity–invasibility relationship remains poorly understood.

Plant litter has long been recognized as a key factor shaping soil nutrient availability, secondary chemical profiles and microbial communities (Bonanomi et al., 2011; Chapman et al., 2006; Veen et al., 2019). These litter-mediated mechanisms have attracted growing attention in the context of plant invasion (Eppinga et al., 2011; Zhang et al., 2019). Invasive plants often produce litter with lower lignin content and C:N ratios (Liao et al., 2008; Luan et al., 2021), which decomposes rapidly and enhances nutrient availability, thereby promoting the growth of invasive plants (Chapman et al., 2006; Davis et al., 2000; Liu et al., 2017; Wardle et al., 2004). Allelopathic compounds released from their litter can further suppress the establishment and performance of native species (Uddin & Robinson, 2017; Zhang et al., 2021). In contrast, evidence also suggests that native litter may act as a natural barrier to invasion (Chen et al., 2019; Ning et al., 2016). For example, Hou et al. (2012) showed that leaf litter extracts from three native subtropical Chinese tree species (*Cryptocarya concinna*, *C. chinensis* and *Machilus chinensis*) significantly suppressed the seedling growth of the invasive weed *Mikania micrantha*. Despite some mechanistic insights from single-species litter studies, the broader mechanisms by which litter mediates diversity–invasibility relationships remain unclear.

Forest ecosystems are intricate mosaics where multiple plant species coexist, each contributing distinct litter types that are intermixed through natural processes (Staelens et al., 2003). Litter mixing can substantially alter chemical composition and reshape soil microbial communities, thereby modifying soil processes (Gessner et al., 2010; Otsing et al., 2018). Mixed litter provides diverse resources for microbial decomposers, enhancing nutrient acquisition and accelerating nutrient release (Chapman et al., 2013; Loreau, 2001). It may increase the diversity of microbial decomposers and stimulate overall decomposition (Handa et al., 2014; Santonja et al., 2017). Mixed litter can also directly shift soil fungal community composition, with consequences for nutrient release patterns (Otsing et al., 2018; Treplin et al., 2013). Saprotrophic soil fungi can suppress plant pathogens and symbiotic fungi by competing for space and nutrients, which directly interact with plants (Rousk & Bååth, 2011). Given its predicted impact on below-ground processes, we hypothesize that plant litter shapes the diversity–invasibility relationship by altering nutrient release patterns through changes in soil microbial community composition and diversity. However, this hypothesis has not yet been tested in the literature.

In fact, relying solely on nutrient supply is insufficient to fully explain the effects of litter mixtures on invasive plants, because the strength and direction of allelopathic effects can also change with litter species richness (Wang et al., 2025; Yuan et al., 2022). Litter species richness may enhance the allelopathic inhibition of invasive plants, as the probability of species with strong allelopathic properties rises. Moreover, allelopathic compounds from different litter types may complement each other, jointly suppressing invasive species. Alternatively, allelopathic substances may be diluted in mixtures to the extent that their inhibitory effects on invaders diminish. Therefore, depending on whether allelopathic inhibition of the invader increases or decreases with litter species richness, the nutrient release-driven diversity–invasibility relationship will be either steeper or shallower. Therefore, understanding how litter species richness regulates the diversity–invasibility relationship through nutrient release and allelopathy is crucial.

To goal of this study was to examine whether nutrient release and allelopathic compounds from litter contribute to the diversity–invasibility relationship. We established three parallel sub-experiments in which we selected 15 native plant species from temperate forests in China to construct litter mixtures spanning a gradient of species richness levels (1, 3, 6, 9, 15) (Figure 1). We used *Phytolacca americana* as a model invader, exposing it to litter treatments. The first experiment focused on the response of litter nutrient release and soil microbial communities to litter species richness. The second assessed the growth of the invader in response to litter species richness. The third evaluated the role of allelopathy by introducing activated carbon, which adsorbs these compounds. Specifically, we addressed the following three questions: (1) Does litter species richness capture the diversity–invasibility relationship? (2) Can the effects of litter species richness on invasion be explained by nutrient release from litter? (3) If so, do allelopathy modify the diversity–invasibility relationship?

2 | MATERIALS AND METHODS

2.1 | Study location and species

The study was conducted in the Kunyu Mountain National Nature Reserve, located in the eastern part of the Shandong Peninsula, China (37.10–37.19° N, 121.37–121.48° E). The reserve covers an area of 154.165 km^2, with an annual precipitation of 900–1000 mm and an average annual temperature of 11.8°C. The region has a temperate monsoon and maritime climate (Wang et al., 2009). According to China's vegetation zoning, the area is classified as a warm-temperate deciduous broadleaf forest type, characterized by high plant species diversity and complex community structures (Wang & Zhou, 2000). The reserve contains large areas of well-preserved secondary natural vegetation and various well-developed plantations.

We selected 15 common native plant species that represent the three most prevalent plant life forms in the region. These species coexist in stratified communities, representing different stages of ecological succession and occupy different ecological roles in the forest layers (Wang & Zhou, 2000). Additionally, these species are major contributors to litter production in temperate forests (Jevon et al., 2022; Schnitzer & Bongers, 2002). The selected species included eight trees: *Quercus glandulifera* Blume, *Q. variabilis* Blume,

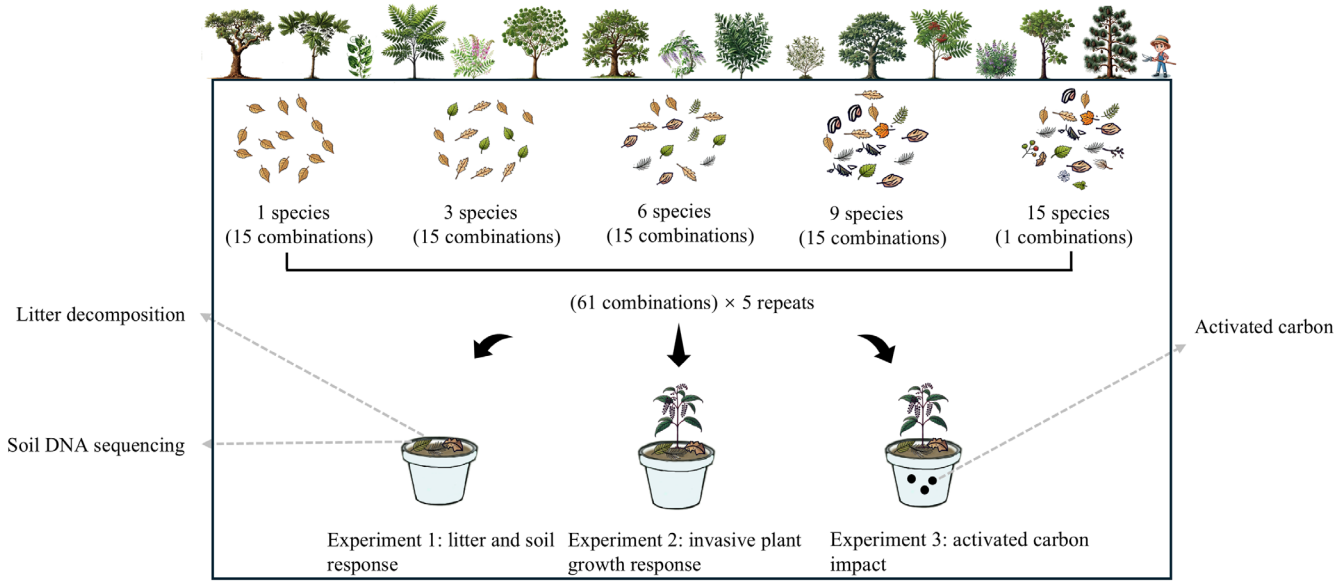


FIGURE 1 Experimental setup to investigate the role of litter in regulating the diversity–invasibility relationship. Litter mixtures were created from 15 native plant species with five levels of species richness (1, 3, 6, 9, 15) and the invasive plant *Phytolacca americana* was used as the test species. Three simultaneous greenhouse experiments were conducted: (1) examining the response of litter nutrient release and soil microbial communities to litter species richness, (2) assessing the growth responses of invasive plants to litter species richness and (3) evaluating the impact of activated carbon addition on the responses of invasive plants to litter species richness.

Q. acutissima Carruth., *Alnus hirsuta* Turcz. ex Rupr., *Kalopanax septemlobus* (Thunb.) Koidz., *Sorbus alnifolia* (Siebold & Zucc.) C. Koch, *Ailanthus altissima* (Mill.) Swingle and *Pinus densiflora* Siebold & Zucc.; five shrubs: *Rhus chinensis* Mill., *Grewia biloba* var. *parviflora*, *Lespedeza bicolor* Turcz., *Indigofera kirilowii* Maxim. ex Palib. and *Prunus japonica* Thunb.; and two vines: *Celastrus orbiculatus* Thunb. and *Smilax china* L.

Phytolacca americana L., a perennial herb introduced to China from North America in 1935 (Xiao et al., 2019), has since spread widely across the country (Huang & Ding, 2016). Since 2000, *P. americana* has rapidly expanded in coastal sandy protective forests in Shandong Province, forming monocultures that significantly inhibit the natural regeneration of understory vegetation, leading to a notable decline in biodiversity (Fu et al., 2012). In recent years, its invasion of natural forests has intensified, particularly in areas such as the Kunyu Mountain National Nature Reserve, where it frequently establishes in the understory, posing a severe threat to biodiversity and ecosystem services within the reserve (Chen et al., 2021). We collected *P. americana* seeds from over 50 maternal plants across 12–15 sampling sites in forest communities near Zhenshan Hill (37.30–37.32° N, 121.19–121.21° E) on 10 November 2022. The seeds were then combined into a bulk sample and stored for future use.

2.2 | Litter addition experiment

Representative litter material was collected in 2023. For each of the 15 plant species, we selected three plots (50 m × 50 m) spaced 500 m apart. Within each plot, we selected healthy individuals with similar diameters at breast height (DBH) according to the selection criteria outlined in Table S1, ensuring a minimum distance of 10 m between individuals. Each plot contained at least 10 trees, resulting in over 30 sampled trees per species. Leaves were collected from branches to avoid the impact of different decomposition stages on chemical composition. The litter was cut into 1-cm fragments, air-dried at room temperature and stored until the experiment began. To ensure equal representation, we used 15 combinations for each richness level except for the unique 15-species combination. Six grams of litter were used for each combination, with equal contributions from each species. In total, 61 litter combinations were used: 15 single-species combinations, 15 three-species combinations, 15 six-species combinations, 15 nine-species combinations, and 1 fifteen-species combination (Table S2). The selection of these five levels of species richness (1, 3, 6, 9, 15) is based on previous surveys indicating that the vegetation in the Kunyu Mountain region is diverse and exhibits a clear spatial gradient (Chen et al., 2019; Wang & Zhou, 2000). At these different richness levels, the litter from these species is more likely to mix under the influence of wind and water, creating various litter combinations that further impact ecosystem functions.

On 15 July 2023, we collected background forest soil from open areas of Zhenshan Hill, removed plant roots and large debris using a 1-cm sieve, and filled 16 × 18 cm pots with 1.5 L of the soil. Meanwhile, *P. americana* seeds were sown in trays (54 × 28 × 4.6 cm) filled with sterilized vermiculite. The pots and trays were placed in a greenhouse in Yantai, China, with temperatures ranging from 18°C to 25°C under natural light. From 1 to 3 August 2023, we initiated three simultaneous experiments (Figure 1). In the first experiment, prepared litter was placed on the soil surface to simulate natural decomposition using 305 pots (61 combinations × 5 replicates). In the

second experiment, the same litter mixtures were used, and the invader *P. americana* seedlings were transplanted into the centre of each pot (61 combinations × 5 replicates). In the third experiment, activated carbon was mixed with soil at 20 mL/L to adsorb allelopathic compounds (Callaway & Aschehoug, 2000; Ning et al., 2016), with the same litter treatment and the invader seedlings (61 combinations × 5 replicates). During cultivation, all 915 pots received natural light, adequate water, regular weeding, and were repositioned weekly to minimize positional effects.

After 10 weeks, all remaining litter in the first experiment was carefully collected from each pot using sterilized tweezers, placed into pre-labelled paper bags, and transported to the laboratory for storage under dry conditions until analysis. Subsequently, the top 0–2 cm soil layer in each pot was carefully removed with a sterilized spatula. Soil from the remaining layer was then collected from each pot and pooled by species combination (excluding the 15-species combination) in a sterile plastic tray, where the samples were thoroughly mixed to create a composite sample. From each composite sample, 10–20 mL of soil was then transferred into a sterile 50-mL centrifuge tube, resulting in a total of 65 samples. All samples were transported on ice to the lab and stored at −80°C for DNA extraction.

In the second and third experiments, we harvested above-ground biomass by cutting the plants at the base and obtained below-ground biomass by washing the roots free of soil. After accounting for 13 deaths, a total of 597 seedlings were collected. The biomass was dried at 65°C for 1 week to a constant mass and weighed to the nearest milligram. Total biomass was calculated as the sum of above-ground and below-ground dry weights.

2.3 | Litter measurements

Litter samples were dried at 65°C to a constant weight and gently cleaned to prevent nutrient loss. Due to the complete decomposition of five replicates within one combination, 300 samples remained to be analysed. Litter mass loss was calculated for each sample by comparing its weight before and after decomposition. We measured the C and N concentrations of litter mixtures before and after decomposition, analysing one randomly selected subsample per combination, except in the 15-species richness treatment, where all five replicates were analysed, yielding a total of 65 samples. The percentages and net amounts of C and N released were then calculated based on the average mass loss of each combination and the corresponding changes in concentration. Percentage release was defined as the proportion of initial C or N lost during decomposition, whereas net release was calculated as the difference in total C or N amount before and after decomposition. To determine initial litter quality, we measured the initial carbon (C), nitrogen (N) and total phenols concentrations using three subsamples for the litter of each species. All samples were ground into fine powder and analysed using an elemental analyser (Vario EL; Elementar Analysensysteme). Total phenols concentrations, which characterize allelochemical content and decomposition potential, were determined by colorimetric analysis using gallic acid as the standard (Singleton & Rossi, 1965).

2.4 | Soil microbial measurements

To identify whether the microbial communities were influenced by plant litter, we conducted molecular analyses, focusing on fungal communities because plant litter influences them more strongly than bacterial communities (Habtewold et al., 2020; Heinen et al., 2020). The DNA extraction, PCR amplification, and amplicon sequencing were performed at Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). Total genomic DNA was isolated from 250 mg of each homogenized soil sample using an EZNA® Soil DNA Kit (Omega Bio-ek). The fungal ITS1 region was amplified using the universal primers CTTGGTCATTTAGAGGAAGTAA (forward) and GCTGCGTTCTTCATCGATGC (reverse) (Ghannoum et al., 2010). PCR amplification was conducted in triplicate using 20 μL reaction mixtures composed of buffer, rTaq polymerase, dNTPs (2.5 mM), primers (5 μM), genomic DNA (10 ng) and bovine serum albumin (BSA). Fungal PCR was run on an ABI GeneAmp 9700 thermal cycler (Applied Biosystems) under the following conditions: 95°C for 3 min; 30 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 45 s; and a final extension at 72°C for 10 min. PCR products were excised from 2% agarose gels and purified using the DNA Gel Extraction Kit (Yuhua, China). The purified products were quantified using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) and pooled in appropriate ratios based on sequencing requirements. Libraries were prepared from the purified PCR products using the NEXTFLEX Rapid DNA-Seq Kit (PerkinElmer, USA). Paired-end sequencing was performed using the Illumina MiSeq platform (Illumina, USA).

Raw paired-end reads were processed using fastp (v0.19.6) for quality control, including trimming low-quality bases and removing short or ambiguous reads. The filtered reads were then merged into single sequences using FLASH (v1.2.11 http://ccb.jhu.edu/software/FLASH/), and demultiplexed according to barcodes and primers with corrected sequence orientation. High-quality sequences were clustered into operational taxonomic units (OTUs) at 97% identity using UPARSE v7.1. Taxonomic assignment was performed against the UNITE database (Abarenkov et al., 2010). Putative functional groups (e.g. plant pathogenic, saprotrophic and mycorrhizal fungi, including arbuscular and ectomycorrhizal fungi) were identified using the FUNGuild database (Nguyen et al., 2016). To account for differences in sequencing depth, the final sequencing depth was rarefied to 41,683 reads.

2.5 | Litter leachate experiment

It has been reported that activated carbon not only adsorbs allelopathic compounds but also soil nutrients (Lau et al., 2008). To avoid incorrect assessment of allelopathic effects due to nutrient adsorption by activated carbon, we further used sterile litter leachate

to assess its allelopathic effects on the germination and seedling growth of *P. americana*. This method is widely used in allelopathy studies (Zhang et al., 2021). The procedure was as follows: On 1 August 2024, each type of air-dried litter was immersed in distilled water (1:25 ratio) for 24 h at room temperature. The leachate was first filtered with Whatman No. 1 paper and then with a 0.2-μm membrane (50 mm in diameter) to remove large particles and microorganisms. Equal volumes of the 15 litter leachates were mixed according to the litter decomposition design, resulting in 61 distinct litter leachate mixtures across five levels of species richness. All leachates were stored at −4°C until use.

We used the same background soil from the litter decomposition experiment as the growth substrate. The soil was sterilized (autoclaved at 121°C, 0.105 MPa for 30 min, once per day for three consecutive days). Each 66-mL pot was filled with 60 mL of soil. On 12 August 2024, 20 *P. americana* seeds were sown on the soil surface, and 5 mL of leachate was subsequently applied to each pot on a weekly basis for 6 weeks. To avoid nutrient interference from different leachates, we added 5 mL of diluted Hoagland nutrient solution to each pot weekly until the plants were harvested. Two weeks after sowing, seedling emergence was recorded and thinned to one robust seedling per pot. In total, 247 pots (60 leachate treatments × 4 replicates, with the 15 species richness treatment group having seven replicates) were set up. On 24 September 2024, the above-ground parts were harvested, dried at 65°C for 1 week to constant weight, and then weighed for biomass determination.

2.6 | Statistical analyses

To investigate the effects of litter species richness and the addition of activated carbon on the invader performance, we used a linear mixed model with the 'lme4' package (Bates et al., 2015) in R 4.0.5 (R Core Team, 2022). The model included litter species richness and the addition of activated carbon as fixed factors, with the above-ground, below-ground or total biomass of *P. americana* as the response variable. For each biomass metric, we conducted post-hoc analyses using the emtrends function in the 'emmeans' package to examine how the invader biomass varied with litter species richness within each activated carbon treatment. To improve normality and homoscedasticity of the residuals, the invader biomass was natural log-transformed.

The soil microbial communities were analysed using the Majorbio Bio-Pharm platform (www.majorbio.com). To test the effects of litter species richness on the community composition of plant pathogenic, saprotrophic and symbiotrophic fungi, separate permutational multivariate analyses of variance (PERMANOVA, 999 permutations, Bray–Curtis distance) were conducted for each fungal group. Nonmetric multidimensional scaling (NMDS) based on Bray–Curtis distances was then performed for each group to visualize the effects of litter species richness. The Shannon index and the relative abundance of each fungal guild, as well as their predominant OTU taxa, were calculated across litter species richness using one-way analysis of variance (ANOVA). The Shannon index was used to assess community diversity. For saprotrophic fungal taxa significantly influenced by litter species richness, linear regression was performed to assess their relationship with the percentage of litter nutrient release. As some important saprotrophic fungal OTU taxa strongly influenced litter nutrient release, an independent *t*-test was used to assess whether the presence of specific plant litter had a significant contribution to these OTU taxa.

Regression analysis was performed to test how litter carbon (C) and nitrogen (N) release percentages depend on litter species richness. Similar analyses were conducted for the C and N release percentages and their net release amounts, revealing a positive correlation (Figure S1). Additionally, because the three biomass metrics showed highly similar response patterns, we only conducted one regression analysis to examine the relationship between net C and N release and the total biomass of the invader (averaged per combination).

Regression analysis was used to examine the relationship between the species richness of the litter leachate, invader above-ground biomass, and seed germination rate in the leachate experiment. We explored whether the presence of specific litter leachates had a significant effect on the invader's above-ground biomass. Relative mixture effect (RME) index was introduced to evaluate the additive, synergistic, or antagonistic effects of species richness (Wardle et al., 1997). Based on the effects of allelopathy in each litter on the invader above-ground biomass, we calculated the expected above-ground biomass (E) for the mixed species effect. The RME value was calculated by comparing the observed above-ground biomass (O) with the expected above-ground biomass, using the formula: $RME = (O - E)/E$. $RME > 0$ indicates a synergistic effect of the allelopathy on the invader above-ground biomass, while $RME < 0$ indicates an antagonistic effect. A one-sample *t*-test was performed to evaluate whether the RME values of mixed leachate at different litter species richness significantly deviated from zero.

3 | RESULTS

3.1 | Relationships between litter species richness and invasibility

Total biomass of *P. americana* significantly decreased with increasing native plant litter species richness ($F = 4.950$, $p = 0.027$; Figure 2; Table S3). There were no significant interaction effects between litter species richness and activated carbon addition ($F = 0.042$, $p = 0.837$; Table S3). The addition of activated carbon reduced the invader total biomass to some extent ($F = 4.167$, $p = 0.041$), likely due to its nutrient adsorption properties. However, this does not affect the main findings of our study. Similar patterns were observed for both above-ground and below-ground biomass (Figure S2; Table S3).

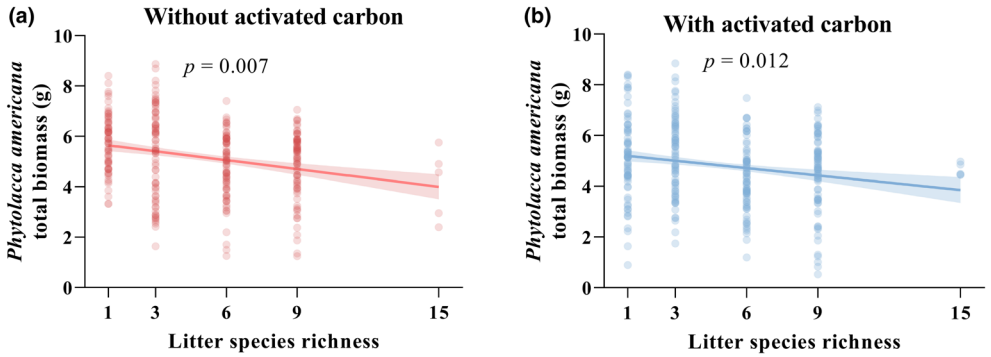


FIGURE 2 Relationship between total biomass of the invader *Phytolacca americana* and native plant litter species richness under different activated carbon treatments in the litter addition experiment: (a) without activated carbon and (b) with activated carbon. Transparent points are the raw data. Lines represent linear regression fits. Shaded areas indicate 95% confidence intervals of fitted lines. All regression lines were significant at $p < 0.05$.

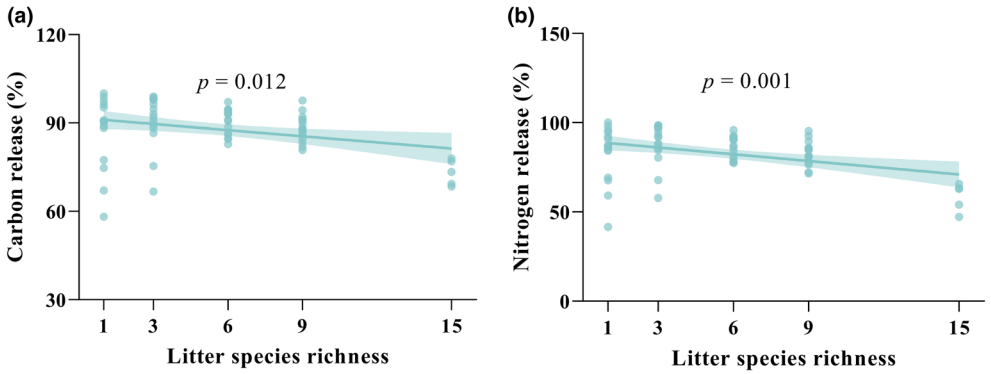


FIGURE 3 Relationships between native litter species richness and the percentages of carbon and nitrogen released in the litter addition experiment: (a) carbon release and (b) nitrogen release. Lines indicate linear regression fits. Shaded areas indicate 95% confidence intervals of fitted lines. All regression lines were significant at $p < 0.05$.

3.2 | Litter C and N release

The percentages of C and N released from litter significantly decreased with increasing litter species richness (Figure 3). Initial analysis of the chemical quality of the 15 litter types revealed that, compared to other litters, those from *Q. glandulifera*, *Q. variabilis*, *Q. acutissima*, *S. alnifolia* and *P. densiflora* exhibited higher C:N ratios and total phenol concentrations (Table S1).

3.3 | Soil microbial properties

Litter species richness had no significant effect on the community structure or diversity of plant pathogenic, saprotrophic or symbiotrophic fungi (Figure 4; Figures S3 and S4). Analysis of the predominant OTU taxa revealed that the proportion of OTU546 (genus: *Bionectria*) in the saprotrophic fungi group decreased with increasing litter species richness, and OTU2427 (genus: *Humicola*) was also affected (Figure 4). In contrast, the predominant OTU taxa of plant pathogenic and symbiotrophic fungi were not influenced by litter species richness (Figures S3 and S4).

3.4 | Relationship between C and N release and invader performance

Among the predominant saprotrophic fungal OTUs, OTU2427 (genus: *Humicola*) showed no correlation with the percentages of C and N released (Figure S5), whereas OTU546 (genus: *Bionectria*) exhibited a significant positive correlation (Figure 5), suggesting that *Bionectria* may play a key role in litter decomposition. Regression analyses revealed a significant positive correlation between the net release of litter C and N and the total biomass of the invader (Figure 6). Further analysis indicated that the presence of *Q. acutissima*, *P. densiflora* and *K. septemlobus* significantly reduced the relative abundance of *Bionectria* (Table S4).

3.5 | The influence of allelopathy on diversity–invasibility relationship

The seed germination rate of *P. americana* was not affected by the species richness of the litter leachate (Figure S6). However, its above-ground biomass significantly decreased with increasing

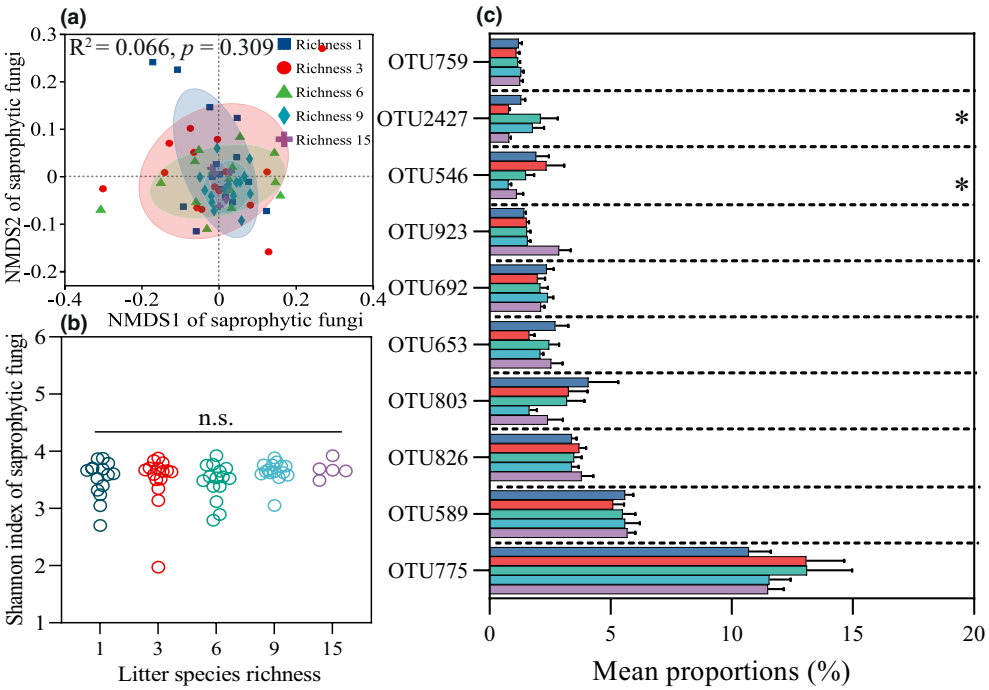


FIGURE 4 Differences in saprotrophic fungi in the litter addition experiment: (a) community structure, (b) Shannon index and (c) mean proportion of dominant OTUs. *Indicates $p < 0.05$, n.s. indicates $p > 0.05$. Error bars represent standard errors.

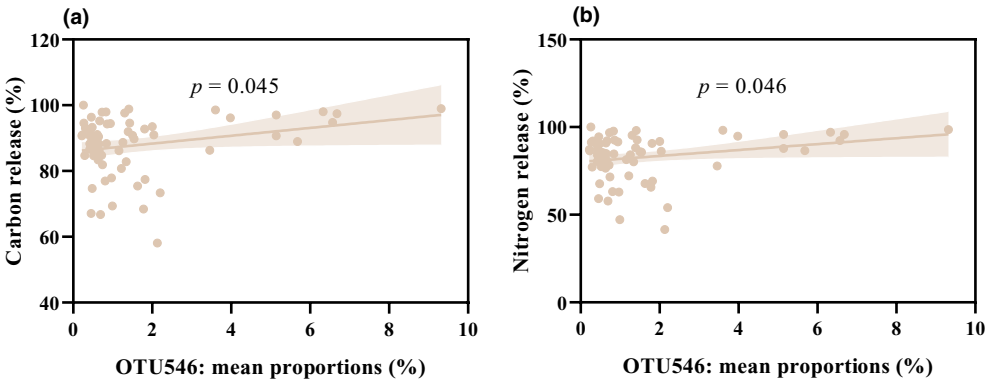


FIGURE 5 Relationships between predominant saprotrophic fungal OTUs and litter nutrient release in the litter addition experiment: (a) relationship between the proportion of OTU546 (genus: *Bionectria*) and carbon release percentage. (b) Relationship between the proportion of OTU546 (genus: *Bionectria*) and nitrogen release percentage. Lines indicate linear regression fits. Shaded areas indicate 95% confidence intervals of fitted lines. All regression lines were significant at $p < 0.05$.

species richness of the litter leachate (Figure 7). Further analysis of species identity revealed that the presence of *Q. acutissima*, *A. hirsuta* and *P. japonica* significantly reduced the invader above-ground biomass (Table S5). RME analysis indicated that the mean RME values at richness levels of 9 and 15 were significantly below 0, indicating an antagonistic effect (Figure S7). Moreover, this antagonistic effect further intensified with increasing richness (Figure S7).

4 | DISCUSSION

Elton's (1958) hypothesis that species-rich communities are more resistant to invasions has gained substantial empirical support across diverse ecosystems (Cheng et al., 2024; Kennedy et al., 2002; Smith & Cote, 2019). This resistance is commonly attributed to intense resource competition in highly diverse plant communities, limiting resource availability for invasive species (Tilman, 1999). Recent breakthroughs in below-ground ecology have revealed mechanisms by which biodiversity affects community invasibility, with soil microbes playing a crucial role (Shen et al., 2021; Wang et al., 2023). Building on this foundation, our study further reveals that plant litter, another essential component of below-ground processes, can mediate negative diversity–invasibility relationships by regulating nutrient release and allelopathy (Figure 8).

Previous results of leaf litter studies suggest that nutrient release may be (though not always) stimulated by plant litter richness, because

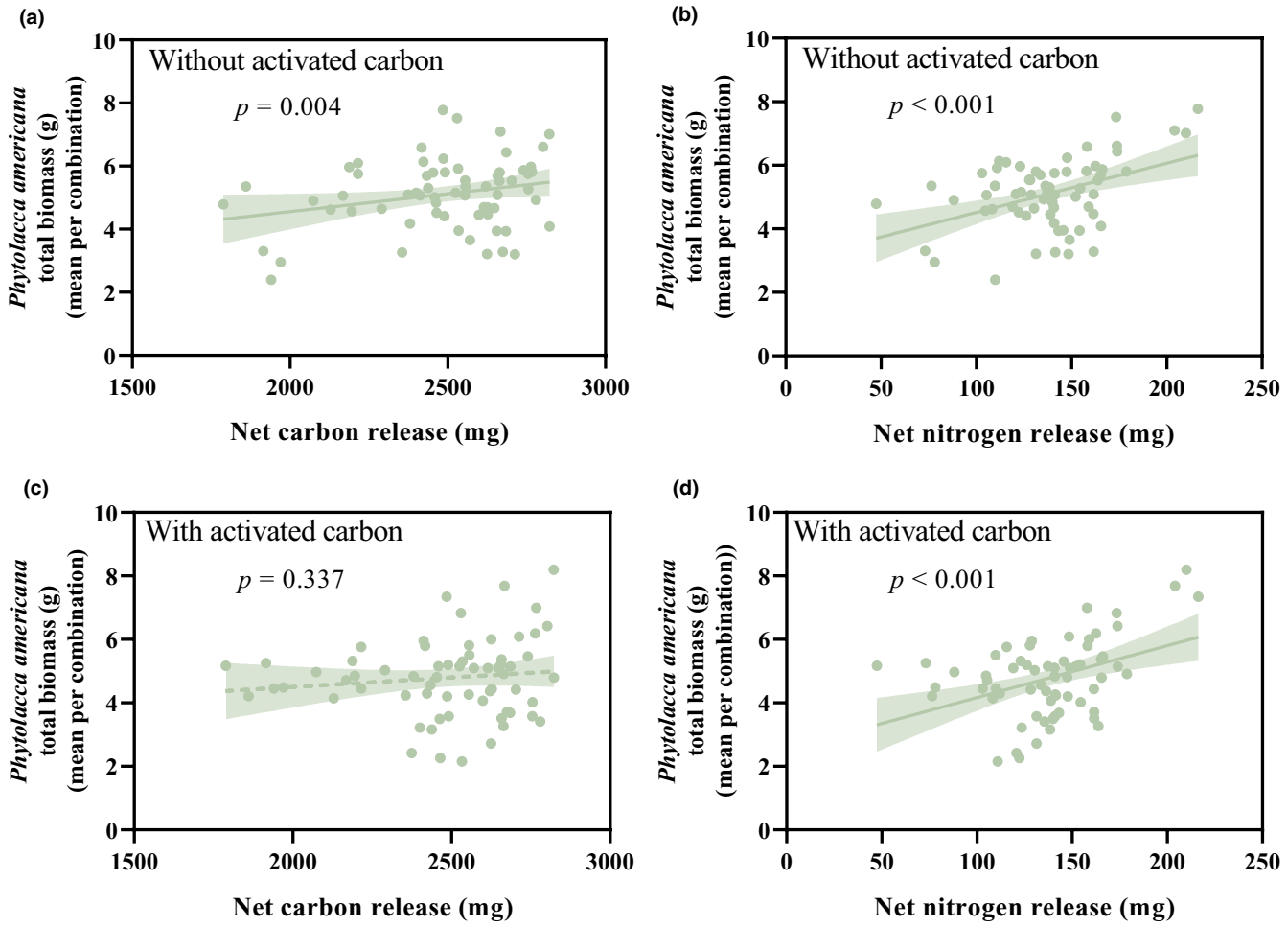


FIGURE 6 Relationships between the net amounts of litter nutrient released and invader performance (total biomass averaged per combination) in the litter addition experiment. Shown are relationships between net carbon or nitrogen release and *Phytolacca americana* total biomass, without activated carbon (a, b) and with activated carbon (c, d). Lines show linear regression fits. Shaded areas indicate 95% confidence intervals of fitted lines. Solid regression lines indicate significant relationships ($p < 0.05$), whereas dashed lines indicate nonsignificant ones.

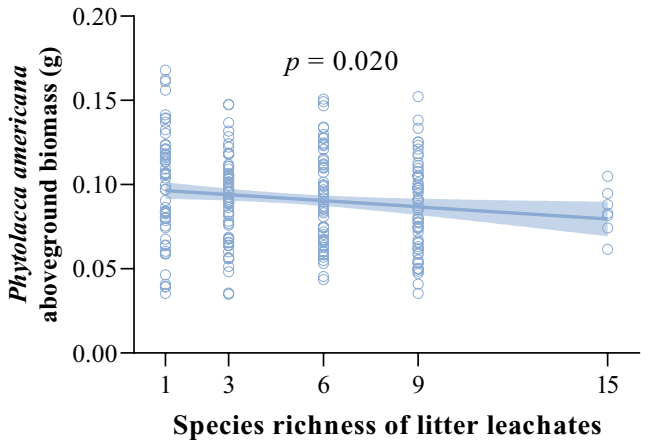


FIGURE 7 Relationship between above-ground biomass of the invader *Phytolacca americana* and native species richness in the litter leachate experiment. Hollow circles represent raw data. Lines indicate linear regression fits. Shaded areas indicate 95% confidence intervals of fitted lines. Solid lines denote significance at $p < 0.05$.

diverse litter mixtures are expected to bolster decomposer diversity and activity (Gessner et al., 2010; Otsing et al., 2018). In contrast to these findings, our results show that increased litter species richness actually limited C and N release, thereby suppressing invasive plant growth. It was observed that the abundance of *Bionectria* decreased with increasing litter species richness, which was the main factor driving the reduction in C and N release rates. The exact mechanism behind this effect remains unclear. One possible explanation is that species repelling this fungus are likely to occur in diverse litter mixtures (Loreau & Hector, 2001). In our experiments, litter from *Q. acutissima* and *P. densiflora* significantly reduced *Bionectria* abundance, supporting this hypothesis. The higher total phenolic concentrations found in these species' litter likely contribute to this suppression, as previous studies have demonstrated that phenolic compounds strongly inhibit decomposers (Chomel et al., 2016; Hättenschwiler & Vitousek, 2000). However, further research is required to confirm this potential mechanism. In conclusion, our findings emphasize the critical role of litter in mediating negative diversity–invasibility relationships by regulating nutrient release.

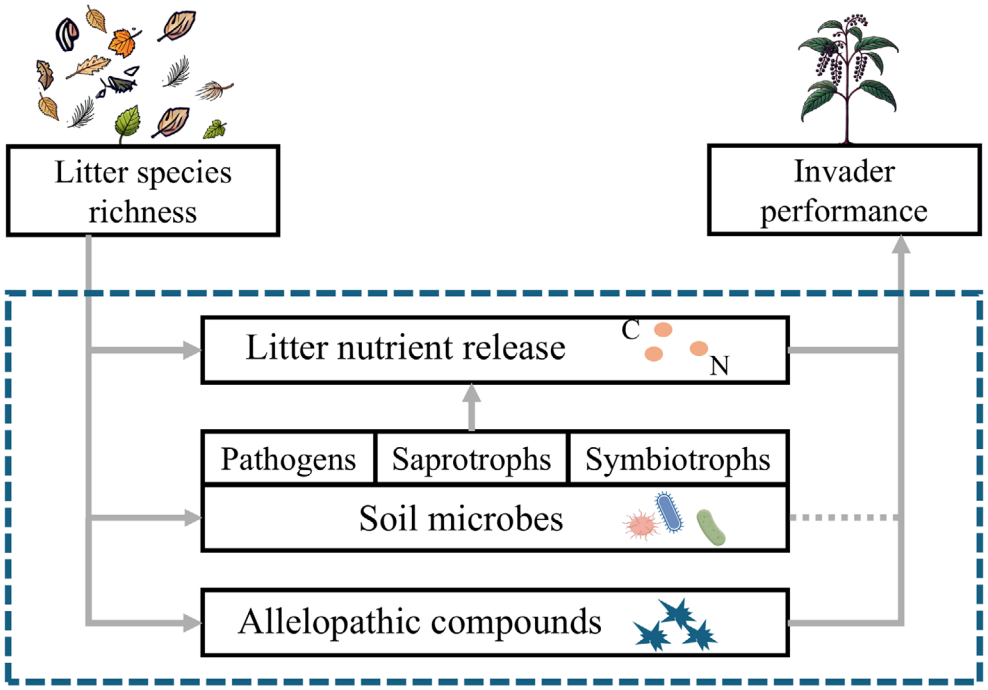


FIGURE 8 Conceptual diagram illustrating potential mechanisms underlying the litter-mediated diversity–invasibility relationship. First, native plant litter species richness can influence decomposer communities, thereby regulating nutrient release to impact invasion resistance. Second, it directly affects invaders by altering pathogen and mutualist dynamics. Third, it modulates allelopathic interactions that impact invasive species performance. The solid line arrow represents the relationship observed in the study, and the dotted line arrow represents the possible relationship not confirmed by the experiment.

We initially expected that allelopathy might alter the effect of litter species richness on invasive plant growth. However, when allelopathic effects were reduced by adding activated carbon, the litter-mediated diversity–invasibility relationship did not change. This means that nitrogen release from litter remained the primary factor influencing this relationship, regardless of whether allelopathy was present. Nevertheless, this does not imply that allelopathy is unimportant; its impact may be masked by nutrient release as decomposition progresses (Bonanomi et al., 2011). Our leachate experiments showed that when nutrient interference was eliminated, the negative effects of allelopathy on the invader intensified with increasing litter species richness. Several recent studies have also demonstrated that allelopathy plays a critical role in the diversity–invasibility relationship (Adomako et al., 2019; Yuan et al., 2022). In addition, the presence of species such as *Q. acutissima* inhibited invasive plant growth through allelopathy, suggesting that their higher occurrence probability in diverse litter mixtures may partly explain the observed suppression. Furthermore, by quantifying the RME of native species on the invader growth, we provided new evidence that increased litter richness can enhance allelopathic suppression of the invader through complementary interactions among native species. Notably, this response was not observed during the seed germination stage, likely due to the minimal competition at this early phase, during which allelopathic interactions have yet to manifest (Inderjit & Duke, 2003). In summary, allelochemicals released from litter play a crucial role in regulating the diversity–invasibility relationship, especially during the competitive growth stage.

The early establishment phase represents a critical window for invasive species success (Blackburn et al., 2011). Meng et al. (2024) highlighted the paramount importance of soil microbial community shifts and nutrient dynamics during the early stages of *P. americana* establishment. In our study, we concentrated on this pivotal early growth period, examining the initial interactions between litter-mediated below-ground processes and the invader. However, this focus inherently limits our ability to assess the long-term impacts of native plant litter decomposition across the entire life cycles of the invader. To construct a more holistic understanding, future research should adopt longitudinal approaches that track these interactions over extended periods, thereby elucidating how nutrient availability and allelopathic compounds influence the invader across different growth stages. Furthermore, the leaves used in our experiments were collected directly from trees rather than naturally senesced litter; thus, their chemical composition may differ slightly from typical leaf litter, potentially affecting the rate of nutrient release as well as the types and intensities of allelopathic compounds. Future studies should compare the effects of litter of different leaf ages on invasive plants to test the applicability of the present findings. Moreover, while our study provides valuable insights into the effects of litter species richness on the invader *P. americana*, the generalizability of these findings to other invaders remains to be examined. Different invaders may exhibit varying sensitivities to nutrient dynamics and allelopathic pressures, necessitating species-specific investigations to fully comprehend the range of litter-induced below-ground processes in invasion ecology. Addressing these limitations will not only

refine our understanding of litter's role in regulating community invasibility but also inform targeted management strategies aimed at mitigating the impacts of a diverse array of invaders, thereby enhancing ecosystem protection and sustainability.

In conclusion, our study underscores the synergistic roles of nutrient dynamics and allelopathy mediated by plant litter in shaping the diversity–invasibility relationship. In ecosystems grappling with invasive species, maintaining or enhancing native litter species richness emerges as a promising strategy to naturally suppress invasive plant proliferation. While challenges such as regional ecological variability and resource constraints exist in scaling litter management practices, our findings offer valuable insights into leveraging native biodiversity as a tool for invasive species control. Future initiatives should integrate these insights into holistic ecosystem management plans, ensuring that the preservation and promotion of native litter richness become cornerstone practices in the fight against biological invasions.

AUTHOR CONTRIBUTIONS

Haokun Li, Xinze Geng and Yuping Hou conceived and designed the study. Haokun Li, Yizhuo Deng, Xiulin Song, Jiaqing Xu and Shuwei Zhang performed the experiments and collected the data. Haokun Li, Chongwei Li, Jinshi Xu, Florencia Tiribelli, Lori Daniels and Guangyan Ni analysed the data. Haokun Li led the writing of the manuscript. Yuping Hou reviewed and edited the manuscript. All authors participated in data interpretation and manuscript revision.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare that are relevant to the contents of this article.

DATA AVAILABILITY STATEMENT

Data are available from Dryad Digital Repository: https://doi.org/10.5061/dryad.2rbnzs82h (Li et al., 2025).

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REFERENCES

Abarenkov, K., Henrik Nilsson, R., Larsson, K.-H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Ursing, B. M., Vrålstad, T., Liimatainen, K., Peintner, U., & Kõljalg, U. (2010). The UNITE database for molecular identification of fungi – Recent updates and future perspectives. *New Phytologist*, *186*, 281–285. https://doi.org/10.1111/j.1469-8137.2009.03160.x

Adomako, M. O., Ning, L., Tang, M., Du, D.-L., van Kleunen, M., & Yu, F.-H. (2019). Diversity-and density-mediated allelopathic effects of resident plant communities on invasion by an exotic plant. *Plant and Soil*, *440*, 581–592. https://doi.org/10.1007/s11104-019-04123-9

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., Wilson, J. R., & Richardson, D. M. (2011). A proposed unified framework for biological invasions. *Trends in Ecology & Evolution*, *26*, 333–339. https://doi.org/10.1016/j.tree.2011.03.023

Bonanomi, G., Incerti, G., Barile, E., Capodilupo, M., Antignani, V., Mingo, A., Lanzotti, V., Scala, F., & Mazzoleni, S. (2011). Phytotoxicity, not nitrogen immobilization, explains plant litter inhibitory effects: Evidence from solid-state ^{13}C NMR spectroscopy. *New Phytologist*, *191*, 1018–1030. https://doi.org/10.1111/j.1469-8137.2011.03765.x

Callaway, R. M., & Aschehoug, E. T. (2000). Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science*, *290*, 521–523. https://doi.org/10.1126/science.290.5491.521

Chapman, S. K., Langley, J. A., Hart, S. C., & Koch, G. W. (2006). Plants actively control nitrogen cycling: Uncorking the microbial bottleneck. *New Phytologist*, *169*, 27–34. https://doi.org/10.1111/j.1469-8137.2005.01571.x

Chapman, S. K., Newman, G. S., Hart, S. C., Schweitzer, J. A., & Koch, G. W. (2013). Leaf litter mixtures alter microbial community development: Mechanisms for non-additive effects in litter decomposition. *PLoS One*, *8*, e62671. https://doi.org/10.1371/journal.pone.0062671

Chen, P., Hou, Y., Zhuge, Y., Wei, W., & Huang, Q. (2019). The effects of soils from different forest types on the growth of the invasive plant *Phytolacca americana*. *Forests*, *10*, 492. https://doi.org/10.3390/f10060492

Chen, P., Huang, Q., Zhuge, Y., Li, C., Zhu, P., & Hou, Y. (2021). The effects of plant–soil feedback on invasion resistance are soil context dependent. *Oecologia*, *197*, 213–222. https://doi.org/10.1007/s00442-021-05004-8

Cheng, C., Liu, Z., Song, W., Chen, X., Zhang, Z., Li, B., van Kleunen, M., & Wu, J. (2024). Biodiversity increases resistance of grasslands against plant invasions under multiple environmental changes. *Nature Communications*, *15*, 4506. https://doi.org/10.1038/s41467-024-48876-z

Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D., Jackson, B. G., & Baldy, V. (2016). Plant secondary metabolites: A key driver of litter decomposition and soil nutrient cycling. *Journal of Ecology*, *104*, 1527–1541. https://doi.org/10.1111/1365-2745.12644

Courchamp, F., Fournier, A., Bellard, C., Bertelsmeier, C., Bonnaud, E., Jeschke, J. M., & Russell, J. C. (2017). Invasion biology: Specific problems and possible solutions. *Trends in Ecology & Evolution*, *32*, 13–22. https://doi.org/10.1016/j.tree.2016.11.001

Davis, M. A., Grime, J. P., & Thompson, K. (2000). Fluctuating resources in plant communities: A general theory of invasibility. *Journal of Ecology*, *88*, 528–534. https://doi.org/10.1046/j.1365-2745.2000.00473.x

Diagne, C., Leroy, B., Vaissière, A.-C., Gozlan, R. E., Roiz, D., Jarić, I., Salles, J.-M., Bradshaw, C. J. A., & Courchamp, F. (2021). High and rising economic costs of biological invasions worldwide. *Nature*, *592*, 571–576. https://doi.org/10.1038/s41586-021-03405-6

Elton, C. S. (1958). *The ecology of invasions by animals and plants*. Methuen.

Eppinga, M. B., Kaproth, M. A., Collins, A. R., & Molofsky, J. (2011). Litter feedbacks, evolutionary change and exotic plant invasion. *Journal*

of Ecology, *99*, 503–514. https://doi.org/10.1111/j.1365-2745.2010.01781.x

Fu, J.-P., Li, C.-R., Xu, J.-W., Cheng, W.-L., Song, R.-F., & Liu, Y. (2012). Prevention and control of invaded plant *Phytolacca americana* in sandy coastal shelter forests. *Chinese Journal of Applied Ecology*, *23*, 991–997.

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., & Hättenschwiler, S. (2010). Diversity meets decomposition. *Trends in Ecology & Evolution*, *25*, 372–380. https://doi.org/10.1016/j.tree.2010.01.010

Ghannoum, M. A., Jurevic, R. J., Mukherjee, P. K., Cui, F., Sikaroodi, M., Naqvi, A., & Gillevet, P. M. (2010). Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. *PLoS Pathogens*, *6*, e1000713. https://doi.org/10.1371/journal.ppat.1000713

Habtewold, J. Z., Helgason, B. L., Yanni, S. F., Janzen, H. H., Ellert, B. H., & Gregorich, E. G. (2020). Litter composition has stronger influence on the structure of soil fungal than bacterial communities. *European Journal of Soil Biology*, *98*, 103190. https://doi.org/10.1016/j.ejsobi.2020.103190

Handa, I. T., Aerts, R., Berendse, F., Berg, M. P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M. O., Jabiol, J., Makkonen, M., McKie, B. G., Malmqvist, B., Peeters, E. T. H. M., Scheu, S., Schmid, B., van Ruijven, J., Vos, V. C. A., & Hättenschwiler, S. (2014). Consequences of biodiversity loss for litter decomposition across biomes. *Nature*, *509*, 218–221. https://doi.org/10.1038/nature13247

Hättenschwiler, S., & Vitousek, P. M. (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution*, *15*, 238–243. https://doi.org/10.1016/S0169-5347(00)01861-9

Heinen, R., Hannula, S. E., De Long, J. R., Huberty, M., Jongen, R., Kielak, A., Steinauer, K., Zhu, F., & Bezemer, T. M. (2020). Plant community composition steers grassland vegetation via soil legacy effects. *Ecology Letters*, *23*, 973–982. https://doi.org/10.1111/ele.13497

Hou, Y., Peng, S., Ni, G., & Chen, L. (2012). Inhibition of invasive specie *Mikania micrantha* HBK by native dominant trees in China. *Allelopathy Journal*, *29*, 307–314.

Huang, W., & Ding, J. (2016). Effects of generalist herbivory on resistance and resource allocation by the invasive plant, *Phytolacca americana*. *Insect Science*, *23*, 191–199. https://doi.org/10.1111/1744-7917.12244

Inderjit, & Duke, S. O. (2003). Ecophysiological aspects of allelopathy. *Planta*, *217*, 529–539. https://doi.org/10.1007/s00425-003-1054-z

Jevon, F. V., Polussa, A., Lang, A. K., William Munger, J., Wood, S. A., Wieder, W. R., & Bradford, M. A. (2022). Patterns and controls of aboveground litter inputs to temperate forests. *Biogeochemistry*, *161*, 335–352. https://doi.org/10.1007/s10533-022-00988-8

Kennedy, T. A., Naeem, S., Howe, K. M., Knops, J. M., Tilman, D., & Reich, P. (2002). Biodiversity as a barrier to ecological invasion. *Nature*, *417*, 636–638. https://doi.org/10.1038/nature00776

Lau, J. A., Puliafico, K. P., Kopshever, J. A., Steltzer, H., Jarvis, E. P., Schwarzländer, M., Strauss, S. Y., & Hufbauer, R. A. (2008). Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytologist*, *178*, 412–423. https://doi.org/10.1111/j.1469-8137.2007.02360.x

Levine, J. M., Adler, P. B., & Yelenik, S. G. (2004). A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, *7*, 975–989. https://doi.org/10.1111/j.1461-0248.2004.00657.x

Li, H., Deng, Y., Geng, X., Song, X., Li, C., Tiribelli, F., Xu, J., Zhang, S., Daniels, L., Xu, J., Ni, G., & Hou, Y. (2025). Data from: *Litter nutrient release and allelopathy jointly contribute to the diversity–invasibility relationship* [Data set]. Dryad. https://doi.org/10.5061/dryad.2rbnzs82h

Li, S., Jia, P., Fan, S., Wu, Y., Liu, X., Meng, Y., Li, Y., Shu, W., Li, J., & Jiang, L. (2021). Functional traits explain the consistent resistance of biodiversity to plant invasion under nitrogen enrichment. *Ecology Letters*, *25*, 778–789. https://doi.org/10.1111/ele.13951

Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J., & Li, B. (2008). Altered ecosystem carbon and nitrogen cycles by plant invasion: A meta-analysis. *New Phytologist*, *177*, 706–714. https://doi.org/10.1111/j.1469-8137.2007.02290.x

Liao, H., Luo, W., Peng, S., & Callaway, R. M. (2015). Plant diversity, soil biota and resistance to exotic invasion. *Diversity and Distributions*, *21*, 826–835. https://doi.org/10.1111/ddi.12319

Liu, Y., Oduor, A. M. O., Zhang, Z., Manea, A., Tooth, I. M., Leishman, M. R., Xu, X., & van Kleunen, M. (2017). Do invasive alien plants benefit more from global environmental change than native plants? *Global Change Biology*, *23*, 3363–3370. https://doi.org/10.1111/gcb.13579

Loreau, M. (2001). Microbial diversity, producer–decomposer interactions and ecosystem processes: A theoretical model. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*, 303–309. https://doi.org/10.1098/rspb.2000.1366

Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, *412*, 72–76. https://doi.org/10.1038/35083573

Luan, J., Liu, S., Li, S., Whalen, J. K., Wang, Y., Wang, J., Liu, Y., Dong, W., & Chang, S. X. (2021). Functional diversity of decomposers modulates litter decomposition affected by plant invasion along a climate gradient. *Journal of Ecology*, *109*, 1236–1249. https://doi.org/10.1111/1365-2745.13548

Meng, Y., Geng, X., Zhu, P., Bai, X., Zhang, P., Ni, G., & Hou, Y. (2024). Enhanced mutualism: A promotional effect driven by bacteria during the early invasion of *Phytolacca americana*. *Ecological Applications*, *34*, e2742. https://doi.org/10.1002/eap.2742

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Ning, L., Yu, F.-H., & van Kleunen, M. (2016). Allelopathy of a native grassland community as a potential mechanism of resistance against invasion by introduced plants. *Biological Invasions*, *18*, 3481–3493. https://doi.org/10.1007/s10530-016-1239-6

Otsing, E., Barantal, S., Anslan, S., Koricheva, J., & Tedersoo, L. (2018). Litter species richness and composition effects on fungal richness and community structure in decomposing foliar and root litter. *Soil Biology and Biochemistry*, *125*, 328–339. https://doi.org/10.1016/j.soilbio.2018.08.006

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rousk, J., & Bååth, E. (2011). Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiology Ecology*, *78*, 17–30. https://doi.org/10.1111/j.1574-6941.2011.01106.x

Santonja, M., Rancon, A., Fromin, N., Baldy, V., Hättenschwiler, S., Fernandez, C., Montès, N., & Mirleau, P. (2017). Plant litter diversity increases microbial abundance, fungal diversity, and carbon and nitrogen cycling in a Mediterranean shrubland. *Soil Biology and Biochemistry*, *111*, 124–134. https://doi.org/10.1016/j.soilbio.2017.04.006

Schnitzer, S. A., & Bongers, F. (2002). The ecology of lianas and their role in forests. *Trends in Ecology & Evolution*, *17*, 223–230. https://doi.org/10.1016/S0169-5347(02)02491-6

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., … Essl, F. (2017). No saturation in the accumulation of alien species worldwide. *Nature Communications*, *8*, 14435. https://doi.org/10.1038/ncomms14435

Shen, C., Wang, J., He, J.-Z., Yu, F.-H., & Ge, Y. (2021). Plant diversity enhances soil fungal diversity and microbial resistance to plant invasion. *Applied and Environmental Microbiology*, *87*, e00251-21. https://doi.org/10.1128/AEM.00251-21

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American*

Journal of Enology and Viticulture, *16*, 144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Smith, N. S., & Cote, I. M. (2019). Multiple drivers of contrasting diversity–invasibility relationships at fine spatial grains. *Ecology*, *100*, e02573. https://doi.org/10.1002/ecy.2573

Staelens, J., Nachtergale, L., Luyssaert, S., & Lust, N. (2003). A model of wind-influenced leaf litterfall in a mixed hardwood forest. *Canadian Journal of Forest Research*, *33*, 201–209. https://doi.org/10.1139/x02-174

Tilman, D. (1999). The ecological consequences of changes in biodiversity: A search for general principles. *Ecology*, *80*, 1455–1474. https://doi.org/10.1890/0012-9658(1999)080[1455:TECOCI]2.0.CO;2

Treplin, M., Pennings, S. C., & Zimmer, M. (2013). Decomposition of leaf litter in a US saltmarsh is driven by dominant species, not species complementarity. *Wetlands*, *33*, 83–89. https://doi.org/10.1007/s13157-012-0353-1

Uddin, M. N., & Robinson, R. W. (2017). Allelopathy and resource competition: The effects of *Phragmites australis* invasion in plant communities. *Botanical Studies*, *58*, 29. https://doi.org/10.1186/s40529-017-0183-9

Van Kleunen, M., Dawson, W., Essl, F., Pergl, J., Winter, M., Weber, E., Kreft, H., Weigelt, P., Kartesz, J., Nishino, M., Antonova, L. A., Barcelona, J. F., Cabezas, F. J., Cárdenas, D., Cárdenas-Toro, J., Castaño, N., Chacón, E., Chatelain, C., Ebel, A. L., … Pyšek, P. (2015). Global exchange and accumulation of non-native plants. *Nature*, *525*, 100–103. https://doi.org/10.1038/nature14910

Veen, G., Fry, E. L., Ten Hooven, F. C., Kardol, P., Morriën, E., & De Long, J. R. (2019). The role of plant litter in driving plant-soil feedbacks. *Frontiers in Environmental Science*, *7*, 168. https://doi.org/10.3389/fenvs.2019.00168

Wang, J., Gao, S., Hong, H., Xue, W., Yuan, J., Wang, X.-Y., van Kleunen, M., & Li, J. (2025). Herbivory and allelopathy contribute jointly to the diversity–invasibility relationship. *Ecology*, *106*, e4490. https://doi.org/10.1002/ecy.4490

Wang, R., & Zhou, G. (2000). *Vegetation of Shandong*. Shandong Science and Technology Press.

Wang, X.-Y., Gao, S., Chen, T., Wang, J., & Yu, F.-H. (2023). Interactions between soil microbes and native species drive a diversity-invasibility relationship. *Biological Invasions*, *25*, 1461–1472. https://doi.org/10.1007/s10530-022-02988-z

Wang, Y.-T., Guo, W., Liu, J., Wang, S.-J., Wang, Q., & Wang, R.-Q. (2009). Value of ecosystem services of Kunyu Mountain Natural Reserve. *Acta Ecologica Sinica*, *29*(1), 523–531.

Wardle, D., Bonner, K., & Nicholson, K. (1997). Biodiversity and plant litter: Experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *Oikos*, *79*, 247–258. https://doi.org/10.2307/3546010

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., & Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, *304*, 1629–1633. https://doi.org/10.1126/science.1094875

Xiao, L., Hervé, M. R., Carrillo, J., Ding, J., & Huang, W. (2019). Latitudinal trends in growth, reproduction and defense of an invasive plant. *Biological Invasions*, *21*, 189–201. https://doi.org/10.1007/s10530-018-1816-y

Yuan, L., Li, J., & van Kleunen, M. (2022). Diversity of resident plant communities could weaken their allelopathic resistance against alien and native invaders. *Biological Invasions*, *24*(3), 607–619. https://doi.org/10.1007/s10530-021-02667-5

Zhang, P., Li, B., Wu, J., & Hu, S. (2019). Invasive plants differentially affect soil biota through litter and rhizosphere pathways: A meta-analysis. *Ecology Letters*, *22*, 200–210. https://doi.org/10.1111/ele.13181

Zhang, Z., Liu, Y., Brunel, C., & van Kleunen, M. (2020). Evidence for Elton's diversity–invasibility hypothesis from belowground. *Ecology*, *101*, e03187. https://doi.org/10.1002/ecy.3187

Zhang, Z., Liu, Y., Yuan, L., Weber, E., & van Kleunen, M. (2021). Effect of allelopathy on plant performance: A meta-analysis. *Ecology Letters*, *24*, 348–362. https://doi.org/10.1111/ele.13627

SUPPORTING INFORMATION

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

Figure S1. The relationship between the percentages of carbon and nitrogen released and their net release in the litter addition experiment: (a) relationship between the percentage of carbon released and net release, (b) relationship between the percentage of nitrogen released and net release. Lines indicate linear regression fits. Shaded areas indicate 95% confidence intervals of fitted lines. All regression lines were significant at $p < 0.05$.

Figure S2. Relationships between the above-ground biomass (a, b) and below-ground biomass (c, d) of the invader *Phytolacca americana* and native plant litter species richness in the litter addition experiment. Transparent points are the raw data. Lines represent linear regression fits for each activated carbon treatment. Shaded areas indicate 95% confidence intervals of fitted lines. All regression lines were significant at $p < 0.05$.

Figure S3. Differences in plant pathogen in the litter addition experiment: (a) community structure, (b) Shannon index and (c) the relative proportion of dominant OTUs. *Indicates $p < 0.05$, n.s. indicates $p > 0.05$. Error bars represent standard errors.

Figure S4. Differences in symbiotic fungi in the litter addition experiment: (a) community structure, (b) Shannon index, and (c) the relative proportion of dominant OTUs. *Indicates $p < 0.05$, n.s. indicates $p > 0.05$. Error bars represent standard errors.

Figure S5. Relationships between predominant saprotrophic fungal OTUs and litter nutrient release in the litter addition experiment: (a) relationship between the proportion of OTU2427 (genus: *Humicola*) and the percentage of carbon released, (b) relationship between the proportion of OTU2427 (genus: *Humicola*) and the percentage of nitrogen released. Lines indicate linear regression fits. Shaded areas indicate 95% confidence intervals of fitted lines. All dashed regression lines were not significant at $p > 0.05$.

Figure S6. Relationships between the seed germination rate of the invader *Phytolacca americana* and litter species richness in the litter leachate experiment. Lines indicate linear regression fits. Shaded areas indicate 95% confidence intervals of fitted lines. Dashed lines indicate non-significance at $p > 0.05$.

Figure S7. Relative mixed effects (RME) of litter leachate on the invader *Phytolacca americana* biomass under different litter species richness in the litter leachate experiment. Black circles represent the RME values of mixed leachates on the invader biomass. Significant differences from '0' are indicated by * at $p < 0.05$. Error bars represent standard errors.

Table S1. Criteria for diameter at breast height of 15 species and the chemical properties of their litter.

Table S2. The effects of litter species richness (1, 3, 6, 9, 15) on invasive plants were tested using 61 different litter mixture types. Grey cells indicate the mitures of each litter species.

Table S3. Output of linear mixed-effects models testing the effects of litter species richness and activated carbon on the log-transformed biomass of the invader *Phytolacca americana*, in the litter addition experiment.

Table S4. Independent-sample *t*-tests were conducted to examine whether litter species identity affected the relative abundance of OTU546 (genus: *Bionectria*), one of the predominant saprotrophic fungal OTUs, in the litter addition experiment.

Table S5. Independent-sample *t*-tests of the effects of individual litter species identities on the above-ground biomass of the invader *Phytolacca americana* in the litter leachate experiment.

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