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RESEARCH ARTICLE

Nutrient availability and invader density regulate the diversity–invasibility relationship mediated by soil microbes

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Abstract

1. Elton's biotic resistance hypothesis, which proposes that diverse plant communities are more resistant to biological invasions, has received considerable experimental support. However, the roles of soil microbes in the diversity–invasibility relationship and how they are influenced by nutrient availability and invader density remain poorly understood in temperate forest ecosystems.
2. We conditioned soils for nearly 1 year using nine individually grown native woody species. We then mixed the soils to create a synthetic diversity gradient of one, three or six species. The invasive plant *Phytolacca americana* was then grown in pots inoculated with these differently conditioned soils. Each soil treatment was crossed with two nutrient availabilities (fertilised vs. non-fertilised) and three invader densities (one, two and four plants) to test invasion success.
3. We found that soil treatments did not significantly affect *P. americana* biomass under low nutrient availability, whereas biomass increased with increasing soil inoculum diversity under high nutrient availability. Soil treatments had no significant effect at invader densities of two and four, whereas *P. americana* biomass increased with increasing diversity levels at a density of one. Furthermore, among all native species, *Quercus acutissima* reduced *P. americana* biomass at an invader density of four, although this suppressive effect disappeared with nutrient addition. Analysis of the soil microbial community suggests that these patterns may reflect diversity-driven pathogen dilution and a reduction in the relative abundance of saprotrophic fungi associated with *Q. acutissima*.
4. Overall, our results indicate that the diversity–invasibility relationship is modulated by soil microbes, and this modulation depends on nutrient availability and invader density. By integrating the interactive effects of plant diversity, nutrient conditions and invasion intensity, this study provides a potential microbial pathway to explain context-dependent shifts in biotic resistance. Furthermore, our findings also highlight the important role that some specific native species

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can play in enhancing community resistance to invasion in particular ecological contexts.

KEYWORDS
biological invasion, biotic resistance, invader density, nutrient availability, plant diversity, soil microbe

1 | INTRODUCTION

Biological invasions pose increasing threats to global biodiversity, economies and human well-being (Diagne et al., 2021), which has led to extensive research on the drivers of ecosystem invasibility (Seebens et al., 2017; Van Kleunen et al., 2015). Elton (1958) proposed that species-rich communities are more resistant to invasions, and a number of studies have since supported the hypothesis at the community level (Case, 1990; Cheng et al., 2024; Delavaux et al., 2023). However, notable exceptions challenge this view (El-Barougy et al., 2020; Emery & Gross, 2006; Ernst et al., 2023), suggesting that the relationship between biodiversity and invasibility is not universally applicable. This contradiction highlights the importance of further understanding the potential factors influencing the relationship between biodiversity and invasibility.

More recently, researchers have shown increasing interest in incorporating soil microbes into the diversity–invasibility relationship (Liao et al., 2015; Wang et al., 2022) as soil microbes play a key role in mediating interspecific plant interactions (Bennett & Klironomos, 2019; Morris et al., 2007). Species-rich communities can reduce pathogen prevalence in a population or community when native plant species vary in their resistance or susceptibility to pathogens (Keesing & Ostfeld, 2021; Schmidt & Ostfeld, 2001). This dilution effect may help to reduce the negative impact on later invasive plants. However, species-rich plant communities can also harbour more diverse pathogen species (Hudson et al., 2006; Keesing et al., 2006). This amplification effect may increase the likelihood that some of these pathogens negatively affect subsequent invasive plants. Mutualistic interactions either align or conflict with these hypotheses (Mitchell et al., 2006; Pyšek et al., 2019; Reinhart & Callaway, 2006). Given these varying predictions, it is crucial to further investigate how soil microbes influence the diversity–invasibility relationship. Although previous research has explored this microbially mediated relationship in the context of short-term soil conditioning by herbaceous species (Zhang et al., 2020), the effect of long-term soil conditioning by woody plants, which leads to persistent microbial legacies, remains poorly understood.

The effects of microbially mediated plant interactions are context-dependent and influenced by environmental conditions, including biotic and abiotic factors (Catford et al., 2022; Van der Putten et al., 2016). In terms of abiotic factors, nutrient availability plays a crucial role in shaping these interactions (Bennett & Klironomos, 2019). Under low nutrient availability, plants often rely on symbiotic soil microbes to defend against soil pathogens (Gille et al., 2024), whereas under high nutrient conditions, this reliance may decrease, potentially weakening plant defences (Gundale & Kardol, 2021). Moreover, high nutrient availability can exacerbate pathogen proliferation and reduce the abundance of mutualistic microbes, which could further increase pathogen pressure (Paungfoo-Lonhienne et al., 2015). However, despite the deterministic effect of nutrient availability on plant–microbe interactions, little is known about whether the microbe-mediated diversity–invasibility relationship would persist or fundamentally change under differences in nutrient availability (Mallon et al., 2015).

In addition to abiotic factors, changes in the biotic environment, such as invasive plant density (an indicator of invasion intensity), significantly shape plant–microbe interactions (Bever et al., 1997; Ke & Wan, 2020). Similar to native plants, invasive species may experience negative density dependence mediated by soil microbes (Flory & Clay, 2013; Johnson et al., 2012; LaManna et al., 2017). For example, the invasive weed *Microstegium vimineum* in the United States accumulates the fungal pathogen *Bipolaris* in dense populations, leading to significant reductions in biomass and seed production (Kleczewski et al., 2012). Therefore, variations in invasive plant density may reshape the role of microbes in the diversity–invasibility relationship (Van der Putten et al., 2016). However, predicting the effect of invader density on this relationship remains a challenge because the interaction between invader density and microbes varies along nutrient gradients (Stone et al., 2023). Thus, nutrient availability must be integrated with invasive plant density to gain a better understanding of the diversity–invasibility relationship.

Species identity is another important factor that cannot be overlooked when discussing community resistance to invasion (Byun et al., 2013). Research shows that local communities dominated by resource-conservative species are less prone to invasion by alien species (Li et al., 2021). This is not only caused by resource availability but also likely due to plant-mediated shaping of soil microbial community composition (Ke & Wan, 2020). For instance, in forests dominated by ectomycorrhizal (ECM) tree species, saprotrophic microbes are typically scarce, limiting nutrient accumulation in the soil (Pellitier & Zak, 2018). Such deficiencies may limit the establishment of invasive species that rely on rapid nutrient uptake (Davis et al., 2000). In addition, Zhang et al. (2020) found that species capable of recruiting rare pathogens in local communities are more effective at resisting plant invasion. These patterns suggest that plants can influence community resistance to invasion by shaping specific soil microbial communities. However, studies integrating

this concept with nutrient availability and invasive plant density remain limited.

In this study, we aimed to examine how soil microbes shape the diversity–invasibility relationship under varying levels of nutrient availability and invader density in a temperate forest ecosystem. To avoid confounding diversity effects with other factors, such as unequal species dominance arising from interspecific competition, we adopted a soil mixing approach (Ren et al., 2023; Zhang et al., 2020). We conditioned soils for nearly 1 year using nine native woody species grown individually in monoculture. These soils were then mixed to establish a synthetic diversity gradient comprising one, three or six species. Each soil treatment was crossed with two nutrient availabilities (fertilised vs. non-fertilised) and three invader densities (one, two and four plants) to test invasion success. Specifically, our study aimed to test three hypotheses: (1) microbial communities from species-rich soil mixtures could suppress invasive plant growth because they likely harbour diverse pathogens; (2) the microbe-mediated diversity–invasibility relationship is regulated by nutrient availability and invader density given the known effects of these parameters on plant–microbe interactions; and (3) species identity plays a key role in improving community invasion resistance owing to species-specific effects on soil microbial communities.

2 | MATERIALS AND METHODS

2.1 | Study location and species

The nine common native plant species selected in this study included four trees, four shrubs and one climbing vine: *Indigofera kirilowii*, *Grewia biloba*, *Lespedeza bicolor*, *Rhus chinensis*, *Smilax china*, *Pinus densiflora*, *Ailanthus altissima*, *Quercus acutissima* and *Quercus variabilis* (Table S1). These species are characteristic of mixed deciduous-coniferous forests in the Kunyu Mountain National Nature Reserve, located on the eastern Shandong Peninsula (37.10°–37.19° N, 121.37°–121.48° E) and represent different stages of ecological succession (Wang & Zhou, 2000). They coexist in stratified communities, with niche differentiation enabling them to occupy distinct ecological roles across successional stages and forest layers (Chen et al., 2019; Wang & Zhou, 2000).

Phytolacca americana, a perennial herb introduced from North America in 1935, has become a highly invasive species that poses severe threats to biodiversity and ecosystem stability across China (Xiao et al., 2019), including the Kunyu Mountain National Nature Reserve (Zhang et al., 2016; Zhou et al., 2013). In this reserve, *P. americana* can invade various forest types and habitats, where it forms high-density populations (Chen et al., 2019; Wei et al., 2020). On 10 November 2021, *P. americana* seeds from over 50 maternal plants were collected from 12 to 15 forest sites near Zhenshan Hill (37.30°–37.32° N, 121.19°–121.21° E), pooled and stored for future use. These sites are located adjacent to the Kunyu Mountain National Nature Reserve and share similar environmental conditions (e.g. vegetation type, climate) (Chen et al., 2019; Wang & Zhou, 2000).

2.2 | Experimental setup

The experiment was conducted in a greenhouse near Zhenshan Hill in China. In Step 1 (i.e. soil conditioning phase; Figure 1), soil was conditioned for nearly 1 year by separately growing nine native species. In Step 2 (i.e. soil mixing phase), soil samples were mixed to generate diversity treatments with one, three or six plant species. In Step 3 (i.e. testing phase), each soil mixture was inoculated onto the sterilised substrate, and a factorial experiment was performed with three invader densities (one, two or four plants) and two nutrient levels (fertilised vs. non-fertilised).

2.3 | Soil conditioning phase

On 1 September 2021, we sowed the seeds of the nine native species into trays (54 × 28 × 4.6 cm) filled with sterilised vermiculite. All trays were placed in a greenhouse under consistent environmental conditions of 18°C–25°C and natural light. On 1 October 2021, we transplanted 15 seedlings of each species into 1-gallon pots, each filled with 25% field soil, 37.5% non-sterilised sand and 37.5% non-sterilised vermiculite. Field soil was collected from grasslands near the greenhouse and sieved through a 1-cm mesh to remove plant roots and large debris. Each pot contained one seedling. We placed 135 pots in individual trays and replaced any dead seedlings within 2 weeks. The pots were watered as required and randomly rearranged every 2 weeks. On 15 August 2022, all above-ground plant parts were removed, and the soil was sieved through a sterilised 5-mm mesh to filter fine roots and debris. Soil samples from 15 pots per species were combined, resulting in a total of 9 soil samples.

2.4 | Soil mixing phase

We mixed soil samples from the conditioning phase to generate three diversity treatments (Figure 1): Diversity 1 consisted of soil conditioned with a single species, while Diversity 3 and 6 involved multiple species combinations. To maintain balance across the treatments, we randomly selected nine combinations for both Diversity 3 and 6, ensuring that each species appeared with equal frequency across the different treatments, resulting in 27 soil samples (Table S2).

2.5 | Testing phase

On 15 August 2022, we collected 25-mL samples from each soil combination (~5% of soil volume) and inoculated them into 700-mL pots filled with 500 mL of substrate soil (grassland soil:river sand,

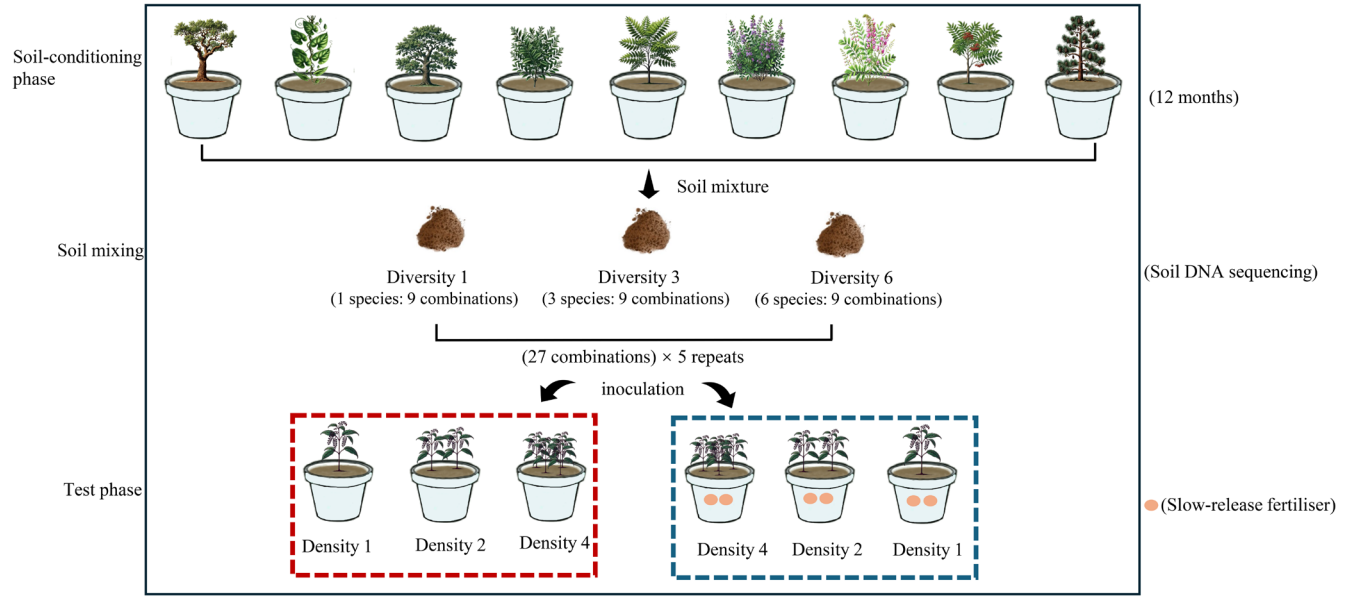


FIGURE 1 Experimental procedure. Step 1: Soil conditioning phase. Nine native plant species were grown separately in pots for nearly 1 year to establish the soil microbial communities. Step 2: Soil mixing phase. Conditioned soils were mixed in various combinations to create three diversity levels: one, three and six species. Step 3: Testing phase. The mixed soil (25 mL) was inoculated into sterilised soil (500 mL per pot) with three diversity levels (one, three or six species) under different nutrient conditions (fertilised vs. non-fertilised) and invader densities (one, two or four plants).

1:1), which was autoclaved (121°C, 0.105 MPa, 30 min) once daily for three consecutive days. The small soil inoculum volume was used to minimise abiotic variation among soil combinations (Kulmatiski & Kardol, 2008). The pots were placed in a greenhouse for 2 weeks to allow the microbial communities to stabilise and re-establish. Simultaneously, we sowed seeds of the invasive plant *P. americana* into trays filled with the same type of sterilised vermiculite used in the soil conditioning phase. On 1 September 2022, we transplanted one, two or four similarly sized *P. americana* seedlings into a prepared pot. One group of pots received 2 g of slow-release fertiliser (N:P:K, 20%:20%:10%), and the other group received no fertiliser. A total of 810 pots were prepared (3 diversity treatments × 9 species combinations per diversity treatment × 2 nutrient availability conditions × 3 density treatments × 5 replicates). Cultivation was performed with adequate natural light exposure, frequent watering and in the absence of weeds. Pots were repositioned randomly every 2 weeks to minimise positional effects. On 1 November 2022 (i.e. 8 weeks after the start of the test phase), we harvested all above-ground plant parts, dried them at 70°C for 72 h until a constant weight was achieved, and measured the biomass. For planting densities of two or four plants per pot, biomass was determined per pot and averaged to calculate the biomass per individual plant for subsequent analyses.

To evaluate the effects of slow-release fertiliser on resource availability, we first analysed the nutrient levels of the sterilised substrate soil before planting (Figure S1). Based on the soil volume and fertiliser nutrient composition, we calculated that adding 2 g of slow-release fertiliser per pot would contribute approximately 0.53 g/kg nitrogen (N), 0.53 g/kg phosphorus (P) and 0.27 g/kg potassium (K) to the soil. Compared with the non-fertilised treatment, the addition of fertiliser nearly doubled the soil phosphorus content and increased the nitrogen content by approximately one third. Because of the difficulty in replicating the absolute nutrient levels of field soils in pots with limited capacity, we selected nutrient levels to ensure that the low-nutrient treatment strongly limited plant growth and the high-nutrient treatment did not severely restrict growth. Previous studies have confirmed the growth-limiting effects of both low and high-nutrient supplies (Liu & Kleunen, 2017).

2.6 | Soil sampling, DNA extraction, amplicon sequencing and bioinformatics

We focused on fungal communities because they are more strongly affected by plants than soil bacterial communities and have profound impacts on plant performance and invasion (Callaway et al., 2011; Lu et al., 2018). We extracted total genomic DNA from 27 soil samples during the mixing phase and amplified the fungal ITS1 region using the universal primer pair (forward: CTTGGTCATTTAGAGGAAGTAA, reverse: GCTGCGTTCTTCATCGATGC) (Ghannoum et al., 2010). Purified polymerase chain reaction products were sequenced on an Illumina MiSeq platform (Illumina). High-quality sequences were clustered into operational taxonomic units (OTUs) at 97% similarity. Fungal ITS sequences were matched against the UNITE database and classified into plant pathogenic, saprotrophic and symbiotrophic sequences using the FUNGuild database (Nguyen

et al., 2016). To account for differences in sequencing depth, the final sequencing depth was rarefied to 40,365 reads.

2.7 | Statistical analysis

To investigate the effects of diversity treatment, nutrient availability and invasive plant density on invasive plant performance, we computed a linear mixed model using the 'lme4' package (Bates et al., 2015) in R 4.0.5 (R Core Team, 2022), with diversity treatment, nutrient availability and invasive plant density as fixed factors and *P. americana* biomass as the response variable. The soil status (i.e. identity of the native species for the Diversity 1 treatment and species combinations for the Diversity 3 and 6 treatments) was included as a random effect. Tukey's test was performed using the 'HSD.test' function in the 'agricolae' package of R to compare *P. americana* biomass across treatments. To evaluate the differences at each treatment level, we treated diversity treatment and invasive plant density as categorical rather than continuous variables.

To assess the effect of species identity on community invasibility, we quantified the impact of individual species based on the log-response ratios for *P. americana* biomass. The log-response ratio was calculated as log ($biomass_{present}/biomass_{absent}$), where $biomass_{present}$ and $biomass_{absent}$ represent the average biomass of *P. americana* when the specific species was present and absent, respectively. Positive values indicate that the species promotes the growth of *P. americana*, while negative values indicate that it inhibits growth. One-sample Student's *t*-tests were performed to determine whether the log-response ratios for each treatment differed significantly from zero.

Soil microbial communities were analysed using the Majorbio Bio-Pharm platform (www.majorbio.com). To evaluate the effects of diversity treatment on fungal community composition, a permutational multivariate analysis of variance (PERMANOVA) was conducted using Bray–Curtis distances with 999 permutations. Nonmetric multidimensional scaling (NMDS) was employed to visualise these compositional changes. One-way ANOVA was used to compare the relative abundance and Shannon index of plant pathogenic, saprotrophic and symbiotrophic fungi across different diversity treatments. For plant pathogenic and symbiotrophic fungi, linear regression was performed to assess their relationships with *P. americana* biomass, and Pearson correlation heatmaps were used to visualise the associations between their predominant OTU taxa and invader biomass. To examine the impact of species identity on fungal community composition, PERMANOVA was used to compare fungal community composition in the presence and absence of specific species, and NMDS was performed to visualise the differences among these communities. Given that *Q. acutissima* significantly influenced the soil microbial community structure, linear regression analysis was performed to assess the relationships between NMDS axis scores (NMDS1 and NMDS2) and *P. americana* biomass. Independent samples *t*-tests were used to analyse the effects of species identity on the Shannon index and relative abundance of the three functional groups.

3 | RESULTS

3.1 | Plant performance

Increased nutrient availability significantly increased *P. americana* biomass, highlighting the plant's ability to rapidly absorb and utilise additional nutrients (Figure 2). Significant two-way interactions were observed among nutrient availability, invasive plant density and the diversity treatment (Table S3). Under low nutrient availability, the diversity treatment had no effect on *P. americana* biomass, whereas under high nutrient availability, *P. americana* biomass increased with increasing diversity levels (Figure 2).

As the density of *P. americana* increased, the average biomass per individual significantly decreased (Figure 3), indicating strong intraspecific competition. At a density of 1, *P. americana* biomass increased significantly with increasing diversity levels, whereas at densities of 2 or 4, the diversity treatment had no effect (Figure 3).

Based on a species-specific presence and absence contrast analysis across native community compositions, species identity significantly affected *P. americana* biomass, and this effect was further modulated by nutrient availability and invasive plant density (Figure 4). Under low nutrient availability, *G. biloba*, *A. altissima* and *Q. variabilis* did not affect *P. americana* biomass, whereas under high nutrient availability, they increased *P. americana* biomass, although this effect diminished or disappeared as the invasive plant density increased (Figure 4). Among all species, *Q. acutissima* significantly reduced *P. americana* biomass, especially at an invasive plant density of four (Figure 4). This suppressive effect disappeared under high nutrient availability (Figure 4).

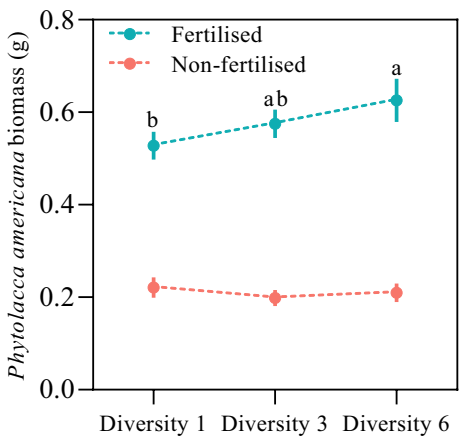


FIGURE 2 Mean biomass (±SE) of *Phytolacca americana* under combinations of three native species diversity levels (one, three or six species) and two nutrient levels (fertilised vs. non-fertilised). Letters identify diversity treatments that differ significantly within each nutrient level ($p < 0.05$). Error bars represent standard errors. *Phytolacca americana* biomass values are averaged across invader densities (one, two or four plants).

3.2 | Soil microbial community

NMDS analysis indicated that diversity treatment did not significantly affect fungal community composition (Figure S2). FUNGuild predictions revealed that diversity treatment did not significantly influence saprotrophic and symbiotrophic fungal diversity (Figure 5b,c). A downward trend was observed in the relative abundance of plant pathogenic fungi, although it was not significant (Figure 5a). Among the 15 most abundant OTUs, most showed a significant decreasing trend, with OTU587 (genus: *Cladosporium*) exhibiting the most significant decline (Figure 5a). Some *Cladosporium* species are symbiotrophic (Figure 5c). In the saprotrophic group, the relative abundance of OTU2095 (genus: *Talaromyces*) significantly increased, while OTU2121 (genus: *Arthrobotrys*) and OTU641 (genus: *Penicillium*) significantly decreased (Figure 5b). Linear regression analysis revealed no significant relationship between plant pathogenic or symbiotrophic fungi and the biomass of *P. americana* (Figure S3).

Species identity largely explained the variation in the fungal community composition. Among the nine native plant species, *Q. acutissima* and *Q. variabilis* significantly altered the fungal community (Figure 6), reducing the relative abundance of plant pathogenic and saprotrophic fungi, while increasing the relative abundance of symbiotrophic fungi (Table S4). In soils shaped by *Q. acutissima*, the fungal community NMDS1 scores were marginally negatively correlated with *P. americana* biomass at an invader density of four (Table S5).

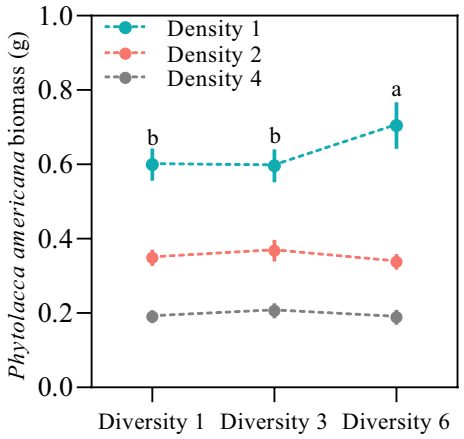


FIGURE 3 Mean biomass (±SE) of *Phytolacca americana* under combinations of three native species diversity levels (one, three or six species) and three invasion densities (one, two or four plants). Letters identify treatments that differ significantly within each invader density ($p < 0.05$). Error bars represent standard errors. *Phytolacca americana* biomass values are averaged across nutrient levels (fertilised vs. non-fertilised).

4 | DISCUSSION

This study investigated the role of soil microbes in mediating the diversity–invasibility relationship and how this effect is influenced by

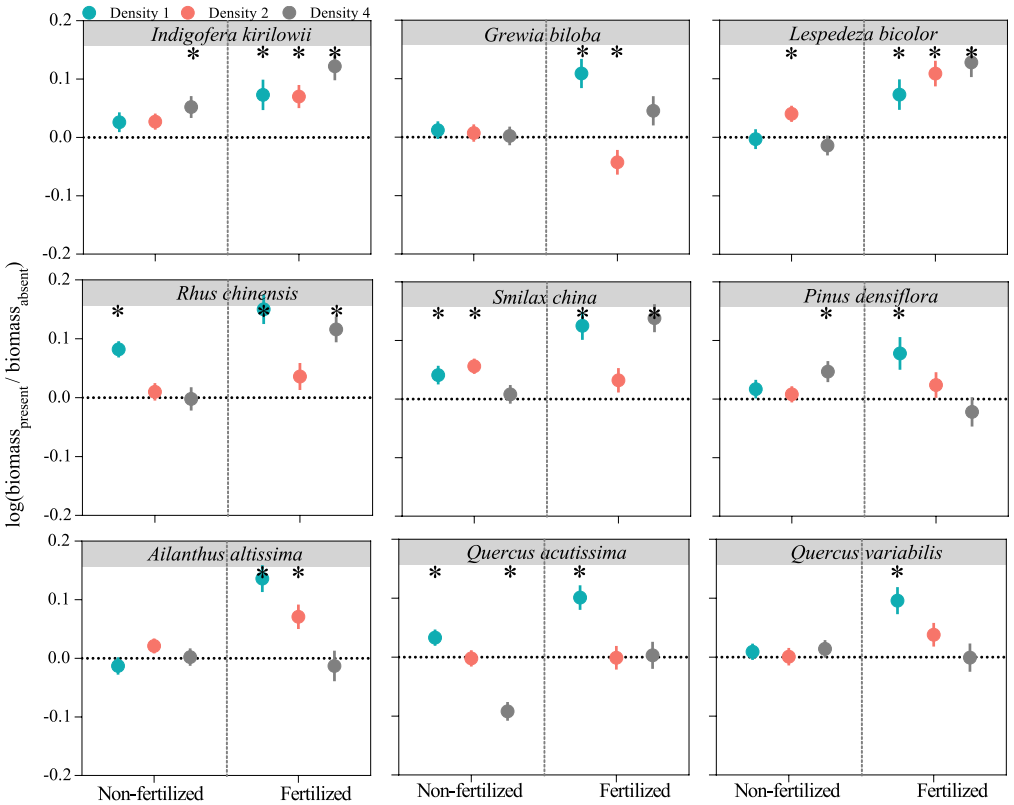


FIGURE 4 Effect of native species identity on *Phytolacca americana* growth. Values represent the log-response ratios of *P. americana* biomass in terms of the presence versus absence of the respective species. *$p < 0.05$, compared with 0. Error bars represent standard errors.

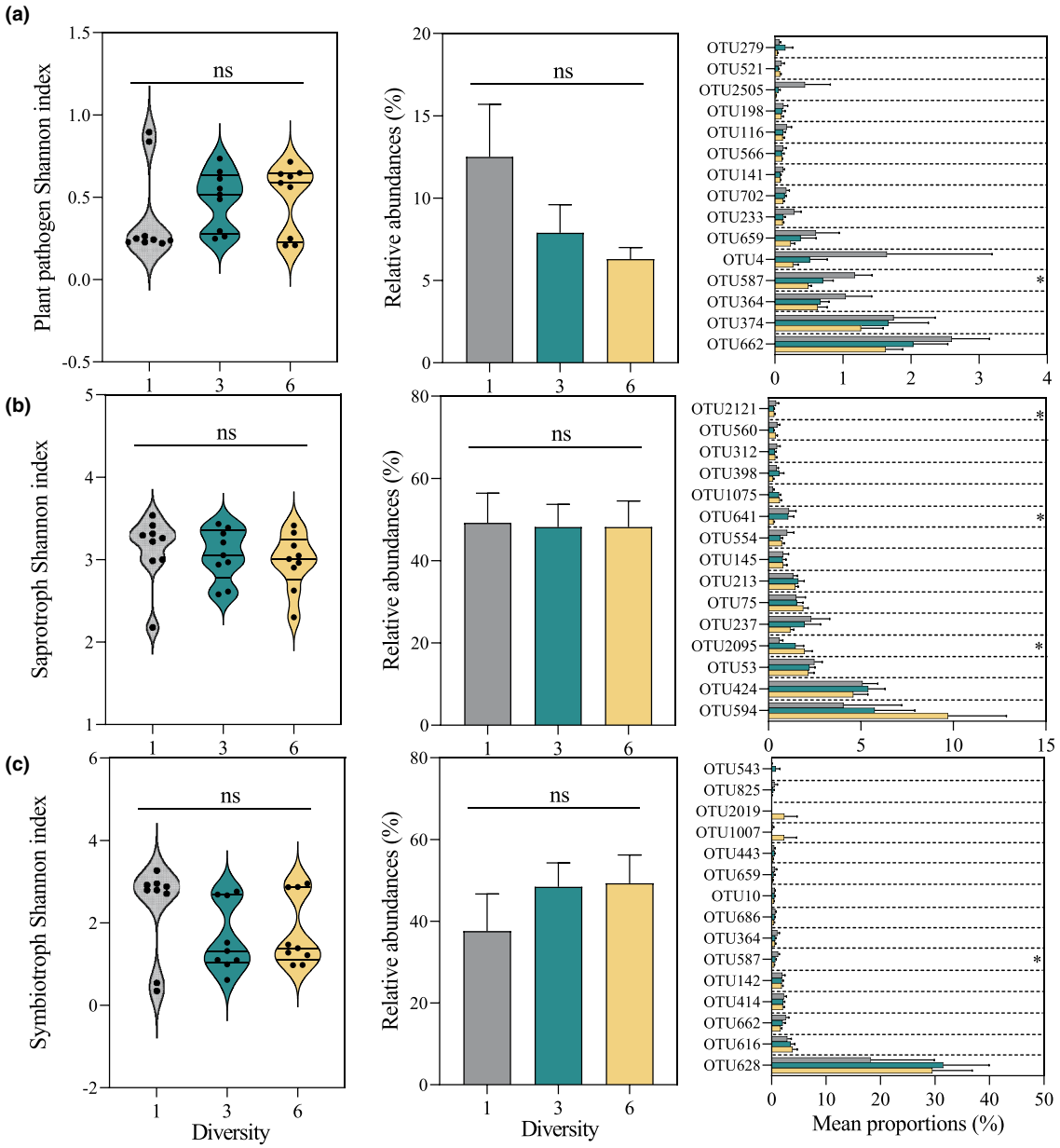


FIGURE 5 Differences in the Shannon index and relative abundance of (a) plant pathogens, (b) saprotrophs and (c) symbiotrophs and relative abundance of dominant OTUs within each functional group. $^{*}p < 0.05$, ns, $p > 0.05$. Error bars represent standard errors.

nutrient availability and invasive plant density. The results showed that the diversity treatment did not significantly affect invasive plant biomass under low nutrient availability or high invasion density. However, under high nutrient availability or low invasion density, invasive plant biomass significantly increased with higher diversity levels. This suggests that within the diversity–invasion resistance framework, the microbial effect is not always suppressive but rather varies with environmental factors, such as nutrient availability and invasive plant density. More importantly, our findings highlight that the microbial effects of specific species may play a key role in enhancing community resistance to invasion.

Contrary to our first hypothesis, we did not observe a negative effect of diversity treatments on the invasive plant *P. americana*. Previous studies have suggested that plant diversity may enhance soil fungal diversity (Shen et al., 2021), which may increase the likelihood that certain harmful microbial groups have a stronger negative impact on alien species, leading to a negative relationship between plant diversity and community invasibility (Zhang et al., 2020). However, this trend was not observed in our study. In contrast, we observed a consistent decline in most major harmful microbial taxa in the conditioned soil samples as diversity levels increased, with the relative abundance of OTU587 (genus: *Cladosporium*) showing the most notable decrease. This pattern is in line with the classic dilution effect (Schmidt & Ostfeld, 2001; Wang et al., 2023), suggesting that more diverse native plant communities may reduce pathogen pressure rather than intensify it.

We found that under high nutrient availability, *P. americana* biomass increased significantly with increasing diversity levels.

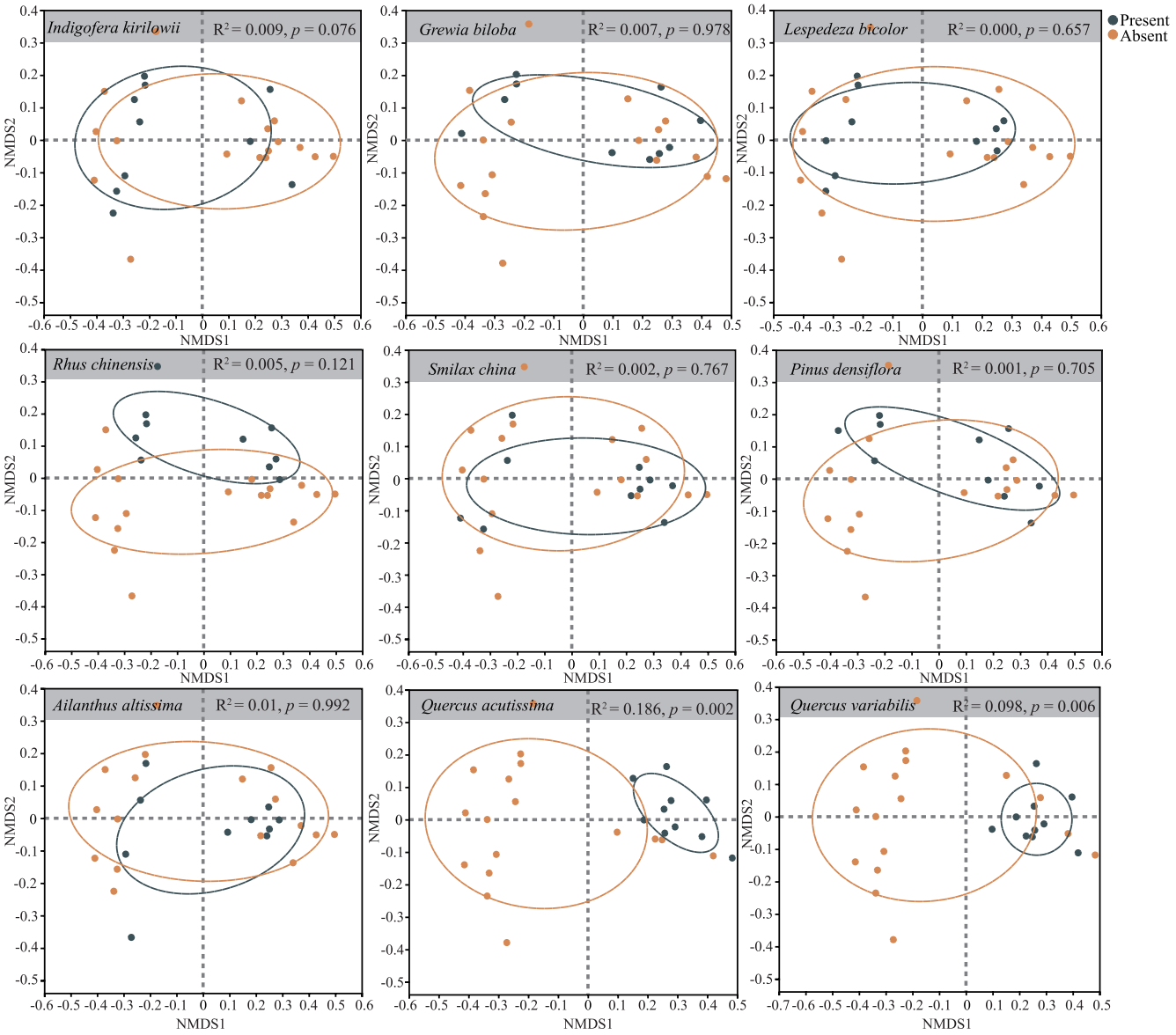


FIGURE 6 Effect of native species identity on the soil fungal community based on nonmetric multidimensional scaling. R^2 values indicate the proportion of community variation explained by the presence or absence of each native species. Statistical significance ($p < 0.05$) indicates a difference in community composition associated with that species.

This likely reflects a continuous decline in the relative abundance of major pathogenic microbes with higher diversity levels, thereby facilitating invasion. Notably, we did not find any significant negative relationships between individual pathogenic microbial groups and plant performance, suggesting that the observed effects may be associated with broader shifts in the pathogenic community rather than with any single group. In contrast, this diversity-driven promotion of invasion was not observed under low nutrient availability. A likely explanation is that under nutrient limitation, mutualistic microbes can effectively assist invasive plants in coping with pathogen pressure (Callaway et al., 2004; Gille et al., 2024; Marchetto & Power, 2018). Supporting this, we observed stronger associations between certain symbiotrophic fungi, such as OTU825 (genus: *Serendipita*), and the invasive plant under low nutrient availability but not under high nutrient conditions (Figure S4). Additionally, Meng et al. (2024) also found that enhancing the relationship with mutualistic soil microbes was key to the successful invasion of *P. americana* in low nutrient sandy loam. Therefore, any benefits from pathogen dilution may be masked under low nutrient availability. These findings support our second hypothesis that nutrient enrichment may reverse the classic 'diversity enhances resistance' pattern, thereby weakening the protective function of native biodiversity even in species-rich temperate forests.

We further observed that the invasive plant biomass increased with higher diversity levels under low invasion densities. This may indicate that lower pathogen pressure in species-rich communities facilitates the initial establishment of invasive species. However, this beneficial effect sharply declined at higher invasion densities. One possible explanation is that higher invader density

promotes soil pathogen accumulation (Crawford et al., 2019; Day et al., 2015), thereby overriding the diversity-driven pathogen dilution effect. This pattern of pathogen accumulation has been frequently documented in forest ecosystems (Augspurger & Kelly, 1984; Bagchi et al., 2010). Additionally, we found that certain mutualistic microbes, such as OTU543 (unclassified genus), were more strongly associated with the invasive plant at the highest invader density, but this association was absent at lower densities (Figure S4). Such shifts in microbial associations may help buffer the negative effects of plant pathogens (Bennett & Klironomos, 2019) and thereby diminish the diversity-driven pathogen dilution effect. Overall, these findings suggest that species-rich communities may promote the establishment of invasive species during early invasion stages through the effects of soil microbes, but this facilitative effect gradually weakens and eventually disappears as invasion intensifies. This interpretation aligns with a recent comprehensive analysis of over 113,800 vegetation samples from the Czech Republic, which found that the resistance of native plant diversity to invasion depends strongly on different invasion stages (Guo et al., 2024). Thus, our findings broaden the invasion paradox framework (Fridley et al., 2007) by showing that the diversity–invasibility relationship can be reversed by shifts in nutrient availability and invader density—two environmental gradients that are intensifying under global change.

Our third hypothesis posited that specific native species could enhance community resistance to invasion by shaping soil microbial communities, which was supported by our results. We observed that soil fungal communities in the presence of *Q. acutissima* clustered towards the positive side of the NMDS1 axis, distinctively separated from communities in pots without this species. Moreover, at high invasion densities, NMDS1 axis scores were negatively correlated with invasive plant biomass, suggesting that fungal community shifts mediated by *Q. acutissima* contributed to increased resistance to invasion. Additionally, *Q. acutissima*, a typical resource-conservative ECM species, reduced the relative abundance of saprotrophic fungi, thereby decreasing the availability of soil nutrients (Zhu et al., 2024). Under high invasion densities, intensified nutrient depletion increases invasive plants' sensitivity to microbially driven changes in resource availability, which may explain why the inhibitory effect appeared only at higher invasion densities (Ke & Wan, 2020). Although we did not quantify nutrient changes across treatments, the disappearance of the inhibitory effect of *Q. acutissima* on invasive plant growth under nutrient addition further supports our hypothesis. Overall, these findings underscore the role of *Q. acutissima* in shaping soil microbial communities to enhance resistance to invasion, with this effect being partially dependent on nutrient availability and invasive plant density.

Our results suggest that soil microbes uniquely shaped by native woody plants are crucial in mediating the diversity–invasibility relationship in temperate forest ecosystems. Unlike herbaceous plants, woody plants require more time to establish soil legacies, including specialised soil microbial communities and root exudates (Xi et al., 2023). In this study, we conditioned soils for nearly 1 year, which enabled us to focus on the initial soil–microbe interactions without the confounding factors that can accumulate over multiple growing seasons. This controlled approach provides valuable insights into the responses of soil microbial communities during the early stages of woody plant establishment (Kulmatiski et al., 2008). In contrast, longer-term observations might capture additional shifts that develop over a plant's full lifespan (Silvertown et al., 1993). Clark et al. (2013) showed through modelling that the relationship between diversity and invasibility can shift from negative to positive over time as communities mature. Building on our findings, future work could supplement experiments with soil samples taken directly from mature forest communities, where plant and microbial communities have coevolved over long periods, thereby improving our understanding of both the short- and long-term dynamics that shape interactions between woody plants and their microbial partners.

Several important points regarding our experimental approach should be clarified. Although mixing equal volumes of soils conditioned by monocultures cannot replicate the interactions among coexisting species (Leff et al., 2018), it provides a practical proxy for testing diversity effects mediated purely by microbes. Growing native plant polycultures for 1 year would have resulted in unequal shoot and root biomass among species across diversity levels, thus confounding diversity with dominance (Ren et al., 2023; Yi et al., 2025). In contrast, our mixing design (i) standardised the total inoculum mass added to each test pot, (ii) removed above- and below-ground competition during the conditioning phase, and (iii) ensured that any subsequent effects on the invasive plant stemmed solely from the combined soil microbial legacies of the species pool (Zhang et al., 2020). Thus, although soil mixing inevitably simplifies some aspects of natural coexistence, it remains a validated approach for disentangling diversity effects from biomass and dominance gradients. Nevertheless, because this method does not fully capture the microbial dynamics that emerge in intact polycultures, generalising the results to natural systems must be performed cautiously. Future research should employ soils conditioned by intact plant polycultures and assess microbial communities directly from established diverse communities to more rigorously test the mechanisms underlying the diversity–invasibility relationship.

In conclusion, by explicitly integrating plant diversity, nutrient availability and invader density within a unified experimental framework, our study offers new insights into how below-ground microbial processes influence invasion outcomes in temperate forest ecosystems. We show that native plant communities may modulate resistance to invaders through soil microbial effects, which are influenced by nutrient availability and invasion density. Moreover, specific native species, such as *Q. acutissima*, play a crucial role in shaping soil microbial communities, thereby enhancing resistance to invasion. We hope that future studies will build on this framework by testing additional invaders and extending it to more complex and realistic communities, thereby contributing to a more mechanistic understanding of diversity–invasibility relationships in the context of global environmental changes.

AUTHOR CONTRIBUTIONS

Haokun Li and Yuping Hou conceived and designed the study. Haokun Li, Yizhuo Deng, Shuwei Zhang and Xiulin Song performed the experiments and collected the data. Haokun Li, Yizhuo Deng, Danli Chen, Xinze Geng, Yanhui Zhuge, Chongwei Li and Guangyan Ni analysed the data. Haokun Li led the writing of the manuscript. Yuping Hou and Jeremy Greenberg reviewed and edited the manuscript. All authors contributed critically to the drafts and gave final approval for the publication.

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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

The data supporting the findings of this study are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.1ns1rn95g (Li et al., 2025).

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SUPPORTING INFORMATION

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

Figure S1. Nutrient content of sterilised basal soil, including total carbon (TC), nitrogen (TN) and phosphorus (TP). Data are presented as the mean (±SE).

Figure S2. Effect of diversity treatments on the soil fungal community based on nonmetric multidimensional scaling. A p-value >0.05 indicates that the difference in community composition associated with diversity treatments is not statistically significant.

Figure S3. Relationships from partial regressions between the relative abundance and Shannon index of plant pathogenic, and symbiotrophic fungi and the biomass of the invasive plant *Phytolacca americana*. Dashed lines represent non-significant correlations ($p > 0.05$).

Figure S4. Heat map showing the correlations between soil fungal communities and the performance of the invasive plant under different treatments. Specifically, panels (a) and (b) show the correlations between the biomass of *Phytolacca americana* and the 15 most abundant OTU taxa classified as plant pathogenic and symbiotrophic fungi, respectively. Asterisks indicate significance levels: $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Table S1. Information on the Chinese native species used in this study.

Table S2. Effects of diversity treatments on invasive plants were tested using 27 soil mixture types. Each column represents one type of soil mixture. Grey cells indicate the species used to condition each soil mixture. Diversity 1: Soils conditioned using one species; Diversity 3: Soils conditioned using three species; Diversity 6: Soils conditioned using six species. Each species contributed to one mixture for Diversity 1, three mixtures for Diversity 3 and six mixtures for Diversity 6.

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Table S3. Results of linear mixed-effects models testing the effects of diversity treatment, nutrient availability, invader density and their interactions on *Phytolacca americana* biomass.

Table S4. Results of independent *t*-tests examining the effects of species identity on plant pathogens, saprotrophic fungi and symbiotrophic fungi.

Table S5. Results of a linear regression on the relationships between fungal community NMDS1/NMDS2 scores and *Phytolacca americana* biomass.

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