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Native Plant Diversity Provides Resistance to Invasion by an Alien Species in Natural and Experimental Settings

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ABSTRACT

It remains uncertain whether native plant diversity increases resistance to plant invasions at meaningful scales in nature due to contradictions between observational and experimental studies. In a field survey of 253 sites spanning 15 latitudinal degrees in China, we found that the relative abundance of the invader *Alternanthera philoxeroides* decreased with increasing native plant richness. Results from a 3-year field experiment and a 2-year mesocosm experiment further demonstrated that native diversity can suppress *A. philoxeroides* population growth (but not dominance) when natives precede the invader, or limit its population size when natives arrive after the invader. Insect herbivores and the soil biota were unlikely to influence diversity-based resistance in the field experiment, as their effects on both *A. philoxeroides* and native species did not change with native richness. Our results provide solid evidence that native plant diversity can provide resistance against plant invasion in natural ecosystems.

1 | Introduction

Over the past two centuries, the proliferation of alien invasive species has become a threat to local biodiversity globally and a substantial economic burden (Bellard et al. 2021; Diagne et al. 2021). Consequently, identifying the factors that promote or hinder species invasion has become a central concern in ecology. High native species diversity often reduces the abundance or richness of alien species (Beaury et al. 2020; Delavaux et al. 2023), formalised as the diversity resistance hypothesis (DRH, also known as the diversity–invasibility relationship) (Elton 1958).

The DRH has been extensively tested with plant species but with mixed results, depending on the approach used (Fridley et al. 2007; Levine 2000). Experimental studies, which have focused mostly on one to four growing seasons (Kennedy et al. 2002; Li et al. 2022; Maron and Marler 2007), and some observational studies (Beaury et al. 2020; Kennedy et al. 2002) have generally revealed that native plant diversity strongly

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Zhen Liu and Biao Zhu contributed equally to this study.

reduces the abundance or richness of alien plants. However, other observational studies, usually at large scales (e.g., landscape scale), have revealed a positive correlation between native plant diversity and the abundance or richness of alien plants (Altieri et al. 2010; Byers and Noonburg 2003; Stohlgren et al. 1999). Thus, integrated field experiments and large-scale surveys are crucial, but no study has explored the DRH with such a synthetic approach. Experiments provide strong mechanistic evidence but are often limited in spatial scale. The latter is particularly important because diversity-based resistance can change across space, possibly due to changes in abiotic and biotic environments (Beaury et al. 2020; Li et al. 2022). Therefore, whether native plant diversity can increase resistance to alien plant invasions at meaningful scales in natural ecosystems remains an open question.

Variation in diversity-based resistance against alien plant invasions can arise from several factors, such as biotic and abiotic environments (Hulvey and Teller 2018; Kennedy et al. 2002; Rejmanek 1989), which might covary with native plant diversity in natural ecosystems. For example, insect herbivores and soil biota can either increase or decrease diversity-based resistance (Liao et al. 2015; Tao et al. 2024; Wang et al. 2025), depending on their host preferences. This may reflect insect herbivory and soil-borne enemies changing asymmetrically with increasing plant diversity due to their effects on natives being more sensitive to native diversity than aliens that experience enemy release (Barnes et al. 2020; Schnitzer et al. 2011). Additionally, the order of species arrival can affect competition between native and alien species (Weidlich et al. 2021; Wolkovich and Cleland 2011), thereby affecting diversity-based resistance to invasion. For example, restoration experiments with seeds have shown that early arrival of native species and the resulting priority effects are crucial for diversity-based resistance (Halassy et al. 2023). However, many alien plants emerge earlier than native species do (Wolkovich and Cleland 2011; Zettlemoyer et al. 2019), potentially weakening or reversing the priority effects for natives over time. Regardless, we know little about the effects of the diversity of natives that arrive after invasions (e.g., via seed dispersal) on extant alien plants, which may be crucial for managing invasive alien plants.

Here, we conducted a comprehensive study of the DRH using one of the world's most harmful invaders of aquatic and terrestrial ecosystems, *Alternanthera philoxeroides* (Tanveer et al. 2018). First, we carried out a large-scale field survey across 253 sites from 21.5°N to 36.7°N (Figure 1a) to explore how *A. philoxeroides* abundance was related to native plant diversity, insect herbivores, soil fungal pathogens and climate. Second, we performed a 3-year field experiment and a 2-year mesocosm experiment (using the same native plant richness gradient in each), in which native plants were established before *A. philoxeroides* or added to existing *A. philoxeroides* monocultures. Third, we compared the amounts of foliar herbivory and the effects of soil biota on *A.*

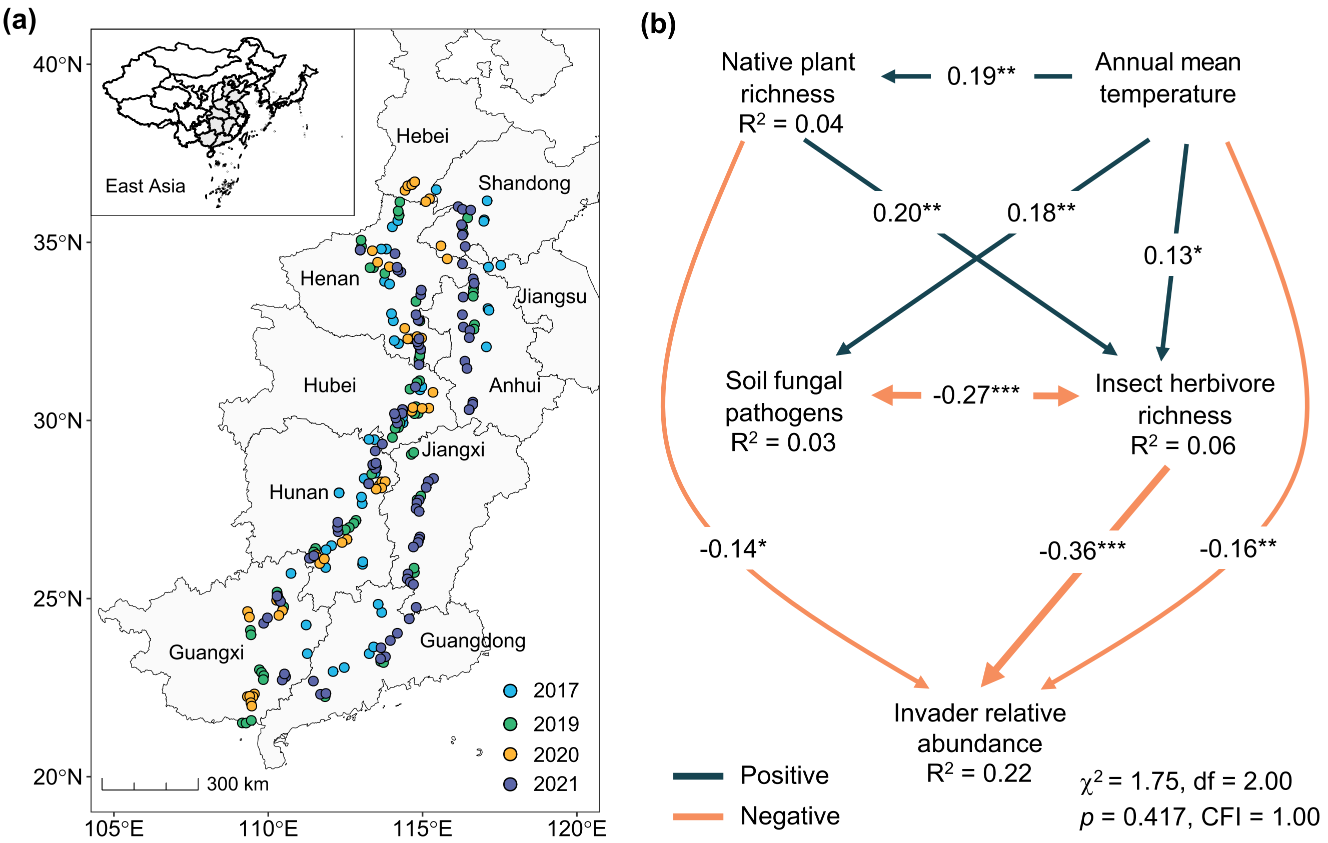

FIGURE 1 | Field survey. (a) The locations for the 4-year field survey spanning 15 latitudinal degrees. (b) The results of an SEM model show how native plant diversity, as well as climate, is related to the relative abundance of *A. philoxeroides* directly and indirectly by affecting herbivore richness and the relative abundance of soil pathogens in the field survey. Blue and orange lines note positive and negative correlations, respectively. The numbers represent standardised coefficients. The width of the lines is positively correlated with standardised coefficients. $^{*}p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$.

philoxeroides and co-occurring native plants at different levels of native plant richness in the 3-year field experiment.

Based on previous studies (discussed above) and a model of scale dependence of the DRH (Byers and Noonburg 2003), we hypothesised that native plant diversity would suppress *A. philoxeroides* in the two experiments, but would be positively correlated with *A. philoxeroides* abundance in the natural field. Insect herbivores preferentially attack and soil fungal pathogens primarily infect co-occurring natives (e.g., *A. sessilis*) over *A. philoxeroides* (Gao et al. 2023; Lu et al. 2018). So, we hypothesised that increasing plant diversity will asymmetrically decrease insect herbivory and effects of soil-borne enemies for native species, thereby contributing to diversity-based resistance to *A. philoxeroides*. By integrating a large-scale field survey and multiyear experiments to explore DRH on a widespread invader, our results offer insights into both invasion mechanisms and invasive species management.

2 | Materials and Methods

2.1 | The Invasive Species

Alternanthera philoxeroides (Mart.) Griseb. (Asteraceae) (Figure S1a), native to South America, is a noxious perennial weed that reproduces solely via vegetative means in Asia, North America, Australia and South America (Tanveer et al. 2018). In China, *A. philoxeroides* has a uniform genetic background (Li et al. 2024), occurs in both aquatic and terrestrial habitats and imposes severe threats to both agricultural and natural ecosystems. Herbicides and the introduced insect *Agasicles hygrophila* Selman and Vogt have failed to control *A. philoxeroides* in terrestrial ecosystems in China (Gao et al. 2023; Lu and Ding 2010). This is because the terrestrial populations of this species have robust root systems and are highly tolerant to abiotic and biotic stresses (Lu and Ding 2010).

3 | Field Survey

To test the hypothesis that native plant diversity is positively correlated with *A. philoxeroides* abundance in the natural field, we performed a comprehensive field survey between October and November 2017 (49 sites), 2019 (72 sites), 2020 (48 sites) and 2021 (84 sites) from 21.5°N to 36.7°N, for a total of 253 randomly selected sites with *A. philoxeroides* (Figure 1a, Figure S1a). These sites were either located on river banks or abandoned farmland. We did not survey sites without *A. philoxeroides* because we cannot rule out other factors, such as a lack of propagules, preventing *A. philoxeroides* invasion at these sites. Detailed information on the first year of *A. philoxeroides* invasion for each site was provided by Prof. Rui Wang from Chinese Academy of Agricultural Sciences.

At each site, we randomly chose three to five 0.5 × 0.5 m quadrats (≥ 2 m apart) along three 5 m transects (> 2 m apart). We identified all plant species in each quadrat and estimated the percentage cover of each species after Gao et al. (2021). The origin (native vs. alien) of all species was assigned based on the Chinese Alien Invasive Species Database (http://www.iplant.cn/ias) and Flora Reipublicae Popularis Sinicae (https://www.iplant.cn/frps). For each site, we also collected annual mean temperature and annual precipitation data, which may influence diversity-based resistance to plant invasion (Cheng et al. 2024), from the WorldClim database (https://www.worldclim.org/). These variables were strongly correlated ($r = 0.87$), so we only used annual mean temperature in our analyses.

To test how aboveground insect herbivores were related to native plant diversity, we collected aboveground insects by sweeping (twice, with 20 sweeps per transect) with a 50-cm-diameter net along each transect (before the plant survey). Insects from each site were pooled and stored in 75% alcohol. We morphologically identified the insects to the family level and assigned some taxa as herbivores based on the dominant feeding strategy within the respective taxon for the life stage sampled, as Gao et al. (2021) did. Six sites were not sampled (2017: three sites; 2021: three sites). In 2019, we visually estimated *A. philoxeroides* foliar defoliation (i.e., percentage of leaf area being removed). Because there were many (often > 1000) leaves within each quadrat, we first classified leaf damage into seven categories: 0 = no damage, 1 = 0%–5%, 2 = 5%–13%, 3 = 13%–25%, 4 = 25%–50%, 5 = 50%–75% and 6 ≥ 75%–100% leaf area removed from each quadrat. We then transformed the categories into median per cent defoliation values (e.g., Category 1 = 2.5%) and averaged these values for each site, as Allen et al. (2021) did. Two sites were not measured.

To test how the relative abundance of fungal pathogens in the rhizosphere of *A. philoxeroides* was related to native plant richness, we sampled *A. philoxeroides* rhizosphere soil cores (diameter: 5 cm; depth: 10 cm) in each quadrat. Three sites were not sampled (2017: one; 2019: two). Soil samples collected from the same site were immediately pooled, stored at 4°C and shipped to Huazhong Agricultural University (HZAU) within 1 week. Two millilitre of pooled rhizosphere soil from each site was stored at −80°C for DNA sequencing. We characterised the fungal community via high-throughput DNA sequencing, as Gao et al. (2021) did, and classified a proportion of fungi as putative pathogens based on the FUNGuild database at the confidence level of probable or highly probable (Nguyen et al. 2016). Given that many *Fusarium* species are important plant pathogens (Ma et al. 2013), we assigned the OTUs belonging to this genus as putative pathogens. The relative abundance of pathogens was calculated as the ratio of pathogen OTU reads to the total number of fungal OTU reads. For detailed information, see Supporting Information Methods S1.

3.1 | Experiments

To test whether native plant diversity affects resistance to *A. philoxeroides* invasion, we performed a field experiment (2021–2023, Figure S1b) and a mesocosm experiment (2022–2023, Figure S1c) in Ezhou (114.71°E, 30.36°N), Hubei Province. The average minimum and maximum temperatures are 1.9°C in January and 33.3°C in July, and the annual precipitation is 1400 mm (http://www.nmc.cn). We introduced native plant species before *A. philoxeroides* invasion in the field experiment and after *A. philoxeroides* invasion in the mesocosm experiment. Due to space limitations, we could only have one order of introduction of native and invasive species in each experiment. Therefore, this design precluded us from directly testing the effects of the timing of native species introduction on diversity-based resistance. We established a gradient of native plant richness of 2, 4, 6, 8 and 12 species, with a forb: grass ratio of 1:1. Native communities were constructed from a pool of 18 native species, including 8 grass species and 10 forb species (Table S2). These species frequently co-occurred with *A. philoxeroides* in the field. The richness and composition of native communities were the same for the two experiments. For each richness level, the 18 native species were used at similar frequencies (Figure S2).

3.2 | Field Experiment

In October 2020, we set up one hundred 1.0 × 1.0 m plots in a field and removed all the aboveground vegetation to perform this experiment. In March 2021, we dug 49 holes (diameter: 5 cm and depth: 5 cm) evenly in each plot (~15 cm apart). Subsequently, 10 surface-sterilised seeds of each species were sown in each hole (48 holes for native plants) and covered with a gamma ray–sterilised mixture of peat and soil (1:1 in volume). We surface-sterilised plant seeds by immersion in 70% ethanol (30 s) and subsequently rinsed them twice with demineralised water. In each plot, the number of holes was the same for all native species (randomly allocated). In total, we had 100 plots (5 native richness levels × 20 assemblages), and native richness levels were randomly positioned in the field (Figure S1b). We installed an open-top mesh cage (height: 1 m; 80 mesh) around each plot to prevent seeds from entering from adjacent plots. One month later, plants were thinned to one individual/hole. Three months later, at the centre of each plot, we planted a 5-cm *A. philoxeroides* stem cutting to mimic invasion.

From October 2021 to 2023, we estimated the cover of each species with the point intercept method for each plot, as Descombes et al. (2020) did, in May and October. In November each year, we harvested and weighed (after drying at 60°C for 48 h) aboveground biomass of *A. philoxeroides* and native plants in each plot separately. Subsequently, the dried aboveground materials of *A. philoxeroides* and native species were placed back in the plot from which they had been harvested to minimise disturbance.

To test the hypothesis that native plant diversity asymmetrically decreases herbivory on native plants in comparison with that on *A. philoxeroides*, we first sampled aboveground insects using a modified vacuum cleaner and sampled soil insects using Moericke traps (Luff 1975) in late September (when plant biomass peaked) each year (Supporting Information Methods S2). All the sampled insects were preserved in 75% alcohol. We morphologically identified the insects to species and classified some species as herbivores following the same approach used in the field survey (Table S3). Second, we visually estimated the defoliation level for each species in each plot with the same method used for the field survey.

To test the hypothesis that native diversity asymmetrically decreases the negative effect of soil biota on native plants, we sampled soil from 18 experimental plots, with varying levels of native plant richness: 2 (3 plots), 4 (2 plots), 6 (3 plots), 8 (5 plots) and 12 species (5 plots). For each plot, we sampled soils (diameter: 5 cm and depth: 5 cm) from 10 randomly selected points (< 20 cm apart) in early March 2023, before plant species had germinated in the field. Initially, we sampled soils from five plots for each richness level. However, seven soil samples were accidentally contaminated and were subsequently excluded from this study. In a greenhouse at HZAU, we performed a plant–soil feedback experiment to test the effects of soil biota on *A. philoxeroides* and on each native species that occurred within each respective plot. Individual plants were grown in 2.3-L experimental pots (diameter: 16 cm and depth: 12 cm) filled with either a mixture of field-collected soil and gamma ray–sterilised topsoil (1: 9 volume) or sterilised topsoil alone (control). To minimise the influence of potential variation in soil abiotic properties among experimental plots, only a small amount (10%) of field-collected soil was included in the mixture. *Alternanthera philoxeroides* was grown in soils sampled from each plot, while native species (16 species in total) were planted in soils sampled from plots containing those specific species. We calculated soil effect as Ln (plant mass in nonsterilised soil/plant mass in sterilised soil). For detailed information, see Supporting Information Methods S3.

3.3 | Mesocosm Experiment

We began the mesocosm experiment in March 2022. We placed 120 pots (diameter: 80 cm and depth: 40 cm, ~200 L) in the field (~1 m apart) and filled them with topsoil collected from the same field (Figure S1c). In early March, forty-eight 5 cm stem cuttings of *A. philoxeroides* were evenly planted (~10 cm apart) vertically in each experimental pot. One month later, the coverage of the invader reached 50%, similar to the average coverage (from 45% to 61%) observed during our field survey. We then sowed 1200 seeds of 2 (600 seeds/species), 4 (300 seeds/species), 6 (200 seeds/species), 8 (150 seeds/species) or 12 (100 seeds/species) native species evenly into each pot. Twenty pots that received no seeds were treated as controls. Each richness level included 20 assemblages (Figure S2), which were randomly positioned in the field. We installed an open-top mesh cage (height: 2 m; 80 mesh) around each pot to limit seed movement from adjacent pots. In early May and October 2022 and 2023, we estimated the coverage of each species with the same method as above. We harvested and weighed the aboveground biomass of each species after drying at 60°C for 48 h. To minimise disturbance, we placed the dried aboveground materials of these species back into the pots from which they had been harvested.

4 | Data Analysis

All analyses were performed with R software (version 4.1.2, R Core Team). Data were log-transformed (e.g., the relative abundance of *A. philoxeroides*) or log(x + 1)-transformed (e.g., the relative abundance of all other alien species) when necessary to improve normality and homogeneity of the residuals.

4.1 | Field Survey

We calculated the relative abundance (cover of alien species/cover of all species) of *A. philoxeroides* and all other alien species in each quadrat and averaged the values for each site. To test how the invasion of *A. philoxeroides* and other alien species relates to native richness and the measured abiotic and biotic factors, we first performed linear models within a multimodel inference approach. The base model included native richness (continuous variable), insect herbivore richness, the relative abundance of rhizosphere fungal pathogens (log-transformed), annual mean temperature, year of measurement and habitat type (farmland vs. natural field) and the interaction of native richness with these five variables. We also included the first year of invasion of a field site by *A. philoxeroides* as a covariate in the *A. philoxeroides* model. These predictors were not highly correlated (variance inflation factor < 5 for all variables). Data for all predictors were Z-transformed before these analyses. We selected the best model with the lowest Akaike information criterion (AIC) value using the 'MuMIn' package (Bartoń 2024). We performed this analysis separately for the relative abundance of *A. philoxeroides* and other aliens, with p values adjusted with the Benjamini and Hochberg method. Second, we performed structural equation modelling (SEM) to explore how native diversity, as well as climate variables, related to the relative abundance of *A. philoxeroides* directly and indirectly by affecting insect herbivores and soil fungal pathogens. For detailed information, see Supporting Information Method S4 and Figure S3.

We performed linear models within a multimodel inference approach using the 'MuMIn' package, to explore the dependence of insect herbivore richness and the relative abundance of fungal pathogens in the rhizosphere of *A. philoxeroides* on native plant richness, temperature, year of measurement, habitat type and the interactions of native richness with these three variables. We selected the best model with the lowest AIC value. We performed a linear model to test the dependence of foliar defoliation in 2019 on native plant richness.

4.2 | Experiments

We performed linear mixed models (LMMs) to test the dependence of *A. philoxeroides* relative abundance or aboveground biomass, along with native plant aboveground biomass, on the fixed factors native richness (continuous variable), time of measurement and their interaction with the data from the field and mesocosm experiments separately. We treated plot (field experiment) or pot (mesocosm experiment) as random factor. We compared the diversity slopes among time points with post hoc contrasts, and p values were adjusted via the Benjamini–Hochberg method.

To test the hypothesis that insect herbivory and pathogen impacts asymmetrically decrease on natives versus *A. philoxeroides* with rising native richness in the field experiment, we first performed an LMM with defoliation as the response variable and the fixed factors native richness (continuous variable), plant origin (*A. philoxeroides* vs. native species), time of measurement and their interactions along with plot as a random factor. We also tested the dependence of soil effects in an LMM on the fixed factors native richness (continuous variable), species origin and their interaction and the random factor pot. We used community-weighted defoliation and soil effects for native plants in these analyses (see Supporting Information Method S5). We examined whether native richness could influence *A. philoxeroides* via soil pathogens with a linear model with *A. philoxeroides* soil effect as the response and its relative abundance in the previous October as the predictor.

5 | Results

5.1 | Large-Scale Patterns of Native Diversity and Invasion

Across the 253 sites, 204 native species and 57 other alien species co-occurred with *A. philoxeroides* (Table S1). The mean relative abundances of *A. philoxeroides* and all other alien species across all the sites were 57.03% and 9.38%, respectively, and they were negatively correlated ($r = -0.24$, $p < 0.001$). The overall richness of alien species (including *A. philoxeroides*) was positively correlated with native plant richness ($r = 0.30$, $p < 0.001$). The richness of other alien species (excluding *A. philoxeroides*) was positively correlated with native plant richness ($r = 0.30$, $p < 0.001$) and negatively correlated with the relative abundance of *A. philoxeroides* ($r = -0.23$, $p < 0.001$).

The best model (with the lowest AIC value, $R^2 = 0.43$, Table S4) indicated that the relative abundance of *A. philoxeroides* was negatively correlated with native plant richness ($r = -0.25$, $p < 0.001$, Figure 2a), insect herbivore richness ($r = -0.41$, $p < 0.001$), annual mean temperature ($r = -0.24$, $p < 0.001$) and varied among years ($p < 0.001$). Notably, the negative correlation between native plant richness and *A. philoxeroides* relative abundance was more pronounced in cold climates than in warm climates (Figure 2b). According to the best model ($R^2 = 0.14$, Table S4), the relative abundance of other aliens was positively correlated with herbivore richness, but did not vary with native plant richness (possibly due to variation among years; native plant richness × year: $F_{3,235} = 2.87$, $p = 0.049$, Table S4). The relative abundance of soil pathogens in the rhizosphere of *A. philoxeroides* across 4 years and defoliation on it in 2019 did not depend on native plant richness (Table S5, Figure 2c,d).

In the SEM model, native plant richness, herbivore richness and soil pathogen relative abundance were positively related to annual mean temperature, but *A. philoxeroides* abundance was negatively related to it (Figure 1b). Native richness was negatively correlated with *A. philoxeroides* abundance both directly and indirectly via a negative correlation with herbivore richness, with the direct correlation twice as strong as the indirect one. There was a negative correlation between herbivores and soil pathogens (Figure 1b).

5.2 | Diversity–Invasibility in the Experiments

In the 3-year field experiment, the relative abundance of *A. philoxeroides* decreased with increasing native richness (Figure 3a), but the slopes were similar at different time points (native richness × time: $F_{4,392} = 2.01$, $p = 0.092$; Table S6). Aboveground biomass of *A. philoxeroides* decreased with native richness in the second and third year, but not in the first year (Figure 3b; native richness × time: $F_{2,196} = 12.07$, $p < 0.001$; Table S6). Nevertheless,

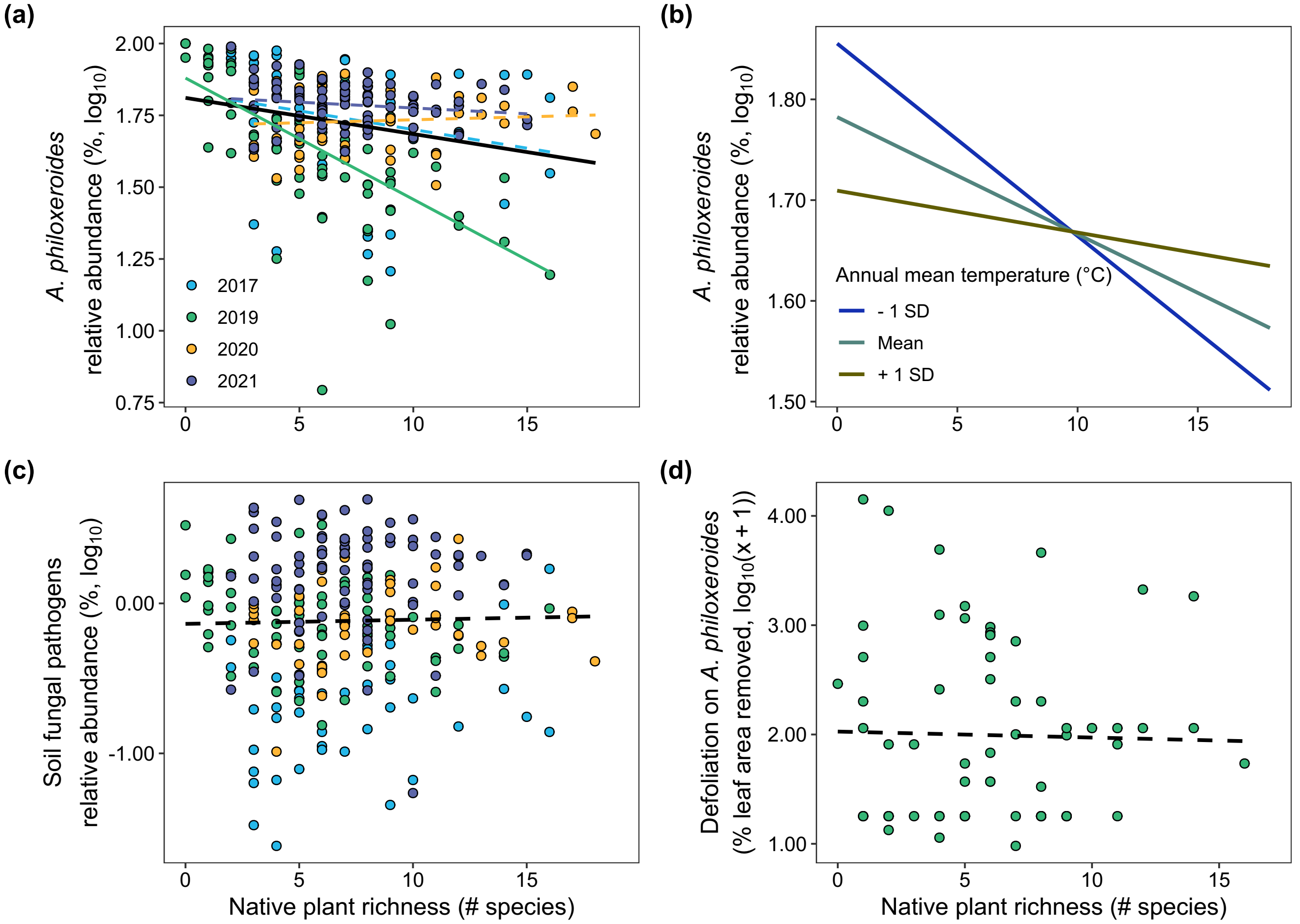

FIGURE 2 | Native plant diversity suppresses species invasion in the field. Relationships between the relative abundance of *A. philoxeroides* and (a) native plant richness for each year and across 4 years (black line), (b) the interaction of annual mean temperature and native plant richness in the 244 survey sites. Relationships between native plant richness and (c) the relative abundance of fungal pathogens in *A. philoxeroides* rhizosphere fungal communities across 4 years, and (d) defoliation level (percentage of leaf area being removed) of *A. philoxeroides* in 2019. The solid and dashed lines indicate significant ($p \leq 0.05$) and nonsignificant ($p > 0.05$) relationships, respectively.

the relative abundance of *A. philoxeroides* increased over time, regardless of native richness (Figure 3a). These results suggest that preestablished diverse native communities can retard *A. philoxeroides* population growth rate, with this resistance strengthening over time, but ultimately *A. philoxeroides* may still become dominant.

In the mesocosm experiment, the relative abundance of *A. philoxeroides* decreased with native richness, with the decrease being more pronounced in the second year (Figure 3c; native richness × time: $F_{3,354} = 56.41$, $p < 0.001$; Table S6). The biomass of *A. philoxeroides* increased with native richness in the first year and decreased with it in the second year (Figure 3d; native richness × time: $F_{1,118} = 37.97$, $p < 0.001$; Table S6). These findings suggest that reintroducing diverse native plants into invaded communities might effectively suppress *A. philoxeroides* invasion.

5.3 | Herbivores and Soil Biota Were Unlikely to Affect Diversity–Invasibility in the Field Experiment

Herbivore richness and abundance increased with native plant richness (richness: $F_{1,98} = 6.08$, $p = 0.046$; abundance: $F_{1,98} = 14.20$, $p < 0.001$; Figure S4) but this did not depend on time (only for richness: $p = 0.299$) or their interaction (richness: $p = 0.814$; abundance: $p = 0.329$; Table S7). Foliar defoliation did not depend on any factor in the LMM (all $p > 0.05$, Table S7, Figure 4a).

Soil biota generally suppressed *A. philoxeroides* and co-occurring native plants. This negative effect did not differ between *A. philoxeroides* and native plants as a group ($F_{1,16} = 2.29$, $p = 0.378$). Notably, soil effects did not change with native richness for either *A. philoxeroides* or native plants (native richness: $F_{1,16} = 1.01$, $p = 0.378$; native richness × origin: $F_{1,16} = 0.82$, $p = 0.378$; Figure 4b). Soil effects were less negative for *A. philoxeroides* for soils with higher relative abundance of the invader the previous October ($F_{1,16} = 8.78$, $p = 0.009$; Figure 4c). In combination with our results showing decreased *A. philoxeroides* abundance with higher native richness, this suggests there may be indirect invasion resistance effects mediated through soil biota.

In the field experiment, native plant aboveground biomass increased with rising native richness in 2021 and 2023 (Figure 4d) and across the 3 years ($r = 0.28$, $p < 0.001$). In

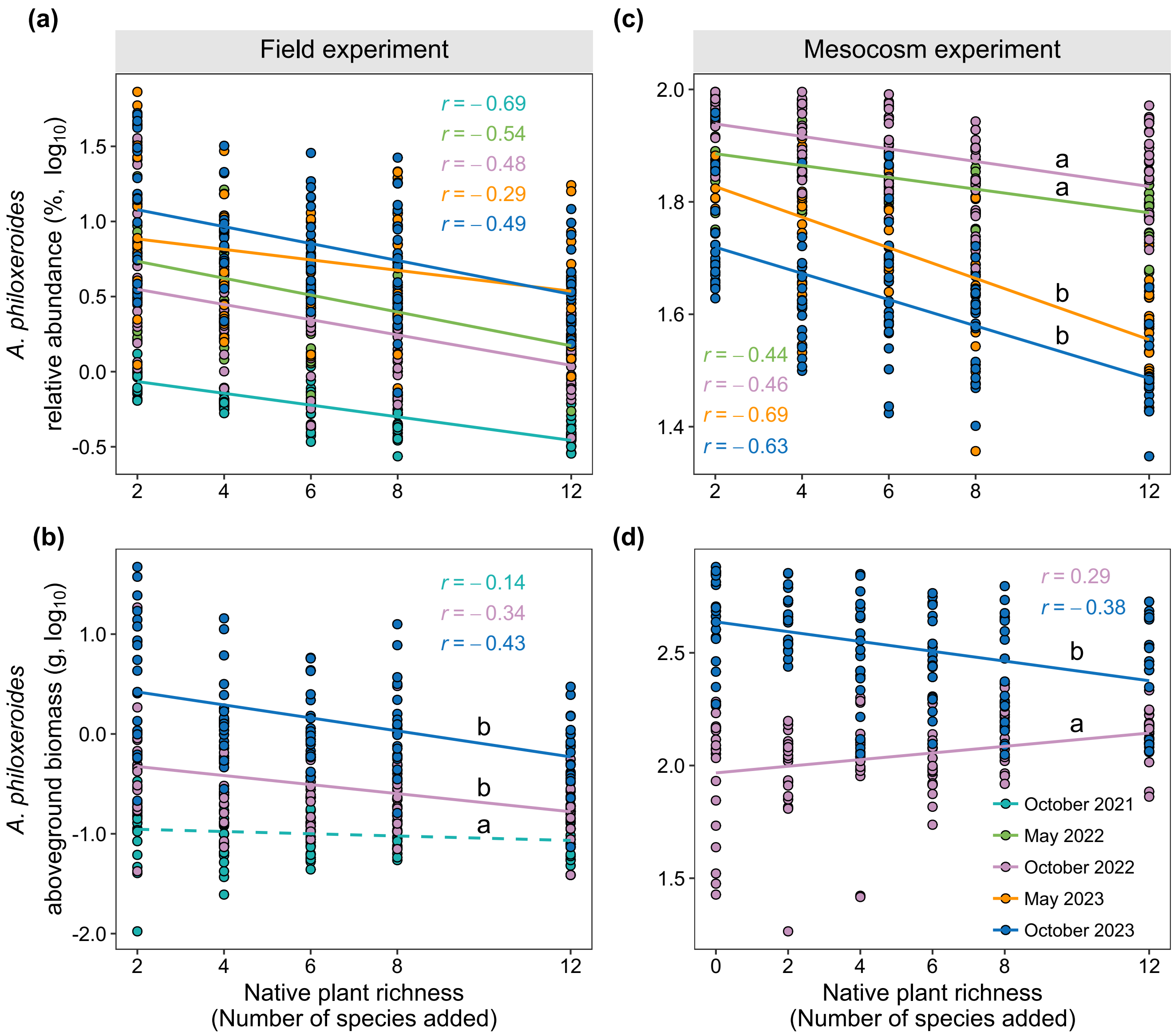

FIGURE 3 | Native plant diversity suppresses invasion in the experiments. Relationships between native plant richness and the relative abundance (a and c) or the aboveground biomass (b and d) of *A. philoxeroides* at each time point in the field experiment (left) and mesocosm experiment (right). The solid and dashed lines indicate significant ($p \leq 0.05$) and nonsignificant ($p > 0.05$) relationships, respectively. Letters indicate slopes that did differ in post hoc tests ($p \leq 0.05$).

the mesocosm experiment, native plant biomass, which was greater in 2023 than in 2022 (time: $F_{1,98} = 88.69$, $p < 0.001$; Table S6), also increased with rising native richness (Figure S5). These findings suggest that competition from native plants could be an important driver of the observed diversity-based resistance. Conversely, neither aboveground insect herbivores nor soil biota were likely to affect diversity-based resistance directly.

6 | Discussion

Our study showed a negative correlation between native plant richness and the relative abundance of one of the world's most noxious plant invaders in the natural setting. Our field and mesocosm experiments further showed that native plant diversity has the potential to provide resistance against the outbreaks of *A. philoxeroides*, regardless of whether the native species arrived earlier or later than the invader. Furthermore, resistance to *A. philoxeroides* invasion via native plant diversity appeared to come from interactions between *A. philoxeroides* and native plants themselves and not indirectly through herbivores or the soil biota. Taken together, the results of our study provide robust evidence for the DRH (Elton 1958) and partially support our first prediction—that native plant diversity suppresses *A. philoxeroides* invasion in the experiments but is positively correlated with *A. philoxeroides* abundance in the field. However, our findings contrast with our second hypothesis—that aboveground herbivory and soil-borne enemies contribute to diversity-based resistance. In combination with two large-scale studies (Beaury et al. 2020; Kennedy et al. 2002), our findings reinforce the notion that promoting native plant diversity can provide resistance against both current and future plant invaders.

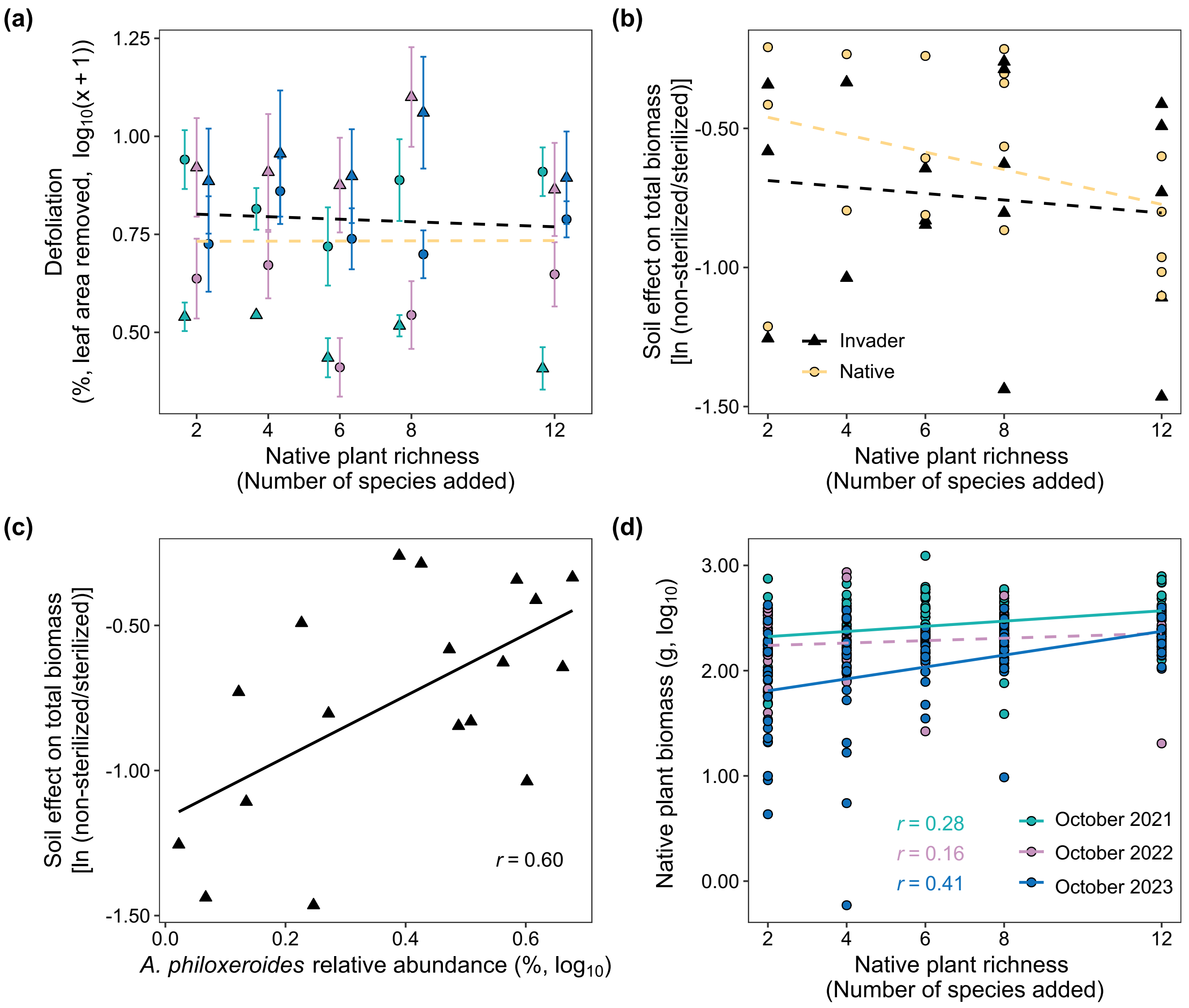

FIGURE 4 | Herbivores and soil biota unlikely affect diversity-based resistance to invasive species. Relationships between native plant richness and (a) defoliation of *A. philoxeroides* and native species (2021–2023), (b) soil effects on *A. philoxeroides* and native species, (c) the dependence of soil effects on *A. philoxeroides* on its relative abundance the previous October and (d) the aboveground biomass of native species (2021–2023), in the field experiment. The solid and dashed lines indicate significant ($p \leq 0.05$) and nonsignificant ($p > 0.05$) relationships, respectively.

We found that diversity-based resistance to *A. philoxeroides* invasion increased over time in the field and the mesocosm experiments. This trend could be related to the complementarity effects, particularly in the mesocosm experiment, in which native biomass increased over the years. Studies have shown that complementarity effects increase over time (Zheng et al. 2024; Zuppinger-Dingley et al. 2014). A more plausible explanation might involve changes in soil biotic properties and/or the interactions of native plants with soil communities, which may have indirectly enhanced the competitive ability of native communities over time, as observed in other studies (Hulvey and Teller 2018; Lankau 2012). The negative relationship between native plant diversity and *A. philoxeroides* abundance, and the positive relationship between the invader's abundance and soil effects, suggest that soil biota may indirectly contribute to the strengthening resistance pattern. Whether these hypotheses hold requires further testing. Regardless, our results suggest that diversity-based resistance against alien species invasion may increase over time. However, most experimental studies have been limited to one growing season (but see Zheng et al. 2018; Li et al. 2022), and observational data were often collected at a single time point. Thus, long-term studies are warranted to fully elucidate the dynamics of diversity-based resistance to plant invasion and explore the underlying reasons.

Knowing the factors that enhance or diminish diversity-based resistance to alien species invasion is crucial for improving the efficacy of diversity-based management of invasive species. The results of our field experiments suggest that neither insect herbivores nor soil-borne enemies alone appeared to affect diversity-based resistance in our system, despite the SEM model (correlative in nature) suggesting that herbivores are likely to enhance diversity effects in the natural field. In contrast, Liao et al. (2015) and Tao et al. (2024) reported positive effects of soil biota on diversity-based resistance in a greenhouse experiment. This discrepancy is reasonable as plant–soil interactions in greenhouse experiments differ from those in natural fields (Forero et al. 2019). However, we cannot rule out the possibility that these above- and belowground organisms might interactively affect diversity-based resistance. Further studies are also needed to test whether our results hold in other regions, as both aboveground herbivores and soil biota associated with *A. philoxeroides* vary across space (Gao et al. 2021).

We also need to note that native plant richness, along with the estimated biotic and abiotic factors, collectively explained only a small proportion of the variation in *A. philoxeroides* abundance in the field. This finding suggests that additional unmeasured factors and their potential interactions with native plant richness likely play important roles in shaping *A. philoxeroides* invasion in natural ecosystems. Indeed, we found that diversity-based resistance against *A. philoxeroides* tends to be more pronounced in cold climates than in warm climates. Similarly, studies have reported that environmental factors (e.g., climate change) and plant diversity can interact to drive diversity-based resistance (Hulvey and Teller 2018; Maron and Marler 2007). As such, future research is needed to understand how native plant diversity interacts with other drivers, such as resource availability (Davis et al. 2000) and allelopathy (Callaway and Ridenour 2004; Wang et al. 2025), affecting alien plant invasion in natural settings. Such investigations will offer new insights into invasion mechanisms and help identify the key factors affecting diversity-based resistance.

In conclusion, our study provides robust evidence that native plant diversity provides resistance against the invasion of a globally distributed major invasive plant in both natural and experimental settings. Notably, we found that a diversity of native plants arriving earlier than the invader can slow the latter's population growth, but *A. philoxeroides* may still become dominant in the long run. This may explain why *A. philoxeroides* dominated the invaded communities in the natural field despite the presence of a diversity of native species. This finding underscores the importance of promoting native plant diversity in already invaded communities. Additionally, our results suggest potential competition among *A. philoxeroides* and other alien species, corresponding to the negative correlation between the richness of other alien species and *A. philoxeroides* abundance. Conversely, other studies reported positive interactions among alien species (e.g., the invasion meltdown hypothesis) (Simberloff and Von Holle 1999). These findings raise the possibility that while native plant diversity may provide resistance against dominant invaders, it may indirectly promote or suppress the invasion of subordinate invaders. Furthermore, the relationship between an invader's abundance and its ecological effects can vary among species and ecological impact metrics (Fenesi et al. 2023). Therefore, the long-term consequences of diversity-based resistance to dominant invaders require further investigation. Nevertheless, our study suggests that promoting native plant diversity can improve resistance against invasion at both large and small scales.

Author Contributions

X.L. conceived and designed the study. Z.L., B.Z., L.G. and C.W. performed the experiments. Z.L., E.S. and B.Z. analysed the data, and X.L. wrote the first draft of the manuscript. All authors contributed to the final version of the paper.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and R-scripts supporting the findings of this study are available on Figshare (https://doi.org/10.6084/m9.figshare.28733072, version 3). The sequence data for soil fungi have been deposited in the NCBI with the accession number PRJNA1247131.

Peer Review

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.