

LETTER

Functional traits explain the consistent resistance of biodiversity to plant invasion under nitrogen enrichment

Shao-peng Li^{1,2}  | Pu Jia³ | Shu-ya Fan¹ | Yingtong Wu⁴ | Xiang Liu⁵  |
Yani Meng¹ | Yue Li¹ | Wen-sheng Shu³ | Jin-tian Li³ | Lin Jiang⁶ 

¹Zhejiang Tiantong Forest Ecosystem National Observation and Research Station, School of Ecological and Environmental Sciences, East China Normal University, Shanghai, China

²Institute of Eco-Chongming, Shanghai, China

³Institute of Ecological Science and Guangdong Provincial Key Laboratory of Biotechnology for Plant Development, School of Life Sciences, South China Normal University, Guangzhou, China

⁴Department of Biology, University of Missouri, St. Louis, Missouri, USA

⁵State Key Laboratory of Grassland Agro-Ecosystems & Institute of Innovation Ecology, Lanzhou University, Lanzhou, China

⁶School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, USA

Correspondence

Jin-tian Li, Institute of Ecological Science and Guangdong Provincial Key Laboratory of Biotechnology for Plant Development, School of Life Sciences, South China Normal University, Guangzhou 510631, China.
Email: lijintian@m.scnu.edu.cn

Lin Jiang, School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia 30318, USA.
Email: lin.jiang@biology.gatech.edu

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Abstract

Elton's biotic resistance hypothesis, which posits that diverse communities should be more resistant to biological invasions, has received considerable experimental support. However, it remains unclear whether such a negative diversity–invasibility relationship would persist under anthropogenic environmental change. By using the common ragweed (*Ambrosia artemisiifolia*) as a model invader, our 4-year grassland experiment demonstrated consistently negative relationships between resident species diversity and community invasibility, irrespective of nitrogen addition, a result further supported by a meta-analysis. Importantly, our experiment showed that plant diversity consistently resisted invasion simultaneously through increased resident biomass, increased trait dissimilarity among residents, and increased community-weighted means of resource-conservative traits that strongly resist invasion, pointing to the importance of both trait complementarity and sampling effects for invasion resistance even under resource enrichment. Our study provides unique evidence that considering species' functional traits can help further our understanding of biotic resistance to biological invasions in a changing environment.

KEYWORDS

biotic resistance, conservative traits, environmental change, functional diversity, grassland experiment, invasibility, nitrogen deposition, phylogenetic distance, resource enrichment

INTRODUCTION

Biological invasions are a growing threat to global biodiversity, economy and human well-being (Diagne et al., 2021). A central challenge in invasion biology has been

to assess the susceptibility of ecological communities to the invasion of exotic species. As one of the most classic theories in invasion biology, Elton's biotic resistance hypothesis predicts that more diverse communities should be more resistant to biological invasions (Elton, 1958).

This negative diversity–invasibility relationship has been well supported by experimental studies that directly manipulated native species diversity, where all other factors were held constant (Kennedy et al., 2002; Smith & Côté, 2019). Yet, it remains unclear whether biodiversity would consistently resist invasion under environmental changes currently affecting many of the Earth's ecosystems, limiting our ability to predict biological invasions under future global change scenarios.

As an important component of global environmental change, nitrogen (N) enrichment has been widely found to cause changes in species diversity and community invasibility (Matson et al., 2002). On the one hand, N enrichment has been identified as one of the major threats to plant diversity (Payne et al., 2017), and numerous experiments have shown that N addition tended to result in plant diversity loss (Midolo et al., 2019). On the other hand, there is accumulating evidence that N addition can facilitate plant invasion (Liu et al., 2019; Seabloom et al., 2015). Nevertheless, little is known about whether the resistance of biodiversity to plant invasion would persist or be fundamentally altered under N enrichment. Remarkably few studies have directly tested the effect of N addition on the diversity–invasibility relationship (Figure S1), and these studies have produced somewhat mixed results (e.g. Heckman et al., 2017; Mattingly et al., 2010).

Biodiversity can resist invasion through both niche complementarity and sampling effects. On the one hand, as proposed by Elton (1958), more diverse communities often result in more complementary resource use among species, leaving less resource available for invaders (Frankow-Lindberg, 2012). From phylogenetic and functional perspectives, this complementarity effect results from the niche pre-emption of resident species with different evolutionary histories and diverse functional traits that translate into broad-spectrum resource use (Cadotte, 2017; Wagg et al., 2017). In this situation, we would expect that species-rich communities resist invasion mainly through high phylogenetic and functional dissimilarity among residents. On the other hand, the sampling effect operates when more diverse communities have greater chances of containing species that strongly resist invasion (Fargione & Tilman, 2005; Wardle, 2001). For instance, dominant species with functional traits that confer strong competitive ability are known to strongly suppress the performance of invaders (Emery & Gross, 2007). In this situation, the community-weighted mean (CWM) of particular traits linked to invasion resistance would largely determine the invasion resistance of resident communities. Furthermore, diverse communities may also have a greater chance of containing resident species that are closely related or functionally similar to the invaders. According to Darwin's naturalisation hypothesis, these residents are likely to compete more strongly with the invaders, and therefore increase the invasion resistance of diverse communities (Cadotte et al.,

2018; Darwin, 1859). Together, we propose that a comprehensive consideration of phylogenetic and functional attributes of resident plant species (e.g. phylogenetic and functional dissimilarity among residents, CWMs of traits), as well as the phylogenetic and functional dissimilarity between invaders and residents, may help to better understand how biodiversity influences invasion resistance.

The relative importance of niche complementarity and sampling effects is likely to change under N enrichment. For example, N enrichment may weaken the importance of N pre-emption but benefit residents with certain traits that compete strongly with invaders (Roscher et al., 2016). In this situation, we would expect the increased importance of CWMs of these traits in determining the diversity–invasibility relationship under N addition. On the other hand, N addition may enhance community biomass, reducing the availability of light and nutrient soil resources, and therefore promote the partitioning of these resources by accentuating differences in species' evolutionary history and functional traits (i.e. coexistence of phylogenetically distantly related species with dissimilar traits; Fridley, 2003). In this situation, we would expect the increasing importance of phylogenetic and functional dissimilarities (i.e. trait complementarity) among residents on biotic resistance under N addition. However, experimental tests of these two different scenarios are lacking.

Here we report on a 4-year grassland experiment that directly manipulated resident species richness, N addition and the initial density of the invader, coupled with phylogenetic and functional analyses, to explore the determinants of community invasibility under elevated N input. Specifically, our study addressed the following three questions: (1) Is resident species diversity a consistent barrier to plant invasion under increased N input? In addition to our experiment, we also resorted to meta-analysis of the few existing empirical studies on this topic to help answer this question. (2) Could the effects of diversity on invasion be captured by species functional and phylogenetic attributes? (3) Is biotic resistance to invasion in the ambient and N enrichment treatments driven by similar or different functional and phylogenetic attributes?

MATERIALS AND METHODS

Study site

Our experiment was carried out in a subtropical arable field (111°48'E, 23°31'N) near Hei-shi-ding Forest Natural Reserve, Guangdong Province, China. The area has a subtropical humid monsoon climate, with a mean annual temperature of 21.0°C, ranging from 10.6°C in January to 28.4°C in July. The study site represents a typical abandoned agricultural field that has

been continuously colonised by diverse plant species. Common ragweed (*Ambrosia artemisiifolia* L.), an agricultural weed native to North America but an invader in China (Xie et al., 2001), is one of the most problematic noxious weeds in our arable fields and strongly reduces crop yield.

Experimental design

Our grassland experiment included 387 individual plots (each 4.0×1.0 m) randomly assigned into three groups, to replicate the diversity treatment three times (Figure S2, Li et al., 2015). Within each group, the 129 plots were randomly assigned to be seeded with 0, 1, 4, 9 or 16 species, with 6, 48, 27, 27 and 21 replicates respectively. The plant composition of each plot was randomly chosen from a set of 16 resident plant species, which are common and abundant species that frequently co-occur nearby. Before sowing, each plot was divided into three subplots (1.0×1.0 m) separated by 0.5 m walkways, and the three subplots were seeded with the same species composition. All subplots received 2,000 seeds in April 2009, with seed numbers divided equally among all the species planted. Diversity treatments were maintained by weeding three times during the first year, removing all species not seeded in the subplots.

In March 2010, invasion treatment was assigned into one randomly selected subplot of each plot. Each of the 369 selected subplots was hand-seeded with 200 seeds of *A. artemisiifolia*. The number of seeds was sufficient to overcome demographic stochasticity associated with the initial invader population. We manually removed *A. artemisiifolia* seedlings in the walkways and nearby fields, to minimise the dispersal of additional seeds into our plots. The detailed experimental design and the primary results of the first 2 years were reported in Li et al. (2015). In 2011, the three groups were randomly assigned to one of the three N treatments, including the control (no N addition), low N addition ($5 \text{ g N m}^{-2} \text{ year}^{-1}$) and high N addition ($10 \text{ g N m}^{-2} \text{ year}^{-1}$). For the two N addition groups, two of the three subplots within each plot (including the invaded subplot and one uninvaded subplot) were amended with NH_3NO_4 , applied once a month from April to September.

Sampling and biomass measurements

Sampling and measurements of all the 1,161 subplots were carried out in early October from 2011 to 2014. The aboveground plant materials were clipped in one 0.3×1.0 m randomly selected strip per subplot each year. Above-ground biomass was sorted for each species at each harvest, then dried at 70°C for at least 72 h and

weighed. The data from the 369 invaded subplots were used in this analysis.

Phylogeny and functional traits

To construct the phylogeny of the 16 resident species and *A. artemisiifolia*, we obtained three commonly sequenced genes (*rbcL*, *matK* and ITS) of these species from GenBank, and generated a Bayesian phylogeny using BEAST version 1.7.5 (Drummond et al., 2012). Complete construction details can be found in Li et al. (2015). We also generated a maximum likelihood phylogeny using PHYML 3.0 (Guindon et al., 2010), and a seed plant supertree by using the R package *V. PhyloMaker* (Jin & Qian, 2019). As the three phylogenies share a similar topology, we only reported results based on the Bayesian molecular phylogeny here.

We measured eight traits that represent multiple functional dimensions (Table 1). Height is a key indicator of plant light utilisation strategy and competitive ability (Díaz et al., 2016; Kunstler et al., 2016). Specific leaf area (SLA), leaf dry matter content (LDMC), leaf carbon to nitrogen ratio (leaf C:N ratio), leaf carbon to phosphorus ratio (leaf C:P ratio) are key traits in the leaf economic spectrum, and have been widely used to indicate plant resource use strategies (conservative versus acquisitive; Díaz et al., 2016; Wright et al., 2004). We also considered three physiological traits, including light-saturated photosynthetic rate (A_{max}), photosynthetic water use efficiency (PWUE) and photosynthetic nitrogen use efficiency (PNUE), which are directly associated with resource-use efficiency. These traits are among the most considered traits in invasion ecology (Funk et al., 2008; van Kleunen et al., 2010; Tecco et al., 2010), benefiting the comparisons of our results to previous studies. Plant height, SLA, LDMC, leaf carbon concentration, leaf nitrogen concentration and leaf phosphorus concentration were measured following standard protocols (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013). These traits were measured on at least 10 mature individuals (between 40 and 165 leaves) per species. Photosynthetic traits were measured *in situ* on two leaves per individual and five individuals per species, using the LI-6400 portable photosynthesis system (LI-COR Inc.). The measurements were taken from 10 am to 2 pm in July. A_{max} , PWUE and PNUE were calculated following Zhou et al. (2021). For all traits, species mean values were used in the analysis.

Predictor variables of invasion

We considered multiple variables belong to three categories: (1) resident richness and biomass, (2) resident community phylogenetic and functional structure and

Abbreviation	Description
Predictor variables	
Planted richness	The number of planted resident species in the subplot
Realised richness	The realised number of resident species in the subplot
Resident biomass	The total biomass of all resident species in the subplot for each year
α MPD_phy	Mean pairwise phylogenetic distance among resident species
α MNND_phy	Mean nearest phylogenetic distance among resident species
α MPD_traits	Mean pairwise functional distance among resident species based on the eight traits
α MNND_traits	Mean nearest functional distance among resident species based on the eight traits
Fd _{is} -all	Functional dispersion of resident community based on the eight traits
CWM_PC1	Community-weighted mean values of the first PCA axis generated by the eight traits
CWM_PC2	Community-weighted mean values of the second PCA axis generated by the eight traits
β MPD_phy	Mean phylogenetic distance between the invader and all resident species in a recipient community
β MNND_phy	Phylogenetic distance between the invader and its nearest relative resident species in the recipient community
β MPD_traits	Mean functional distance between the invader and all resident species based on the eight traits
β MNND_traits	Functional distance between the invader and its nearest relative resident species based on the eight traits
Functional traits	
Height	Mean plant height
SLA	Specific leaf area
LDMC	Leaf dry matter content
C:N ratio	Leaf carbon to nitrogen ratio
C:P ratio	Leaf carbon to phosphorus ratio
A _{max}	Light-saturated photosynthetic rate
PWUE	Photosynthetic water use efficiency
PNUE	Photosynthetic nitrogen use efficiency

TABLE 1 The variables and functional traits used in the analysis

(3) phylogenetic and functional dissimilarity between the invader and residents (Table 1). For resident richness, we considered the planted resident richness, as well as the realised resident richness of each subplot. Resident community biomass was obtained by summing the biomass of all resident species in each subplot.

For resident community phylogenetic structure, we calculated the mean pairwise phylogenetic distance (α MPD_phy) and mean nearest phylogenetic distance (α MNND_phy) among resident species from the Bayesian phylogeny, based on the realised species composition of each subplot in each year (Tatsumi et al., 2019). For resident community functional structure, we constructed a Gower distance matrix of all species using the eight plant traits, and calculated mean pairwise functional distance (α MPD_traits) and mean nearest functional distance (α MNND_traits) among resident species. We also calculated the functional diversity of resident communities as functional dispersion (FD_{is}), which is the mean-weighted

distance of each species to the community-weighted centroid in the multivariate trait space (Laliberté & Legendre, 2010). These indices provide multiple ways to quantify potential niche complementary among residents, as represented by measured phylogenetic and functional dissimilarity metrics. To reduce the dimension of community-level traits, we performed a principal component analysis (PCA) on the eight traits, and used the first and second principal components (PC1 & PC2), which accounted for 61.2% and 15.5% of the total variation in the data respectively (Table S1; Figure S3), in the ensuing analyses involving multiple traits. We then calculated the community-weighted mean trait values of PC1 and PC2 (CWM_PC1 & CWM_PC2), weighted by the abundance of each resident species in the community. Besides considering multiple traits together, we also quantified the effect of individual traits on ragweed invasion. To do so, we constructed Gower distance matrices of species based on each individual trait, and

calculated the values of FD_{is} and CWM for each trait (Luo et al., 2019).

The phylogenetic dissimilarity between the invader and the resident communities was calculated as the mean phylogenetic distance between the invader and all residents (βMPD_{phy}), as well as the phylogenetic distance between the invader and its nearest relative resident ($\beta MNND_{phy}$) in each subplot (Li et al., 2015). An identical framework was used to calculate the mean functional distance (βMPD_{traits}) and mean nearest functional distance ($\beta MNND_{traits}$) between the invader and resident communities, using the Gower distance matrix generated by multivariate combinations of traits. The phylogenetic and functional dissimilarity indices, as well as the indices mentioned in the above paragraph, were calculated using the *Picante* (Kembel et al., 2010) and *FD* (Laliberté et al., 2014) packages in R 4.0.2 (R Core Team, 2020). All predictive variables were standardised to have a mean of zero and a standard deviation of one to account for the different scales among them.

Statistical analyses

To explore the effect of planted species richness and N addition on the performance of the resident species and the invader, we used linear mixed-effects models to fit realised resident richness, resident biomass and invader biomass as a function of planted species richness, N addition and their interaction, with the sampling year treated as a random factor. The same linear mixed-effects models were used to assess the effects of planted species richness, N addition and their interaction on the phylogenetic and functional variables. To assess the relative importance of predictive variables (i.e. variables in Table 1) on invader biomass under different N addition treatments, we fitted a series of linear mixed-effects models with each predictive variable as a single fixed factor, and the sampling year as a random factor. The standardised coefficient was calculated and compared for each variable. We then constructed a multivariate linear mixed-effects model with all predictive variables as potential fixed factors, and generate a full set of models using the dredge function in the *MuMIn* package in R (Bartoń, 2021). These models were ranked using the Akaike information criterion corrected for small sample size (AICc); the model with the lowest AICc value was selected as the best model.

We further performed piecewise structural equation modelling (SEM) to disentangle the important pathways through which planted richness and N addition influence invader biomass. SEMs were fit using linear mixed-effects models where sampling year was treated as a random factor. We first formulated an *a priori* model that included all possible pathways (Figure S4), and then simplified the initial model by eliminating non-significant pathways until we attained the final model.

Model adequacy was assessed using the χ^2 test and AIC in the *piecewiseSEM* package (Lefcheck, 2016) in R 4.0.2.

Meta-analysis

To further examine the diversity–invasibility relationship under N addition across multiple studies, we conducted a meta-analysis of existing studies on this topic, following the PRISMA guidelines (Page et al., 2021). Briefly, we first performed a systematic search of the relevant papers using the ISI Web of Science database (Figure S1), and manually searched for additional references included in several major reviews (e.g. Liu et al., 2017; Peng et al., 2019; Smith & Côté, 2019; Tomasetto et al., 2019). We then selected studies to be included in our meta-analysis based on the following criteria: (1) studies must have directly manipulated or quantified the species diversity of resident communities; (2) studies must have directly manipulated N addition, or there was a significant N gradient among the studied sites (e.g. low versus high fertile sites), making it possible to compare the effect of diversity on invader performance under different N treatments; (3) at least one aspect of invader performance should have been measured as a metric of community invasibility, such as the emergence, density, cover or biomass of the invaders. To minimise issues arising from non-independence, we only used the metrics representing invader overall performance (e.g. biomass) if multiple performance metrics were reported (e.g. reproductive potential, emergence, density, cover and biomass) for the same study. We then obtained Pearson's correlation coefficients between resident species richness and invader performance, and used Fisher's z-transformed correlation coefficient as the effect size. To control for non-independence of data from the same study, we used mixed-effects models to assess the overall relationships between resident species richness and invader performance under both control and nitrogen addition treatments. We then assessed the significance of mean effect sizes using bias-corrected 95% bootstrap confidence intervals based on 9,999 iterations. Potential publication bias was checked using the regression test for funnel plot asymmetry (Egger et al., 1997) and Rosenthal's Fail-Safe N test (Rosenthal, 1979). We conducted the meta-analyses using the *metafor* package (Viechtbauer, 2010) in R 4.0.2.

RESULTS

Consistently negative diversity–invasibility relationships

As expected, N addition reduced realised resident species richness and increased both resident and invader biomass (Table S2). Nevertheless, planted species

richness had a consistently negative effect on invader biomass, irrespective of N addition, across the 4 years of the experiment (Figure 1). The effect size of planted species richness on invader biomass did not vary among different N addition treatments (Figure 2), indicating that N addition did not alter the diversity–invasibility relationships.

Our meta-analysis showed that there was a limited number of studies that directly examined the diversity–invasibility relationship under both ambient and N addition conditions, and these studies showed somewhat mixed results (Figure 3). No significant publication bias was detected in our meta-analysis (Egger's regression test, $t = -0.17$, $p = 0.86$; Fail-Safe number = 3559). However, when all studies were considered together, the overall mean effect size of

resident diversity on invader performance was significantly negative in both control and N addition treatments (Figure 3).

The effects of predictor variables on invader biomass

Invader biomass showed consistently negative relationships with planted richness, realised richness and total biomass of resident communities (Figure 2). Invader biomass also showed consistently negative relationships with the functional dissimilarity of the residents. More specifically, invader biomass was negatively related to FD_{is} and αMPD of multiple traits (αMPD_{traits} and Fd_{is-all}), as well as FD_{is} of most individual

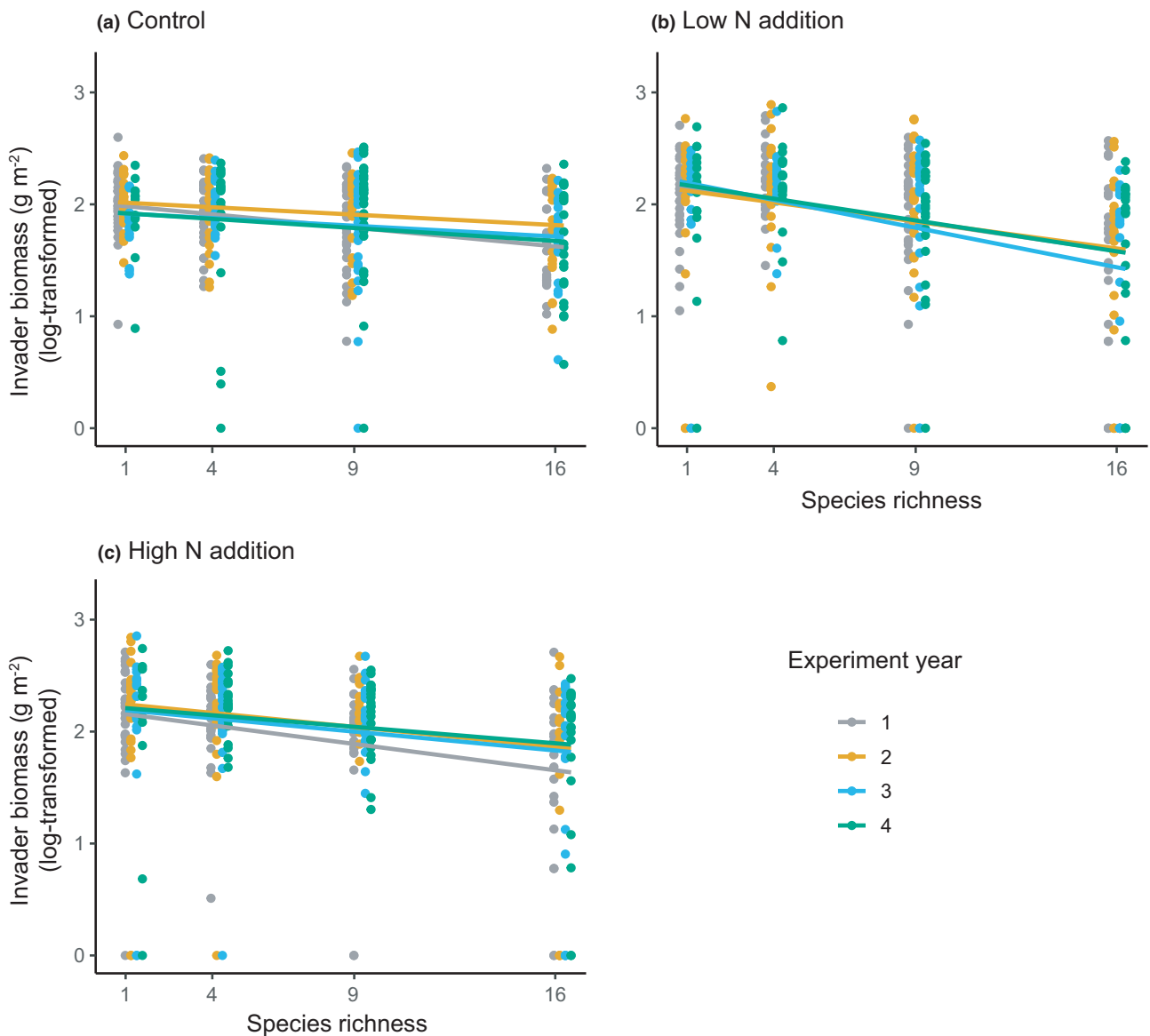


FIGURE 1 Invader biomass consistently decreased with increasing planted species richness under different nitrogen (N) addition treatments. Lines represent linear regression fits for each year. All regression lines were significant at the $p < 0.05$ level

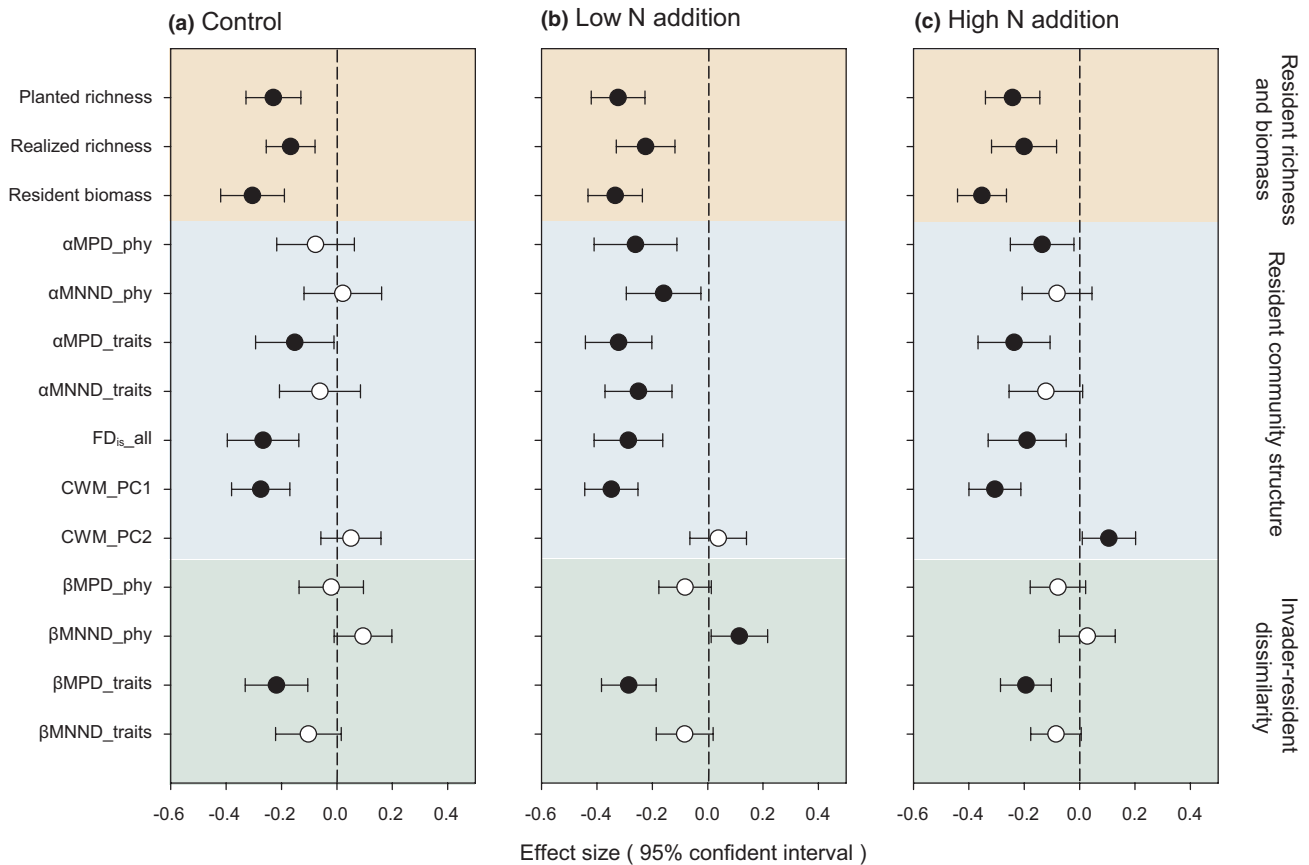


FIGURE 2 Relative influences of resident richness and biomass, resident community structures and invader-resident dissimilarity on invader biomass under different N addition treatments. Effect sizes are standardised coefficients from linear mixed-effects models estimated separately for each predictor variable. Solid circles indicate significant effects and open circles indicate non-significant effects. The descriptions of the indexes are shown in Table 1

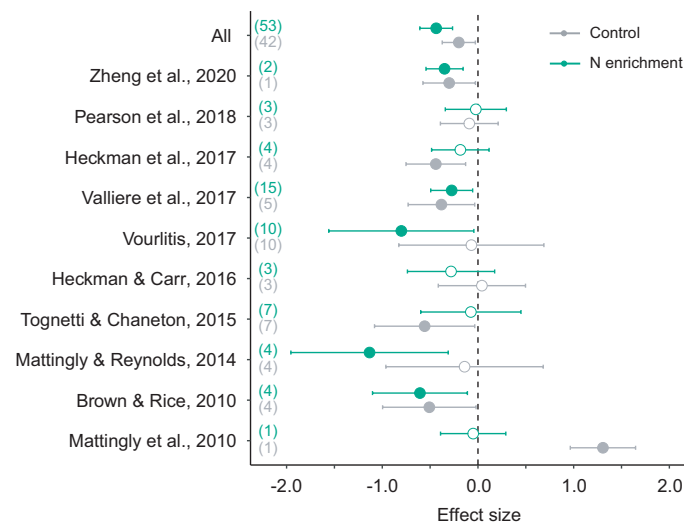


FIGURE 3 A meta-analysis shows the effects of resident richness on the invader performance. Data represent mean effect sizes with bias-corrected 95% bootstrap confidence intervals, and solid and open circles indicate values significantly and non-significantly different from zero respectively. Values in parentheses represent the sample sizes

traits (including C:N ratio, C:P ratio, A_{\max} , PWUE, PNUE), across N addition treatments (Figure 2 & Figure S5). Phylogenetic dissimilarity of the residents,

measured as α MPD_phy or α MNND_phy, was a generally weaker predictor of invader biomass than functional dissimilarity (Figure 2).

Invader biomass was also strongly influenced by community-weighted mean trait values. In particular, invader biomass was significantly negatively related to CWM_PC1 (Figure 2). Large PC1 scores corresponded to large values of LDMC, C:N ratio, C:P ratio, A_{max} , PWUE, PNUE, but low value of SLA (Table S1; Figure S3a), indicating a conservative resource-use strategy. Resident species with higher PC1 scores, such as *Pennisetum alopecuroides* and *Paspalum notatum*, showed stronger resistance on invasion in monocultures and became more dominant in polycultures (Figure S3), indicating that the conservative resource-use strategy favoured dominance and invasion resistance of residents. Similar to the results based on multiple traits, we also found that invader biomass was negatively related to CWM of LDMC, C:N ratio, C:P ratio, A_{max} , PWUE, PNUE, but positively related to CWM of SLA of resident communities (Figure S5). Community-weighted mean of PC2 scores, which was largely associated with plant height (Table S1; Figure S3), was a weak predictor of invader biomass (Figure 2).

Compared to the strong influence of functional dispersion and community-weighted mean trait values of the residents on invader biomass, the influence of invader-resident phylogenetic and functional dissimilarity was generally weaker. Only β MPPD_traits had a negative effect on invader biomass across the three N levels, while the effects of β MPPD_phy, β MNND_phy and β MNND_traits were not significant in most N treatments (Figure 2).

Multiple regression and structural equation modelling results

Similar to univariate results, planted richness, N addition, resident biomass, Fd_{is_all} and CWM_PC1 were always retained as significant predictors of invasion biomass in the top 10 multivariate models (Table S3). The best multivariate model showed that invader biomass was positively associated with N addition and β MPPD_phy, but negatively associated with planted richness, resident biomass, Fd_{is_all} and CWM_PC1 (Table 2). Except for β MPPD_phy, these variables were also retained in the final SEM model in predicting invader biomass (Figure 4). The SEM model revealed that N addition had opposite effects on invader biomass. On the one hand, N addition increased invader biomass directly as well as via decreasing the Fd_{is_all} of the resident communities. On the other hand, N addition also increased the CWM_PC1 and the total biomass of the residents, which in turn, decreased invader biomass. In contrast, planted richness always had negative effects on invader biomass. Plant richness decreased invader biomass directly, as well as via its positive effects on Fd_{is_all} , CWM_PC1 and the total biomass of the resident communities (Figure 4). Together, the increased functional dispersion, increased

TABLE 2 The best multivariate linear mixed-effects model for predicting invader biomass, selected using Akaike Information Criteria corrected for small sample size (AIC_c). Sampling year was included as a random factor

Fixed effects	Standardised coefficient	Standard error	t-value	p-value
Planted richness	-0.16	0.04	-4.02	<0.0001
N addition	0.16	0.04	4.36	<0.0001
Resident biomass	-0.22	0.04	-5.17	<0.0001
Fd_{is_all}	-0.17	0.04	-4.45	<0.0001
CWM_PC1	-0.19	0.04	-4.44	<0.0001
β MPPD_phy	0.15	0.04	3.79	0.0002

community-weighted mean of resource-conservative traits, and increased biomass of resident species in more diverse communities contributed most importantly to invasion resistance.

DISCUSSION

Elton's prediction that species-rich communities could better resist invasion has been well supported by experimental studies (Kennedy et al., 2002; Smith & Côté, 2019). However, the robustness of these results in changing environments has been questioned (Heckman et al., 2017; Mallon et al., 2015). It has been suggested that resource addition could reduce the importance of niche pre-emption in diverse communities, and therefore weaken the negative diversity–invasibility relationship (Mallon et al., 2015). Contrary to this prediction, our 4-year grassland experiment, coupled with the meta-analysis, showed that resident diversity significantly decreased invader biomass under both ambient and N addition conditions (Figures 1, 3), indicating that diversity could serve as a consistent barrier to plant invasion regardless of resource enrichment. More importantly, our experiment showed that plant diversity resisted invasion mainly through increases in the biomass, trait dissimilarity, as well as community-weighted means of resource-conservative traits, of the resident communities, underscoring the importance of functional traits in determining invasion resistance under changing environments.

Complementarity and sampling effects have been recognised as two important mechanisms in explaining the negative diversity–invasibility relationships (Fargione & Tilman, 2005; Wardle, 2001). However, it remains difficult to separate their effects in practice. Recent studies suggest that the consideration of functional traits could help disentangle their roles (Cadotte, 2017; Luo et al., 2019; Tobner et al., 2016). In accordance with these studies, our study revealed that both trait dissimilarity and community-weighted mean trait values of resident

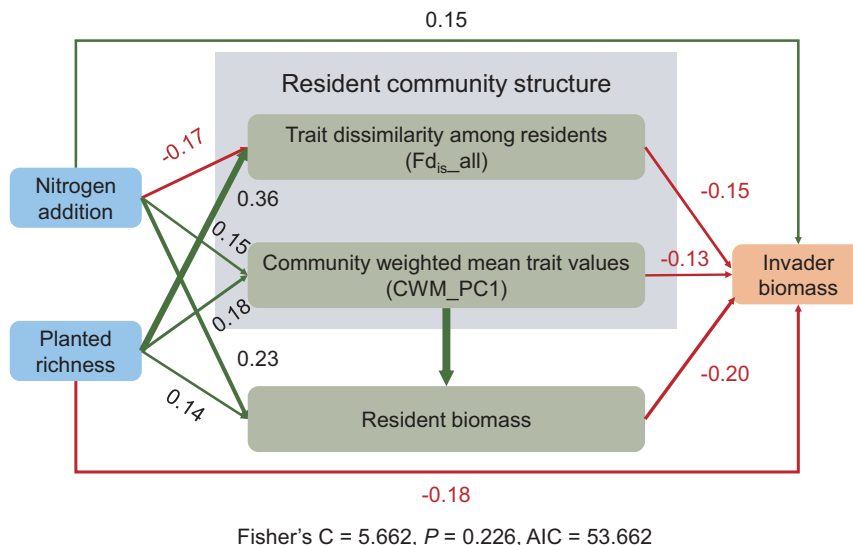


FIGURE 4 Structural equation model showing the direct and indirect effects of planted richness and N addition on invader biomass. Only the significant pathways are included in this final model. Green and red arrows reflect positive and negative pathways respectively. Numbers along the arrows, as well as the width of the arrows, indicate standardised path coefficients

species contributed to the resistance of diversity to invasion. There is increasing evidence that functional dispersion, which measures the trait dissimilarity among species, could capture the niche difference and complementarity among species (Cadotte, 2017; Kraft et al., 2015). Therefore, greater functional dispersion would reflect an increase in niche complementarity among residents, which leads to fewer unoccupied niches available for invaders. In support of this interpretation, we found that functional dispersion of multiple traits, as well as functional dispersion of most individual traits, significantly increased in more diverse communities (Table S4). The increased functional dispersion in turn strongly suppressed invader performance (Figure 2 and Figure S6a). These results support trait complementarity as an important mechanism for the resistance of biodiversity to invasion (Eisenhauer et al., 2013). Conversely, community-weighted mean trait values provide insight into the effect of dominant species traits on invasion resistance, and are therefore closely linked to the sampling effect. In our study, we found residents with higher LDMC, C:N ratio, C:P ratio, A_{max} , PWUE and PNUE, but lower SLA, which were represented by high PC1 values, showed stronger resistance to invasion (Figure S3). These traits have been shown to represent conservative resource-use strategy associated with long lifespan and slow growth (i.e. long-lived, high-density leaves with low nutrient content; Tecco et al., 2010; Wright et al., 2004). Therefore, our results provided strong evidence that residents with more conservative traits could better resist invasion. Moreover, we also found that community weight means of PC1 significantly increased in more diverse communities, indicating the increased dominance of resource-conservative species, which resulted in increased resident biomass and decreased invader

performance in more diverse communities (Figure 4 and Figure S6b). Together, the strong resistance of residents with resource-conservative traits, as well as the increased dominance of these species in more diverse communities, jointly provide strong evidence for the importance of the sampling effect.

Since both complementarity and sampling effects contribute to negative diversity–invasibility relationships, an important question is whether these two effects would persist under N enrichment. N enrichment is known to often decrease resident diversity (Midolo et al., 2019) and increase community invasibility (Seabloom et al., 2015). It has thus been suggested that N addition could reduce the niche complementarity among residents in diverse communities, and, therefore, alleviate the negative effect of diversity on invasion (Mallon et al., 2015). In our study, although N addition reduced the realised richness of resident communities and increased the biomass of the invader, the negative relationship between diversity and invasibility persisted (Figure 1 and Table S2). Our results suggest two possible explanations. First, although resident richness and functional dispersion decreased under N addition (Table S2, S4), functional dispersion still provided invasion resistance and contributed to the negative diversity–invasibility relationships (Figures 2, 4). Second, N addition promoted the dominance of residents with resource-conservative traits, which played an important role in invasion resistance (Figures 2, 4), in diverse communities (Figure 4 and Table S4). Together, we found consistent negative effects of functional dispersion and conservative trait means on invasion under N addition (Figure S6), indicating that biodiversity could resist invasion through both complementarity and sampling effects irrespective of N

addition. As time proceeded, the long-term N deposition would continuously drive down native diversity and feed back to decrease overall invasion resistance. If the results from small-scale studies like ours are applicable, we would expect the maintenance of biodiversity, especially the functional diversity and residents with conservative traits, would play crucial roles in community resistance to invasion. The generality of our results, however, would need to be assessed by long-term experimentation in more realistic systems.

In contrast to the strong effects of trait dissimilarity and community trait means of residents on invasion, we found that the effects of invader-resident phylogenetic and functional dissimilarity were generally weak or nonsignificant (Figure 2). This result is at odds with Darwin's naturalisation hypothesis, which proposes that residents that are phylogenetically and functionally more similar to the invaders could better impede invasion (Cadotte et al., 2018; Darwin, 1859). One possible explanation for this discrepancy is that phylogenetic and functional dissimilarity between residents and the invader could have various influences on invader performance (Divíšek et al., 2018; Malecore et al., 2019). Our previous analysis of the first-year data from the same experimental grassland revealed that the invader *A. artemisiifolia* established better in its closely related resident communities but grew better in its distantly related resident communities (Li et al., 2015). These two mechanisms cancelled each other out, making the overall effect of invader-resident phylogenetic distance on invader biomass nonsignificant. Here, the results of our subsequent 4-year experiment were in accordance with this prior finding, highlighting that the residents more closely related or more functionally similar to the invader were not more effective in reducing invader biomass. More generally, phylogenetic and functional dissimilarity between residents and invaders have been found to promote, inhibit or have no effects on invasion outcome (Bennett, 2019; Cadotte et al., 2018; Ma et al., 2016). Therefore, rather than simply focusing on the invader-resident phylogenetic and functional dissimilarity, our results emphasised that the comprehensive consideration of functional structures and biomass of resident communities could improve our predictive ability on invasion outcome under changing environments.

Several limitations of our study are worth noting. First, while experimental studies like ours often support negative diversity–invasibility relationships, observational studies have reported positive or more complex relationships (i.e. the invasion paradox, Fridley et al., 2007; Peng et al., 2019; Smith & Côté, 2019). While reconciling this paradox is beyond the scope of our study, an important next step is to quantify the influences of phylogenetic relatedness and functional traits on invasion in natural communities across multiple spatial scales. Second, our grassland

experiment, as well as the studies we could find for our meta-analysis, are all relatively short-term in nature and considered a limited number of invader species, environmental change factors and propagule pressure levels. An important question to ask next is whether our findings can be generalised to other invaders and resident communities experiencing long-term environmental change. Third, our study considered eight traits associated with species' competitive ability and resource use strategies (Table 1). Unfortunately, other important traits, such as root traits and traits related to allelopathic compound production, were not collected in our study. Logistical constraints also prevented us from considering the role of intraspecific trait variation. It will be of interest to consider additional traits as well as trait plasticity in future analyses. Finally, our study was designed to understand through which phylogenetic and functional attributes that resident richness could resist invasion under N addition, and therefore resident richness was directly manipulated. It is possible that other unmeasured variables, including light, water, soil nutrients and other diversity dimensions, would correlated with the functional attributes we considered and influence invasion resistance. Future experiments should consider directly assembling communities that vary in functional dispersion and community trait means, as well as invader-native dissimilarity, to fully resolve their relative importance on community invasibility.

By explicitly considering both resident community structure and invader-resident dissimilarity, our study provided a comprehensive framework to assess the contributions of different aspects of phylogenetic and functional attributes to invasion resistance. By applying this framework to the invasion of *A. artemisiifolia* under N addition, our study provides empirical evidence that biodiversity could resist invasion through biomass accumulation, functional dispersion and community trait means of residents, demonstrating the importance of functional traits in regulating invasion resistance. We hope further studies would aim at scaling-up our framework to other invaders in more complex communities and ecosystems, which may help gain a more mechanistic understanding of the diversity–invasibility relationships in a changing environment.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHORSHIP

SPL, WSS, JTL and LJ developed and framed research questions. SPL, PJ, YW and JTL collected the data used in this analysis. SPL, SYF, YW and XL analysed the data. YM and YL performed the meta-analysis. SPL wrote the first draft of the manuscript and all authors contributed to discussing the results and editing the manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data and code that support the findings of this study can be accessed via the Dryad Digital Repository (<https://doi.org/10.5061/dryad.ghx3ffbqk>).

ORCID

Shao-peng Li  <https://orcid.org/0000-0002-1730-3433>

Xiang Liu  <https://orcid.org/0000-0003-3914-5824>

Lin Jiang  <https://orcid.org/0000-0002-7114-0794>

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