

Soil Microbes Alter the Diversity-Invasibility Relationship by Promoting a Complementarity Effect on Community Invasion Resistance

Xiao-Yan Wang (✉ wxy3470117@163.com)

Taizhou University <https://orcid.org/0000-0002-2771-7680>

Song Gao

Taizhou University

Tong Chen

Taizhou University

Jiang Wang

Taizhou University

Fei-Hai Yu

Taizhou University

Research Article

Keywords: Biomass, Diversity effect, Over-invasion resistance index, Phylogenetic distance, Species richness

Posted Date: December 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1136323/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background Soil microbes can affect both the invasiveness of exotic plants and the invasibility of native plant communities, but it still remains unclear whether soil microbes can influence the relationship between native plant diversity and community invasibility.

Methods We constructed native plant communities with three levels of species richness (one, three, or six species) in unsterilized or sterilized soil (i.e., with or without soil microbes) and either prevented their invasion by exotic plants or allowed them to be invaded by each of three exotic species (*Solidago canadensis*, *Erigeron canadensis* or *Symphytotrichum subulatum*), which are highly invasive in China. The soils conditioned by the native plant communities that were not invaded by the exotic species were used as soil microbe inocula to test whether species richness-induced differences in soil microbes affected the growth of each of the three invasive species.

Results Compared with soils containing microbes, the absence of soil microbes weakened the negative species richness-invasibility relationship, indicating that soil microbes can contribute to higher invasion resistance in more diverse native plant communities. In the presence of soil microbes, the higher invasion resistance of more diverse communities was mainly ascribed to the complementarity effect. However, soil microbes from communities with a higher species richness did not have a stronger negative effect on the growth of any of the three invasive species.

Conclusion Soil microbes can alter the diversity-invasibility relationship by promoting the complementarity effect on community invasion resistance. Our results highlight the importance of integrating the role of soil microbes when testing the diversity-invasibility hypothesis.

Introduction

The ability of native plant communities to resist alien plant invasions has received a great deal of attention (Dawson and Schrama 2016; Hess et al. 2020; Urza et al. 2019) due to the broad spread of alien species and their threats to native ecosystems (Cazetta and Zenni 2020; Pearson et al. 2018; Pyšek et al. 2020). Elton (1958) proposed that species-richer communities are more resistant to alien plant invasions. Although many experimental studies have supported this hypothesis (Adomako et al. 2019; Hector et al. 2001; Howeth 2017; Martignoni et al. 2020; Naeem et al. 2000; Selmants et al. 2012), species richness has also been found to have either no or a negative impact on the invasion resistance of native plant communities (Crawley et al. 1999; Lannes et al. 2020; Smith et al. 2004; Zeiter and Stampfli 2012). Soil microbes can affect both the invasiveness of alien species and the invasibility of native plant communities (Beckstead and Parker 2003; Dawson and Schrama 2016; Klironomos 2002; van der Putten et al. 2007). However, it remains unclear how soil microbes affect the plant diversity-invasibility relationship and what the underlying mechanisms may be.

Exotic plants can suppress native plants via their impacts on soil microbes (Inderjit et al. 2021; Lankau 2011; Vogelsang and Bever 2009). For instance, exotic plants can enhance native plant pathogen levels

(Beckstead et al. 2010; Lankau 2011; Mangla et al. 2008), inhibit soil microbes that have a mutualistic relationship with native plants (e.g., some arbuscular mycorrhizal fungi; Lankau 2011; Stinson et al. 2006; Vogelsang and Bever 2009) or produce allelochemicals that are toxic to these beneficial soil microbes (Inderjit and van der Putten 2010; Inderjit et al. 2021; Lankau 2010). Increasing species richness in native plant communities can provide more diverse food resources (root exudates and litter) and increase the available habitats for soil microbes (Hooper et al. 2000; Millard and Singh 2010; Prober et al. 2015; Wardle 2006), thus increasing their biomass, activities and diversity (Hiiesalu et al. 2014; Liu et al. 2020; Mitchell et al. 2010; Prober et al. 2015; Wang et al. 2017). Consequently, native plant communities that are more species-rich may be more likely to harbor soil microbes that can help the native plants to resist invasion-mediated pathogen infection (Klironomos 2002; Kulmatiski et al. 2008; Mitchell 2003; Schnitzer et al. 2011), are beneficial to native plants (Simard and Durall 2004; Sikes et al. 2009; Schnitzer et al. 2011) or can degrade the harmful allelochemicals produced by alien plants (Blum 1998; Ehlers 2011; Li et al. 2015). Therefore, increasing the species richness of native plant communities may suppress exotic plant invasions more strongly via the increased diversity and/or activity of soil microbial communities. If the activity of the beneficial soil microbial communities is suppressed and their diversity and abundance are reduced, the negative relationship between species diversity and community invasibility will be weakened.

In invaded communities, alien species may be strongly negatively affected by some of the native soil pathogens because they have not co-evolved with those pathogens (Fries 2016; Zhang et al. 2020). As an increase in native plant species richness may increase the diversity and abundance of native soil pathogens (Hudson et al. 2006; Keesing et al. 2006), more species-rich native plant communities will likely contain native soil pathogens that negatively affect the growth of exotic plants, thus increasing their resistance to exotic plant invasions. If the activity of soil pathogens is highly suppressed and their diversity and abundance are strongly reduced, the negative relationship between species diversity and community invasibility will again be weakened.

To examine how soil microbes influence the relationship between species diversity and community invasibility, we first constructed experimental native plant communities containing different levels of species richness in sterilized vs. unsterilized soils and allowed them to be invaded by each of three alien species (*Solidago canadensis*, *Erigeron canadensis* and *Symphytotrichum subulatum*), which are highly invasive in China (Guo and Fang 2003; He et al. 2019; Qiang 2001). Then, the soils conditioned by the native plant communities were used as soil inocula to investigate how the soil microbes from native plant communities with different levels of species richness directly affected the growth of the three invasive plants. Specifically, we tested the following hypotheses: (1) the negative relationship between native species richness and community invasibility, indicated by invader biomass, is stronger in the presence vs. absence of soil microbes; (2) soil microbes in more diverse plant communities lead to a higher level of community invasion resistance, as measured by the biomass ratio of the native communities invaded or not invaded by the alien plant; (3) soil microbes from communities with a higher species richness have a stronger negative effect on the growth of the invasive species.

Materials And Methods

Native and invasive species

The plant species pool consisted of eight native species and three invasive species that commonly grow on grasslands around Taizhou city, Zhejiang Province, China (Table S1). Six of the native species were perennials and two were annuals. The three invasive species (*Solidago canadensis*, *Erigeron canadensis* and *Symphotrichum subulatum*) were all Composite, as most invasive species in China are from this family (Ma 2013). *Solidago canadensis* is a perennial and can reproduce both sexually and clonally by producing rhizomes; *E. canadensis* and *S. subulatum* are non-clonal annuals. All three invasive species originated from South America and can produce plenty of viable seeds every year (Ma 2013).

Seeds from the native and invasive species were collected from field sites around Taizhou city in 2013 (Table S1). On March 18–19, 2014, seeds from each species were sown into three plastic containers (52 cm long × 35 cm wide × 15 cm high) filled with peat (Kuheng Co., Shanghai). The plastic containers were placed in a growth chamber at 25°C and a relative humidity of 70% with 16 h of daylight at 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The seedlings were used to construct native plant communities that were to be invaded or not invaded by each of the three invasive species.

Experimental design

To test the hypotheses, we carried out two experiments. For the first experiment, we constructed native plant communities with three levels of species richness (1, 3 and 6 species) in pots (each 27.5 cm in diameter × 31 cm in height) filled with either sterilized soil (without soil microbes) or nonsterilized soil (with soil microbes) and allowed them to be invaded by each of the three alien invasive species (*S. canadensis*, *E. canadensis* and *S. subulatum*) or prevented invasion by these three species. For the treatments with soil microbes, 1.65 L soil was added to the middle layer of 14.85 L of a sterilized soil mixture, and for the treatment without soil microbes, 16.5 L sterilized soil only was added. The sterilized soil mixture consisted of a mixture of soil, peat, sand and vermiculite at a volume ratio of 2:1:1:1, containing $1.60 \pm 0.32 \text{ g kg}^{-1}$ total N, $0.58 \pm 0.18 \text{ g kg}^{-1}$ total P and $13.2 \pm 0.5 \text{ g kg}^{-1}$ organic matter ($\text{mean} \pm \text{SE}$, $n = 5$). The soil was collected from a mountainous area near Taizhou city and sieved through a 1-cm mesh to remove larger roots and stones. The peat, sand and vermiculite were bought from Kuheng Co. (Shanghai).

We constructed three replicates of monocultures for each of the eight native species, five three-species mixtures with different species compositions and five six-species mixtures with different species compositions (Table S2). The species in each of the three- and six-species mixtures were randomly chosen from the native species pool. For the treatment without the invasive species, each pot contained six seedlings of the same native species (for monocultures), including two seedlings of each of the three species (for three-species mixtures) or one seedling of each of the six species (for six-species mixtures). The six seedlings in each pot were arranged in a circular pattern. For the treatment with the invasive species, one seedling of one of the three invasive species was grown in the center of a pot, surrounded by

six seedlings of native species (Fig. 1). The experiment thus consisted of 34 species compositions · 2 soil microbe treatments · 4 invasion treatments, resulting in a total of 272 pots.

The experiment was started on May 26, 2014. The pots were placed randomly in a greenhouse (25°C during the daytime and 18°C at night) at Taizhou University in Taizhou city, Zhejiang Province, China. The aboveground parts of each plant species in each pot were harvested on August 25–28, 2014. All plant material was oven-dried at 70°C for 48 h and weighed. The soils in each of the 34 pots without the invasive species were also harvested, stored at -80°C and used as inocula for the second experiment described below.

On May 25, 2015, 14.85 L of the same sterilized soil mixture as used in the first experiment was inoculated with 1.65 L of the soil from each of the 34 pots previously grown with the native species communities but without the invasive species. Three pots (27.5 cm in diameter × 31 cm in height) containing each of these 34 soil mixtures were prepared, and each pot contained one seedling of *S. canadensis*, *S. subulatum* or *E. canadensis*. There were 102 pots in total. The plant from each pot was harvested on September 16–17, 2015, and the biomass was determined by drying the plant in an oven at 70°C for 48 h and weighing it.

Data analysis

The invasion resistance of a plant community was calculated as the ratio of the aboveground biomass of the community invaded by an alien species to that of the community comprising the same initial species composition but without the invasive species (Pfisterer and Schmid 2002; Tilman 1996; Wang et al. 2007). Complementarity and selection effects were calculated using the invasion resistance data and the additive partitioning method described by Loreau and Hector (2001). The complementarity effect of a

mixture was calculated as $\frac{\overline{\Delta RY}}{N \overline{M}}$, where N is the number of species in the mixture, $\overline{\Delta RY}$ is the mean value of the change in the relative invasion resistance across all species in the mixture and \overline{M} is the mean value of the invasion resistance of the monocultures across all species. The selection effect was calculated as $N \text{cov}(\Delta RY, M)$, where N is the number of species, $\text{cov}(\Delta RY, M)$ is the covariance between the invasion resistance of species in monocultures (M) and their change in the relative invasion resistance in the mixture (ΔRY).

If complementarity between species plays a major role in the invasion resistance, then the invasion resistance of mixtures will be higher than that of the most resistant species in the mixtures. The over-invasion resistance index (OI) was calculated as follows (Hector et al. 2002): $OI = Y / \text{MAX}(M_i)$, where Y is the invasion resistance of a mixture, and M_i is the invasion resistance of species i in the monoculture. If $\log(OI) > 0$, the mixture should have a higher invasion resistance than that of the highest resistant species.

As the damage caused by soil pathogens to invasive species may be influenced by the phylogenetic distance between native and invasive species (Strauss et al. 2006, Zheng et al. 2018), we calculated the phylogenetic distances between the eight native and three invasive species using three commonly sequenced genes from the GenBank: rbcL, matK and ITS (Table S3). Of the 11 species, eight had three genes represented in GenBank. For three native species, *C. arvensis* var. *integrifolium*, *I. japonica* and *P. asiatica* whose sequence data of the three genes were not available or incomplete in GenBank, we used the sequence data from their congeneric relatives (i.e., *C. arvensis*, *I. britannica* and *P. depressa*) as proxies. Sequences were aligned for each region independently using MUSCLE (Edgar 2004) and combined into a single supermatrix. Analyses were conducted using the Maximum Composite Likelihood model (Tamura et al. 2004). The rate variation among sites was modeled using a gamma distribution (shape parameter = 1). All ambiguous positions were removed for each sequence pair (pairwise deletion option). Codon positions included were 1st+2nd+3rd+Noncoding. Evolutionary analyses were conducted in MEGA-X version 10.1.8 (Kumar et al. 2018; Nei and Kumar 2000). The weighted phylogenetic distance was calculated as follows:

$$\text{Weighted phylogenetic distance} = \sum_{i=1}^n R_i D_i$$

where R_i is biomass ratio of native species i in the pot, and D_i is phylogenetic distance between native species i and the invasive species.

Linear regressions were performed to test the relationships between native species richness and the biomass of the native species, the biomass of the invasive species and invasion resistance, and the diversity effects (i.e., complementarity effect and selection effect) and the invasion resistance. The regression slope difference between the sterile and non-sterile treatments was tested using ANCOVA. The differences between the sterile and non-sterile treatments at the three- and six-species levels were analyzed using the t -test. The effects of soil microbes and plant species richness on the biomass of native and invasive species were also analyzed by two-way ANOVA. Linear regressions were also performed to evaluate the soil legacy effect of native species richness on the biomass of the invasive species (for the second experiment). The relationships between the weighted phylogenetic distances and the biomass of invasive species were analyzed by linear regression. All analyses were carried out using SPSS 19.0 for Windows (IBM, Armonk, NY, USA).

Results

Effects of soil microbes on the species richness-invasibility relationship

Consistent with our first hypothesis, the presence of soil microbes changed the relationship between native species richness and the biomass of two of the three invasive species (Fig. 2a, b), as indicated by the significant difference in the regression slopes (Fig. 2a, ANCOVA for *S. canadensis*: $F = 4.30$, $P = 0.042$; Fig. 2b, ANCOVA for *E. canadensis*: $F = 6.01$, $P = 0.017$). For *S. subulatum*, although the presence of soil microbes had no significant effect on the regression slopes of the species richness-invasibility relationships (Fig. 2c, ANCOVA: $F = 1.34$, $P = 0.252$), the biomass of this species in the unsterilized soil was significantly lower than that in the sterilized soil in the mixtures containing three native species ($t = 4.38$, $n = 10$, $P = 0.002$) and six native species ($t = 6.91$, $n = 10$, $P < 0.001$). Two-way ANOVA showed that soil microbes and species richness had significant effects on the biomass of all three invasive species, and they also interacted to significantly affect the biomass of two invasive species (*S. canadensis* and *E. canadensis*, Table 1).

Table 1
Two-way ANOVA for effects of soil microbes and species richness on biomass of the three invasive species

Species	Microbes (M)		Richness (R)		M × R	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>Solidago canadensis</i>	10.12	0.002	16.48	<0.001	3.44	0.038
<i>Erigeron canadensis</i>	12.85	0.001	26.96	<0.001	3.40	0.040
<i>Symphyotrichum subulatum</i>	7.70	0.007	25.51	<0.001	0.72	0.492

Effects of soil microbes on the invasion resistance of native plant communities

Consistent with our second hypothesis, the presence of soil microbes in more diverse plant communities resulted in a higher community invasion resistance (Fig. 3). In the unsterilized soil, the community invasion resistance increased with species richness for all three invasive species (Fig. 3a-c). However, in the sterilized soil, the community invasion resistance and species richness had no relationship for *S. canadensis* and *E. canadensis* and a positive relationship for *S. subulatum* (Fig. 3a-c).

In the unsterilized soil, the community invasion resistance had a positive relationship with the complementarity effect for all three invasive species (Fig. 4a-c). However, in the sterilized soil, the community invasion resistance had a positive relationship with the complementarity effect for *S. subulatum*, but the positive relationship was absent for *S. canadensis* and *E. canadensis* (Fig. 4a-c). Except for *S. subulatum* in the unsterilized soil, the community invasion resistance was not significantly related to the selection effect (Fig. 4d-f). In the unsterilized soil, most mixtures had positive values of the over-invasion resistance index (Fig. 5), indicating that these mixtures had a higher resistance than that of the most highly resistant species in the mixtures.

Direct effects of soil microbes conditioned by native plant communities on invader growth

Inconsistent with our third hypothesis, the biomass of neither *S. canadensis* nor *S. subulatum* was significantly affected by the different soil microbes trained in the native plant communities of different species richness (Fig. 6a, c) and had no significant relationship with the weighted phylogenetic distance (Fig. 7a, c). In addition, *E. canadensis* produced more biomass in the soil inoculated with soil microbes from the more diverse plant communities (Fig. 6b), and the biomass of *E. canadensis* was positively correlated with the weighted phylogenetic distance (Fig. 7b).

Discussion

We found that the absence of soil microbes weakened the negative species richness-invasibility relationship, indicating that soil microbes can contribute to the higher invasion resistance of more diverse native plant communities. In the presence of soil microbes, the higher invasion resistance of plant communities in more diverse communities was mainly ascribed to the complementarity effect. However, soil microbes from communities with a higher species richness did not have a consistently stronger negative effect on the growth of the three invasive species.

Effects of soil microbes on the species richness-invasibility relationship

We found significant negative relationships between native plant species and community invasibility (as measured by the invader biomass), supporting the diversity-invasibility hypothesis proposed by Elton (1958) and also agreeing with the findings of many previous studies (Adomako et al. 2019; Hector et al. 2001; Howeth 2017; Martignoni et al. 2020; Naeem et al. 2000; Selmants et al. 2012). More importantly, we found that the absence of soil microbes weakened the negative plant species richness-invasibility relationship, indicating that this relationship may be partly ascribed to the role of soil microbes. Thus, Elton's diversity-invasibility hypothesis may be associated with the underground bio-systems.

We found that the biomass of native species was significantly positively related to native species richness (Fig. S1, Table S4), as also reported in other studies (Cardinale et al. 2007; Cardinale et al. 2011; Han et al. 2021; Venail et al. 2015). However, the presence vs. absence of soil microbes did not change the relationship between the biomass and richness of native plant species (Fig. S1). These results suggest that the impact of soil microbes on the species richness-invasibility relationship was not due to its effect on the growth of native species.

Effect of soil microbes on the community invasion resistance

Compared with the absence of soil microbes, the presence of soil microbes led to a higher invasion resistance in the more diverse plant communities. Exotic plant species can affect native plants by enhancing their pathogen infection (Beckstead et al. 2010; Mangla and Callaway 2008), inhibiting mutualistic interactions (Stinson et al. 2006; Vogelsang and Bever 2009) or producing allelochemicals that are toxic to soil microbes (Inderjit and van der Putten 2010). The higher invasion resistance of the more diverse plant communities induced by soil microbes may be attributed to two mechanisms. One mechanism is that a higher level of diversity and/or abundance of microbial species in more diverse

communities may lead to the stability of the underground bio-system in response to disturbance (Lankau 2010; Li et al. 2015; Zhu et al. 2011), which can be ascribed to a higher insurance or portfolio effect (Isbell et al. 2009; Wang et al. 2021). The other mechanism is that more diverse communities have a higher probability of including more resistant soil microbial species that influence exotic plants, i.e., a selection effect (Isbell et al. 2015; Wilsey et al. 2014). For example, the more plant species the community contains, the more microbial species the plants carry and thus the higher the probability of harboring microbial species that can degrade allelochemicals (Hiiesalu et al. 2014; Mitchell et al. 2010; Prober et al. 2015). Based on the results of this experiment, the complementarity effect determined the community invasion resistance. Moreover, the positive values of the over-invasion resistance index indicated the existence of facilitation among the plant species induced by soil microbes. Although the specific mechanism underlying the role of soil microbes in the diversity-resistance relationship was not further explored, we can at least be sure that the diversity-resistance relationship can be influenced by the underground bio-system.

Direct effects of soil microbes on the growth of invasive plants

It is well known that the species richness of native plant communities can affect the diversity, abundance and activity of soil microbes (Chen et al. 2019; Dassen et al. 2017; Schmid et al. 2019), including pathogens (Cappelli et al. 2020; Liu et al. 2021; Zhang et al. 2020). If the diversity, abundance and/or activity of soil pathogens increase with species richness in native plant communities, then soils from native plant communities with higher species richness should have a stronger negative effect on the growth of invasive plants. For instance, in a recent study, Zhang et al. (2020) showed that more species-rich plant communities contained a greater diversity of plant pathogens and thus had strong negative impacts on invasive species. However, we found that *S. canadensis* and *S. subulatum* produced a similar amount of biomass when they were grown in the soils inoculated with the soil microbes from the native plant communities with different levels of species richness, and *E. canadensis* even produced more biomass in the soil inoculated with the soil microbes from the more diverse plant communities. These results suggest that the effects of soil pathogens cannot explain the negative species diversity-invasibility relationship detected in the current study systems.

In the present study, the effect of soil microbes on the growth of invasive species also showed no general relationship with the phylogenetic distance between the native and invasive species. There may be two reasons for the lack of impact on the richness-mediated effects of soil microbes on the growth of invasive species. One may be that increasing the species richness of native plant communities in our study did not increase the diversity, abundance or activity of soil pathogens that were harmful to the three invasive plant species. However, increasing plant species richness may increase the diversity of pathogens through amplification (Hudson et al. 2006; Keesing et al. 2006), and it may also reduce the prevalence of pathogens through dilution (Ostfeld and Keesing 2012; Schmidt and Ostfeld 2001; Zhang et al. 2020). The other reason may be that the effect of soil microbes on invasive plants was the net effect of the interaction between pathogenic and mutualistic soil microbes (Reinhart and Callaway 2006). Exotic plants have been proposed to encounter novel but strong soil mutualists, such as arbuscular

mycorrhizal fungi, and benefit disproportionately from these symbiotic mutualists (Reinhart and Callaway 2006; Richardson et al. 2000). As infections with both pathogens and arbuscular mycorrhizal fungi are phylogenetically conserved, the relationship between the effect of soil microbes on invasive species and the phylogenetic distance between native and invasive species may depend on the relative role of the pathogen or arbuscular mycorrhizal fungi.

Conclusions

We conclude that soil microbes can alter the diversity-invasibility relationship by promoting the complementarity effect on the community invasion resistance. However, we did not find a significant role for the richness-induced difference in soil pathogens in explaining the change in the relationship. Soil pathogens and arbuscular mycorrhizal fungi in the invaded area were proven to influence invasive plants (Inderjit 2005; Klironomos 2002; Reinhart and Callaway 2006; Richardson et al. 2000). Therefore, further studies could test the way in which plant species richness alters the impact of soil pathogens vs. arbuscular mycorrhizal fungi on the growth of invasive plants to obtain a deeper understanding of the richness-invasibility relationship. Our results highlight the importance of integrating the role of soil microbes when testing the diversity-invasibility hypothesis.

Declarations

Author contributions Material preparation, data collection and analysis were performed by X-Y Wang, S Gao, J Wang and T Chen. The first draft of the manuscript was written by X-Y Wang and all authors commented on previous versions of the manuscript. J Wang and F-H Yu contributed significantly to approved the final manuscript.

Acknowledgements This work was supported by the National Natural Science Foundation of China (31870504, 42177274), Basic Public Welfare Research Projects in Zhejiang Province (LGN19C150004), Taizhou University National Funds for Distinguished Young Scientists (2017JQ005, 2019JQ005), Natural Science Foundation of Zhejiang Province (LY22C030005, TY22C030179), Taizhou Science and Technology Project (1801ny06, 21hba02).

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

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

2. Beckstead J, Parker IM (2003) Invasiveness of *Ammophila arenaria*: Release from soil-borne pathogens. *Ecology* 84:2824–2831. <http://doi.org/10.1890/02-0517>
3. Beckstead J, Meyer SE, Connolly BM, Huck MB, Street LE (2010) Cheatgrass facilitates spillover of a seed bank pathogen onto native grass species. *J Ecol* 98:168–177. <http://doi.org/10.1111/j.1365-2745.2009.01599.x>
4. Blum U (1998) Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. *J Chem Ecol* 24:685–708. <http://doi.org/10.1023/A:1022394203540>
5. Cappelli SL, Pichon NA, Kempel A, Allan E (2020) Sick plants in grassland communities: a growth-defense trade-off is the main driver of fungal pathogen abundance. *Ecol Lett* 23:1349–1359. <http://doi.org/10.1111/ele.13537>
6. Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS, Loreau M, Weis JJ (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proc. Natl. Acad. Sci. U.S.A.* 104:18123–18128. <http://doi.org/10.1073/PNAS.0709069104>
7. Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, Balvanera P, O'Connor MI, Gonzalez A (2011) The functional role of producer diversity in ecosystems. *Am J Bot* 98:572–592. <http://doi.org/10.3732/ajb.1000364>
8. Cazetta AL, Zenni RD (2020) Pine invasion decreases density and changes native tree communities in woodland Cerrado. *Plant Ecol Divers* 13:85–91. <http://doi.org/10.1080/17550874.2019.1675097>
9. Chen C, Chen HYH, Chen XL, Huang ZQ (2019) Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nat Commun* 10:10. <http://doi.org/10.1038/s41467-019-09258-y>
10. Crawley MJ, Brown SL, Heard MS, Edwards GR (1999) Invasion-resistance in experimental grassland communities: species richness or species identity? *Ecol Lett* 2:140–148. <http://doi.org/10.1046/j.1461-0248.1999.00056.x>
11. Dassen S, Cortois R, Martens H, de Hollander M, Kowalchuk GA, van der Putten WH, de Deyn GB (2017) Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. *Mol Ecol* 26:4085–4098. <http://doi.org/10.1111/mec.14175>
12. Dawson W, Schrama M (2016) Identifying the role of soil microbes in plant invasions. *J Ecol* 104:1211–1218. <http://doi.org/10.1111/1365-2745.12619>
13. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. <http://doi.org/10.1093/nar/gkh340>
14. Ehlers BK (2011) Soil microorganisms alleviate the allelochemical effects of a Thyme monoterpene on the performance of an associated grass species. *PLoS ONE* 6:e26321. <http://doi.org/10.1371/journal.pone.0026321>
15. Elton CS (1958) *The ecology of invasion by animals and plants*. Methuen, London

16. Fries A (2016) Damage by pathogens and insects to Scots pine and lodgepole pine 25 years after reciprocal plantings in Canada and Sweden. *Scand J For Res* 32:1–42. <http://doi.org/10.1080/02827581.2016.1247463>
17. Guo SL, Fang F (2003) Physiological adaptation of the invasive plant *Solidago canadensis* to environments. *Acta Pharmacol Sin* 27:47–52
18. Han W, Chang J, Jiang H, Niu S, Liu Y, Xu J, Wu J, Ge Y (2021) Plant species diversity affects plant nutrient pools by affecting plant biomass and nutrient concentrations in high-nitrogen ecosystems. *Basic Appl Ecol* 56:213–225. <http://doi.org/10.1016/J.BAAE.2021.08.002>
19. He P, Deng Y-J, Hu X-Y, Pan H-M, Deng H-P (2019) Potential allelopathic effect of *Aster subulatus* on *Triticum aestivum* and *Brassica chinensis*. *Acta Prataculturae Sin* 28:101–109
20. Hector A, Dobson K, Minns A, Bazeley-White E, Lawton JH (2001) Community diversity and invasion resistance: an experimental test in a grassland ecosystem and a review of comparable studies. *Ecol Res* 16:819–831. <http://doi.org/10.1046/j.1440-1703.2001.00443.x>
21. Hector A (2002) Biodiversity and the functioning of grassland ecosystems: multi-site comparisons.. In: Kinzig AP, Pacala S, Tillman D (eds) *The functional consequences of biodiversity*. Princeton University Press, Princeton, N.J., pp 71–95
22. Hess MCM, Buisson E, Jaunatre R, Mesleard F (2020) Using limiting similarity to enhance invasion resistance: theoretical and practical concerns. *J Appl Ecol* 57:559–565. <http://doi.org/10.1111/1365-2664.13552>
23. Hiiesalu I, Pärtel M, Davison J, Gerhold P, Metsis M, Moora M, Öpik M, Vasar M, Zobel M, Wilson SD (2014) Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. *New Phytol* 203:233–244. <http://doi.org/10.1111/nph.12765>
24. Hooper DU, Bignell DE, Brown VK, Brussard L, Dangerfield JM, Wall DH, Wardle DA, Coleman DC, Giller KE, Lavelle P, Van Der Putten WH, De Ruiter PC, Rusek J, Silver WL, Tiedje JM, Wolters V (2000) Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. *Bioscience* 50:1049–1061. [http://doi.org/10.1641/0006-3568\(2000\)050\[1049:ibaabb\]2.0.co;2](http://doi.org/10.1641/0006-3568(2000)050[1049:ibaabb]2.0.co;2)
25. Howeth JG (2017) Native species dispersal reduces community invasibility by increasing species richness and biotic resistance. *J Anim Ecol* 86:1380–1393. <http://doi.org/10.1111/1365-2656.12733>
26. Hudson PJ, Dobson AP, Lafferty KD (2006) Is a healthy ecosystem one that is rich in parasites? *Trends Ecol Evol* 21:381–385. <http://doi.org/10.1016/j.tree.2006.04.007>
27. Inderjit (2005) Soil Microorganisms: Soil microorganisms: an important determinant of allelopathic activity. *Plant Soil* 274:227–236. <http://doi.org/10.1007/s11104-004-0159-x>
28. van der Inderjit WH (2010) Impacts of soil microbial communities on exotic plant invasions. *Trends Ecol Evol* 25:512–519. <http://doi.org/10.1016/j.tree.2010.06.006>
29. Inderjit, Simberloff D, Kaur H, Kalisz S, Bezemer TM (2021) Novel chemicals engender myriad invasion mechanisms. *New Phytol* 232:1184–1200. <http://doi.org/10.1111/nph.17685>

30. Isbell FI, Polley HW, Wilsey BJ (2009) Biodiversity, productivity and the temporal stability of productivity: patterns and processes. *Ecol Lett* 12:443–451. <http://doi.org/10.1111/j.1461-0248.2009.01299.x>
31. Isbell F, Craven D, Connolly J, Loreau M, Schmid B, Beierkuhnlein C, Bezemer TM, Bonin C, Bruelheide H, de Luca E, Ebeling A, Griffin JN, Guo Q, Hautier Y, Hector A, Jentsch A, Kreyling J, Lanta V, Manning P, Meyer ST, Mori AS, Naeem S, Niklaus PA, Polley HW, Reich PB, Roscher C, Seabloom EW, Smith MD, Thakur MP, Tilman D, Tracy BF, van der Putten WH, van Ruijven J, Weigelt A, Weisser WW, Wilsey B, Eisenhauer N (2015) Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature* 526:574–577. <http://doi.org/10.1038/nature15374>
32. Keesing F, Holt RD, Ostfeld RS (2006) Effects of species diversity on disease risk. *Ecol Lett* 9:485–498. <http://doi.org/10.1111/j.1461-0248.2006.00885.x>
33. Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417:67–70. <http://doi.org/10.1038/417067a>
34. Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant-soil feedbacks: a meta-analytical review. *Ecol Lett* 11:980–992. <http://doi.org/10.1111/j.1461-0248.2008.01209.x>
35. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549. <http://doi.org/10.1093/molbev/msy096>
36. Lankau R (2010) Soil microbial communities alter allelopathic competition between *Alliaria petiolata* and a native species. *Biol Invasions* 12:2059–2068. <http://doi.org/10.1007/s10530-009-9608-z>
37. Lankau RA (2011) Resistance and recovery of soil microbial communities in the face of *Alliaria petiolata* invasions. *New Phytol* 189:536–548. <http://doi.org/10.1111/j.1469-8137.2010.03481.x>
38. Lannes LS, Karrer S, Teodoro DAA, Bustamante MMC, Edwards PJ, Venterink HO (2020) Species richness both impedes and promotes alien plant invasions in the Brazilian Cerrado. *Sci Rep* 10:11365. <http://doi.org/10.1038/s41598-020-68412-5>
39. Li Y-P, Feng Y-L, Chen Y-J, Tian Y-H (2015) Soil microbes alleviate allelopathy of invasive plants. *Sci Bull* 60:1083–1091. <http://doi.org/10.1007/s11434-015-0819-7>
40. Liu L, Zhu K, Wurzbürger N, Zhang J (2020) Relationships between plant diversity and soil microbial diversity vary across taxonomic groups and spatial scales. *Ecosphere* 11:e02999. <http://doi.org/10.1002/ecs2.2999>
41. Liu X, Zhang L, Huang M, Zhou S (2021) Plant diversity promotes soil fungal pathogen richness under fertilization in an alpine meadow. *J Plant Ecol* 14:323–336. <http://doi.org/10.1093/jpe/rtaa099>
42. Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76. <http://doi.org/10.1038/35083573>
43. Ma S-J (2013) The checklist of the Chinese invasive plants. Higher Education press, Beijing
44. Mangla S, Callaway RM (2008) Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *J Ecol* 96:58–67. <http://doi.org/10.1111/j.1365-2745.2007.01312.x>

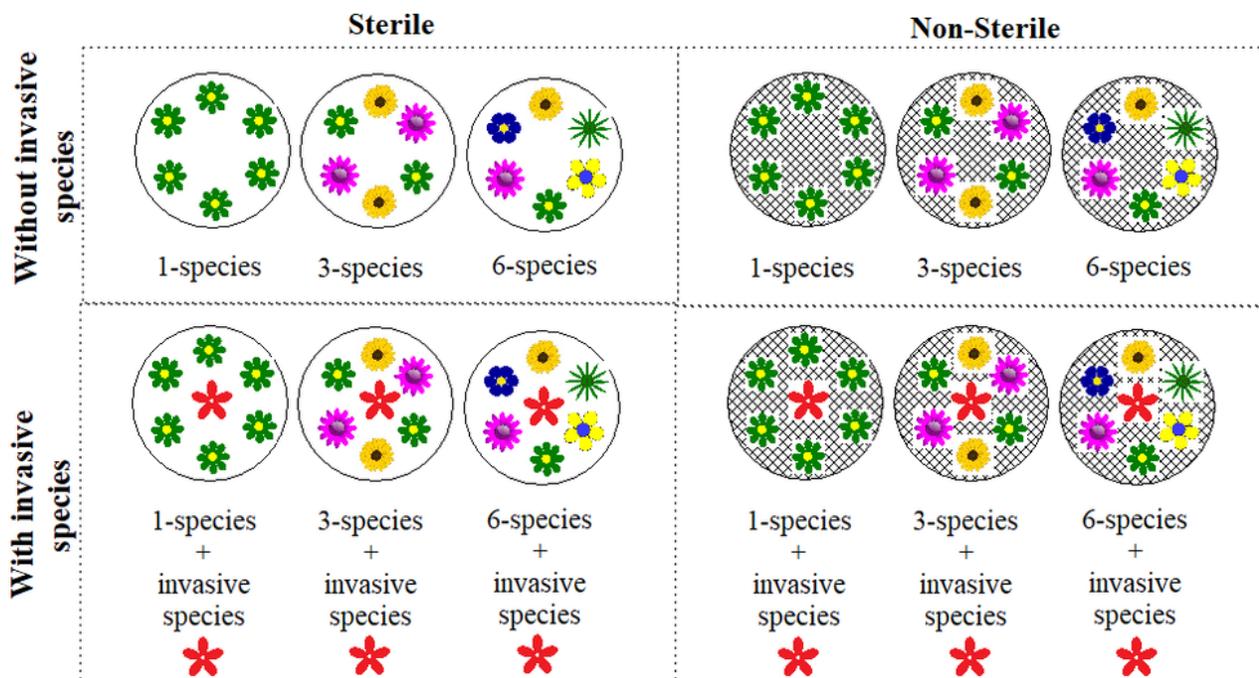
45. Martignoni MM, Hart MM, Tyson RC, Garnier J (2020) Diversity within mutualist guilds promotes coexistence and reduces the risk of invasion from an alien mutualist. *Proc. Royal Soc. B* 287:20192312. <http://doi.org/doi:10.1098/rspb.2019.2312>
46. Millard P, Singh BK (2010) Does grassland vegetation drive soil microbial diversity? *Nutr. Cycl Agroecosystems* 88:147–158. <http://doi.org/10.1007/s10705-009-9314-3>
47. Mitchell CE (2003) Trophic control of grassland production and biomass by pathogens. *Ecol Lett* 6:147–155. <http://doi.org/10.1046/j.1461-0248.2003.00408.x>
48. Mitchell RJ, Hester AJ, Campbell CD, Chapman SJ, Cameron CM, Hewison RL, Potts JM (2010) Is vegetation composition or soil chemistry the best predictor of the soil microbial community? *Plant Soil* 333:417–430. <http://doi.org/10.1007/s11104-010-0357-7>
49. Naeem S, Knops JMH, Tilman D, Howe KM, Kennedy T, Gale S (2000) Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *Oikos* 91:97–108. <http://doi.org/10.1034/j.1600-0706.2000.910108.x>
50. Nei M, Kumar S (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York
51. Ostfeld R, Keesing F (2012) Effects of host diversity on infectious disease. *Annu Rev Ecol Evol Syst* 43:157–182. <http://doi.org/10.1146/annurev-ecolsys-102710-145022>
52. Pearson DE, Ortega YK, Eren Ö, Hierro JL (2018) Community assembly theory as a framework for biological invasions. *Trends Ecol Evol* 33:313–325. <http://doi.org/10.1016/j.tree.2018.03.002>
53. Pfisterer AB, Schmid B (2002) Diversity-dependent production can decrease the stability of ecosystem functioning. *Nature* 416:84–86. <http://doi.org/10.1038/416084a>
54. Prober SM, Leff JW, Bates ST, Borer ET, Firn J, Harpole WS, Lind EM, Seabloom EW, Adler PB, Bakker JD, Cleland EE, DeCrappeo NM, DeLorenze E, Hagenah N, Hautier Y, Hofmockel KS, Kirkman KP, Knops JM, La Pierre KJ, MacDougall AS, McCulley RL, Mitchell CE, Risch AC, Schuetz M, Stevens CJ, Williams RJ, Fierer N (2015) Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecol Lett* 18:85–95. <http://doi.org/10.1111/ele.12381>
55. Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kühn I, Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, van Kleunen M, Vilà M, Wingfield MJ, Richardson DM (2020) Scientists' warning on invasive alien species. *Biol Rev* 95:1511–1534. <http://doi.org/10.1111/brv.12627>
56. Qiang S (2001) *Weed science*. China Agricultural Press, Beijing
57. Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. *New Phytol* 170:445–457. <http://doi.org/10.1111/j.1469-8137.2006.01715.x>
58. Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. *Divers Distrib* 6:93–107. <http://doi.org/10.1046/j.1472-4642.2000.00083.x>
59. Schmid MW, Hahl T, van Moorsel SJ, Wagg C, De Deyn GB, Schmid B (2019) Feedbacks of plant identity and diversity on the diversity and community composition of rhizosphere microbiomes from a long-term biodiversity experiment. *Mol Ecol* 28:863–878. <http://doi.org/10.1111/mec.14987>

60. Schmidt KA, Ostfeld RS (2001) Biodiversity and the dilution effect in disease ecology. *Ecology* 82:609–619. [http://doi.org/10.1890/0012-9658\(2001\)082\[0609:BATDEI\]2.0.CO;2](http://doi.org/10.1890/0012-9658(2001)082[0609:BATDEI]2.0.CO;2)
61. Schnitzer SA, Klironomos JN, HilleRisLambers J, Kinkel LL, Reich PB, Xiao K, Rillig MC, Sikes BA, Callaway RM, Mangan SA, van Nes EH, Scheffer M (2011) Soil microbes drive the classic plant diversity-productivity pattern. *Ecology* 92:296–303. <http://doi.org/10.1890/10-0773.1>
62. Selman PC, Zavaleta ES, Pasari JR, Hernandez DL (2012) Realistic plant species losses reduce invasion resistance in a California serpentine grassland. *J Ecol* 100:723–731. <http://doi.org/10.1111/j.1365-2745.2011.01949.x>
63. Sikes BA, Cottenie K, Klironomos JN (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *J Ecol* 97:1274–1280. <http://doi.org/10.1111/j.1365-2745.2009.01557.x>
64. Simard SW, Durall DM (2004) Mycorrhizal networks: a review of their extent, function, and importance. *Botany* 82:1140–1165. <http://doi.org/10.1139/b04-116>
65. Smith MD, Wilcox JC, Kelly T, Knapp AK (2004) Dominance not richness determines invasibility of tallgrass prairie. *Oikos* 106:253–262. <http://doi.org/10.1111/j.0030-1299.2004.13057.x>
66. Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS Biol* 4:e140. <http://doi.org/10.1371/journal.pbio.0040140>
67. Strauss SY, Webb CO, Salamin N (2006) Exotic taxa less related to native species are more invasive. *Proc. Natl. Acad. Sci. U.S.A.* 103:5841–5845. <http://doi.org/10.1073/pnas.0508073103>
68. Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. U.S.A.* 101:11030–11035. <http://doi.org/10.1073/pnas.0404206101>
69. Tilman D (1996) Biodiversity: population versus ecosystem stability. *Ecology* 77:350–363. <https://doi.org/10.2307/2265614>
70. Urza AK, Weisberg PJ, Chambers JC, Board D, Flake SW (2019) Seeding native species increases resistance to annual grass invasion following prescribed burning of semiarid woodlands. *Biol Invas* 21:1993–2007. <http://doi.org/10.1007/s10530-019-01951-9>
71. van der Putten WH, Kowalchuk GA, Brinkman EP, Doodeman GTA, van der Kaaij RM, Kamp AFD, Menting FBJ, Veenendaal EM (2007) Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity. *Ecology* 88:978–988. <http://doi.org/10.1890/06-1051>
72. Venail P, Gross K, Oakley TH, Narwani A, Allan E, Flombaum P, Isbell F, Joshi JR, Reich PB, Tilman D, van Ruijven J, Cardinale BJ (2015) Species richness, but not phylogenetic diversity, influences community biomass production and temporal stability in a re-examination of 16 grassland biodiversity studies. *Funct Ecol* 29:615–626. <http://doi.org/10.1111/1365-2435.12432>
73. Vogelsang KM, Bever JD (2009) Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology* 90:399–407. <http://doi.org/10.1890/07-2144.1>

74. Wang S, Isbell F, Deng W, Hong P, Dee LE, Thompson P, Loreau M (2021) How complementarity and selection affect the relationship between ecosystem functioning and stability. *Ecology* 102:e03347. <http://doi.org/10.1002/ecy.3347>
75. Wang X-Y, Ge Y, Wang J (2017) Positive effects of plant diversity on soil microbial biomass and activity are associated with more root biomass production. *J Plant Interact* 12:533–541. <http://doi.org/10.1080/17429145.2017.1400123>
76. Wang Y, Yu S, Wang J (2007) Biomass-dependent susceptibility to drought in experimental grassland communities. *Ecol Lett* 10:401–410. <http://doi.org/10.1111/j.1461-0248.2007.01031.x>
77. Wardle DA (2006) The influence of biotic interactions on soil biodiversity. *Ecol Lett* 9:870–886. <http://doi.org/10.1111/j.1461-0248.2006.00931.x>
78. Wilsey BJ, Daneshgar PP, Hofmockel K, Polley HW (2014) Invaded grassland communities have altered stability-maintenance mechanisms but equal stability compared to native communities. *Ecol Lett* 17:92–100. <http://doi.org/10.1111/ele.12213>
79. Zeiter M, Stampfli A (2012) Positive diversity-invasibility relationship in species-rich semi-natural grassland at the neighbourhood scale. *Ann Bot* 110:1385–1393. <http://doi.org/10.1093/aob/mcs193>
80. Zhang Z, Liu Y, Brunel C, van Kleunen M (2020) Evidence for Elton's diversity-invasibility hypothesis from belowground. *Ecology* 101:e03187. <http://doi.org/10.1002/ecy.3187>
81. Zheng Y-L, Burns JH, Liao Z-Y, Li Y-P, Yang J, Chen Y-J, Zhang J-L, Zheng Y-G (2018) Species composition, functional and phylogenetic distances correlate with success of invasive *Chromolaena odorata* in an experimental test. *Ecol Lett* 21:1211–1220. <http://doi.org/10.1111/ele.13090>
82. Zhu X, Zhang J, Ma K (2011) Soil biota reduce allelopathic effects of the invasive *Eupatorium adenophorum*. *PLoS ONE* 6:e25393. <http://doi.org/10.1371/journal.pone.0025393>

Figures

Experiment I



Experiment II

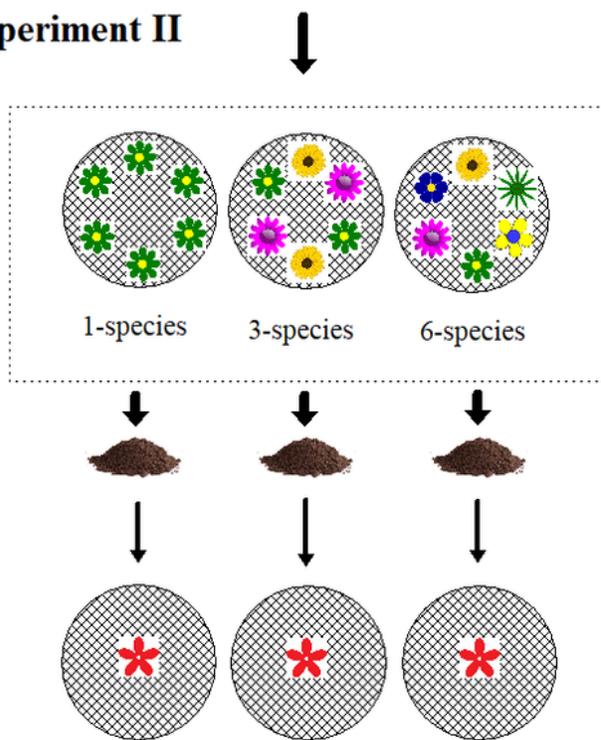


Figure 1

Graphical illustration of the experimental design

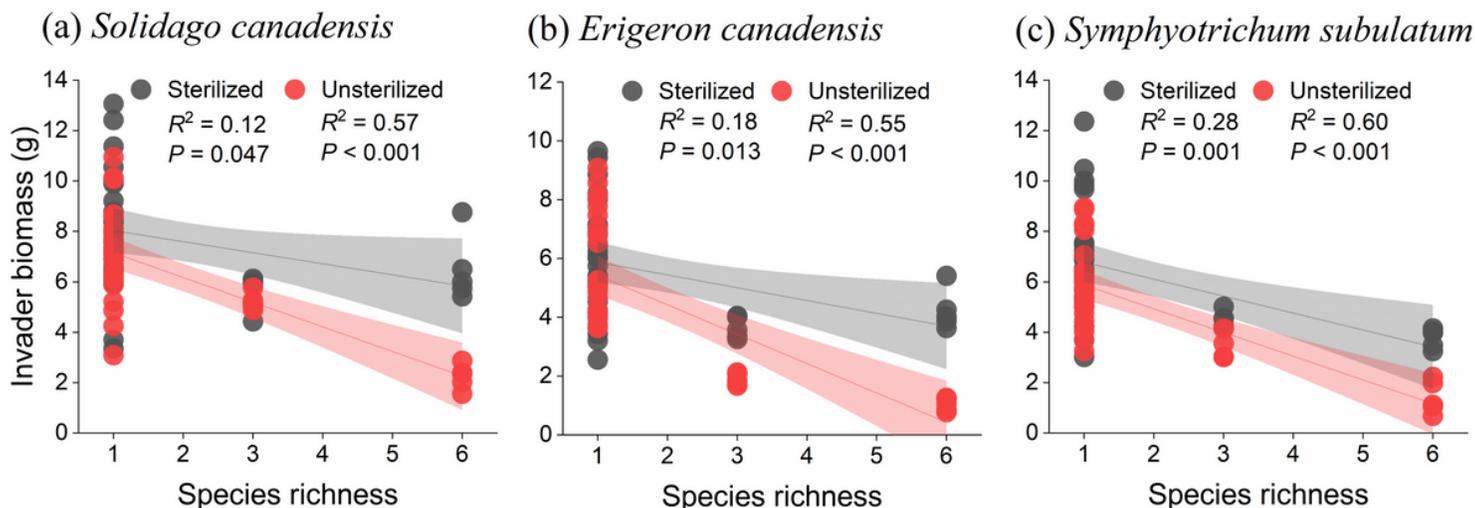


Figure 2

Relationships between species richness and biomass of each of the three invasive species in sterilized or unsterilized soil. (a) *Solidago canadensis*, (b) *Erigeron canadensis* and (c) *Symphyotrichum subulatum*.

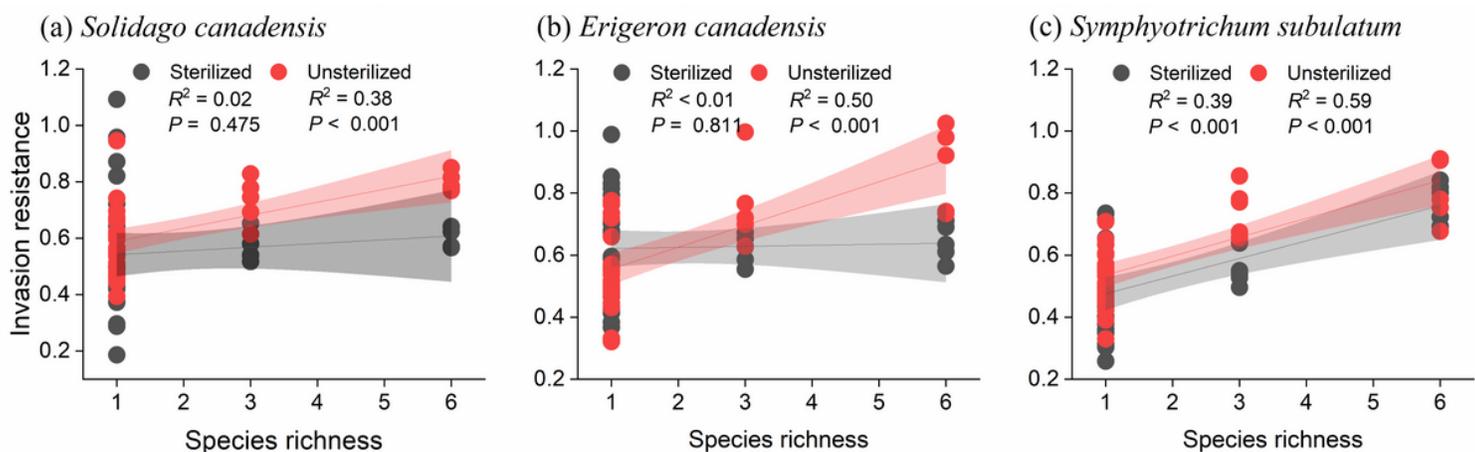


Figure 3

Relationships between species richness and the community invasion resistance in sterilized or unsterilized soil. (a) *Solidago canadensis*, (b) *Erigeron canadensis* and (c) *Symphyotrichum subulatum*.

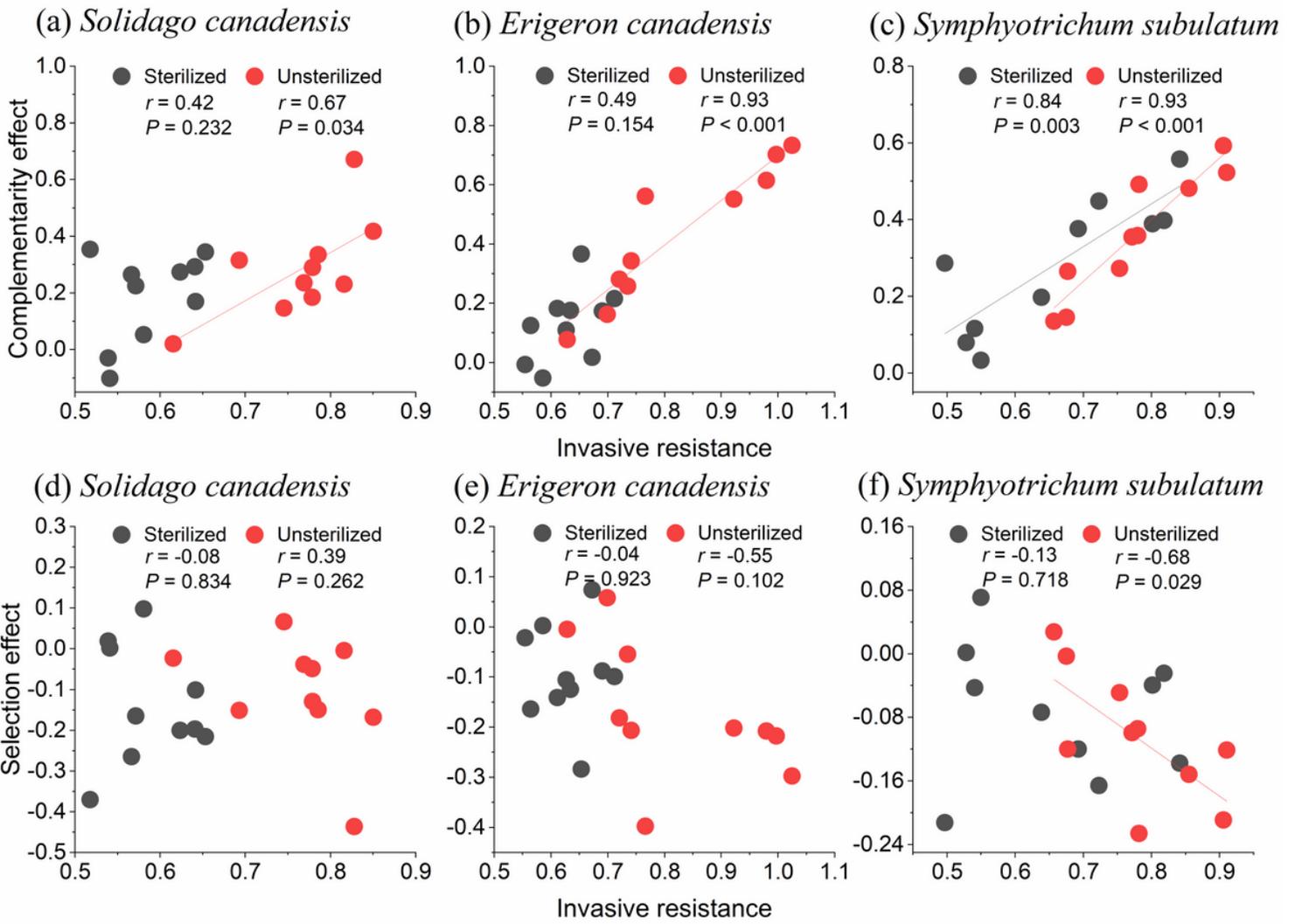


Figure 4

Relationships of the complementarity effect and the selection effect with the community invasion resistance in sterilized or unsterilized soil. (a) and (d) *Solidago canadensis*, (b) and (e) *Erigeron canadensis*, (c) and (f) *Symphyotrichum subulatum*.

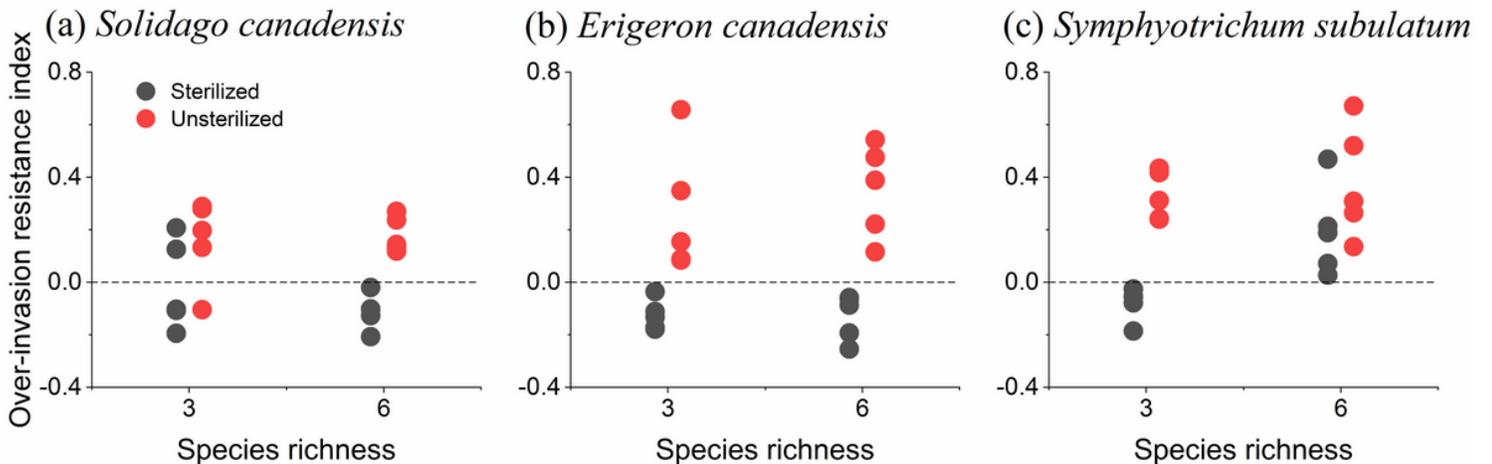


Figure 5

Effects of species richness on the over-invasion resistance index of plant communities in sterilized or unsterilized soil.

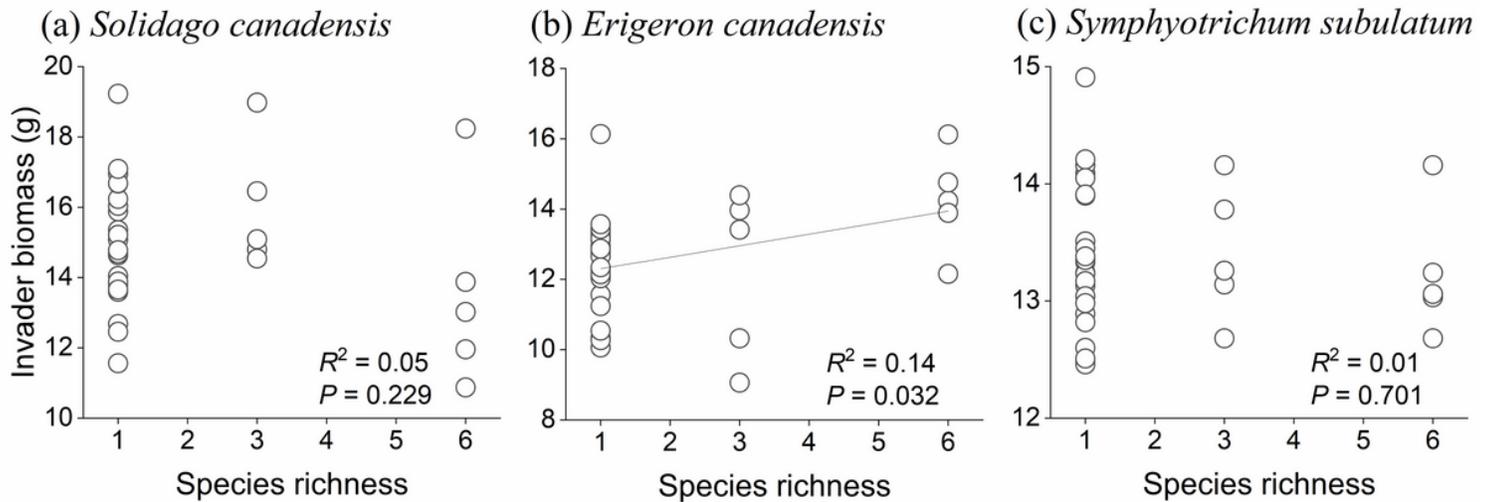


Figure 6

Effects of trained soil microbes by plant communities with different species richness on biomass of each of the three invasive species. (A) *Solidago canadensis*, (B) *Erigeron canadensis* and (C) *Symphyotrichum subulatum*.

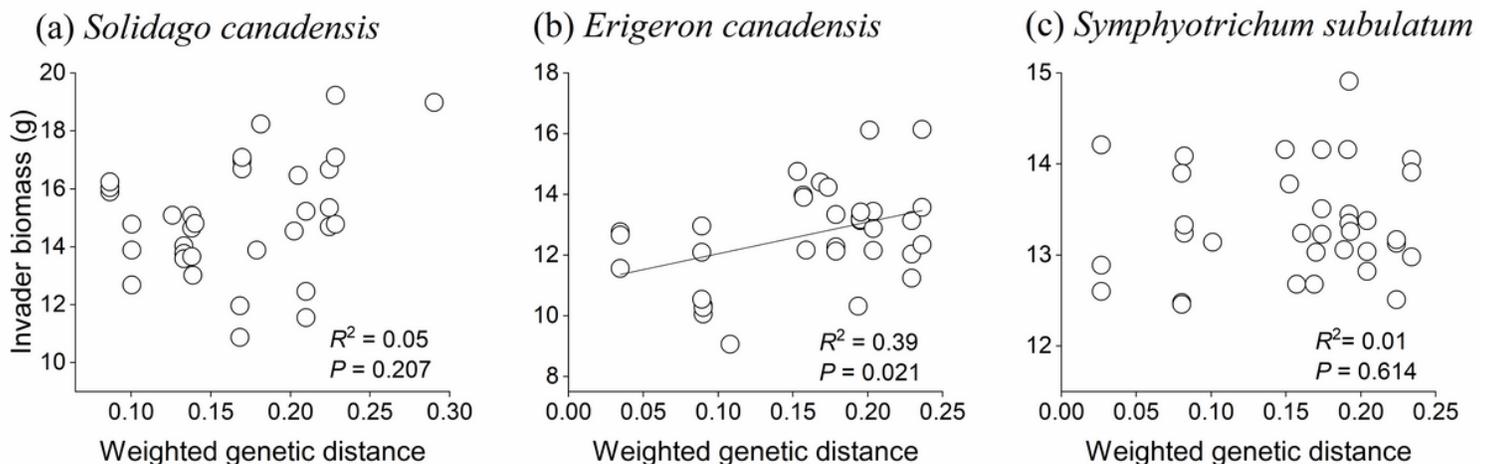


Figure 7

Relationships between phylogenetic distance and biomass of each of the three invasive species. (a) *Solidago canadensis*, (b) *Erigeron canadensis* and (c) *Symphyotrichum subulatum*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [PlantandSoilSupplementarymaterials.docx](#)