Performance of alien and native species in plant-plant and plant-soil interactions

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Summary

Human activities have actively facilitated the expansion and establishment of a multitude of species beyond their native ranges. The continued increase in the number of these alien species poses a huge challenge to global biotic homogenization. Alarmingly, some alien species can become invasive, rapidly spreading and becoming dominant within their naturalized ranges, often at the expense of native species diversity. The competition and coexistence of alien and native species have thus emerged as a persistent concern in ecology.

In invaded ecosystems, the coexistence of multiple alien species is a common occurrence that can facilitate the subsequent establishment of additional alien species (i.e. invasion meltdown hypothesis). Consequently, the complex interactions between species become more intricate, intriguing, and important to understand. This phenomenon has engendered a growing interest among biologists, prompting them to explore the potential for positive interactions between alien species.

Towards the end of the last century, the theoretical framework of plant-soil interactions was proposed, paving the way for studies that focus on the effects of the belowground components of a plant's environment (e.g. soil organisms such as pathogens or mutualists, soil structure, or the amount and distribution of nutrients) on growth and competition. When it comes to alien plant species, their performance in naturalized ranges are jointly shaped by both the species they grow with and the soil they grow on. Despite advancements in this field, many questions remain as to how multiple characteristics of the plant species and soil environment interact to influence invasion success.

To address these issues, in my first chapter, I tested the relationship between the performance of alien species and the richness of native communities, and their dynamic

interactions over time. I used five alien and five native species as invaders in a total of 21 assemblages of one, two or four native competitor species and harvested plants at the early, middle and late growth stages, respectively. Overall, invaders and communities had negative effects on each other. Although alien invaders had better performance than the native invaders in later growth stages, multi-species communities were more resistant to negative effects from invaders.

Based on these results, in my second chapter, I further tested whether the success of subsequent invaders can be affected by the legacy-effects from previous invaders and communities. I used trained soils from the first study (i.e. soil occupied by various previous invaders and/or communities of different diversities) to separately grow five alien and five native plant species. Although subsequent plants were overall negatively affected by soil legacies, their growth loss was reduced when they grew on soil that had been conditioned by previous heterospecific alien invaders. However, this effect was decreased when the soil was also trained by the multi-species communities. These findings suggest that the previous alien plant species can promote the establishment of the subsequent alien plant species, but community diversity may increase resistance against subsequent invaders through interactions with the soil.

In my third chapter, I performed a combined plant-soil-feedback and competition experiment to test how soil legacy affect alien-native species competition, and whether this effect is influenced by the presence of additional competitors (i.e. the third alien or native species). I first conditioned the soil with four alien and four native plant species grown separately or without plant conditioning. In the test phase, I then grew all 16 pairwise alien-native plant species on unconditioned soil or soils conditioned by the alien species, the native species or a mixture of both, and with or without a third alien or native plant species.

On average, both the presence of an additional plant species and soil-legacy effects reduced the growth of the test species. Alien test species grew the worst on the soil conditioned by conspecifics. However, the negative soil-legacy effects on alien test species were partly alleviated when an additional alien species was present. This suggests that alien plants can benefit from other co-growing alien plants, and this may thus result in a competitive advantage of the alien over the native plants.

In my fourth chapter, I further included soil spatial heterogeneity formed by soil legacies and tested whether the performance of alien species is affected by the soil-legacy heterogeneity and the origin of their competitors (alien or native). I conducted a combined heterogeneous plant-soil-feedback and competition experiment. The soil was first conditioned with five alien and five native plant species grown individually. Then, I grew 45 pairwise combinations of alien-alien, alien-native and native-native species on unconditioned soil or homogeneous conditioned soils or heterogeneous conditioned soils. Although soil-legacy effects reduced the growth of the test species, alien species performed better in competition with native species on heterogeneous-legacy soils than on homogeneous-legacy soils. This suggests that the performance of alien plants depends on the legacy of the soil they grow on as well as the origin of their competitors.

Taken together, my thesis reveals that the performance of alien plant species is constrained by the diversity of recipient communities, the historical origins (alien or native) of their competitors, and plant-soil interaction processes. The dominance of some alien plants, as well as the positive feedbacks between invaders that are mediated by soil legacy, can be reduced by the presence of more diverse communities. However, co-occurrence of other alien species and soil-legacy spatial heterogeneity can still relatively favor the establishment of subsequent alien plants. My thesis highlights the importance of combining both plant-plant and plant-soil interactions in understanding the coexistence of alien and native plant species.

Zusammenfassung

Menschliche Aktivitäten haben die Ausbreitung und Etablierung einer Vielzahl von Arten außerhalb ihrer angestammten Verbreitungsgebiete aktiv erleichtert. Die kontinuierliche Zunahme dieser gebietsfremden Arten stellt eine große Herausforderung für die globale biotische Homogenisierung dar. Alarmierend ist, dass einige gebietsfremde Arten invasiv werden, sich schnell ausbreiten und ihre Dominanz in ihren eingebürgerten Verbreitungsgebieten behaupten, oft auf Kosten der einheimischen Artenvielfalt. Die Konkurrenz und Koexistenz von gebietsfremden und einheimischen Arten haben sich daher zu einem anhaltenden Anliegen in der Ökologie entwickelt.

In Ökosystemen, wo natürliche Artenausbreitung passiert, ist die Koexistenz mehrerer gebietsfremder Arten ein häufiges Ereignis, das oft mit der anschließenden Ansiedlung weiterer gebietsfremder Arten einhergeht. Folglich werden diese komplexen Interaktionen zwischen den Arten komplizierter und faszinierender. Das Aufkommen der Invasions-Meltdown-Hypothese hat ein wachsendes Interesse unter Biologen geweckt, was sie dazu veranlasst hat, die positiven Wechselwirkungen zwischen gebietsfremden Arten zu untersuchen.

Gegen Ende des letzten Jahrhunderts wurde der theoretische Rahmen der Pflanzen-Boden-Interaktionen vorgeschlagen und ebnete den Weg für Studien, die sich auf die Auswirkungen des unterirdischen Teils (d.h. Bodenorganismen, Krankheitserreger und räumliche Heterogenität des Bodens) auf das Pflanzenwachstum und die Konkurrenz konzentrieren. Wenn es um gebietsfremde Pflanzenarten geht, werden ihre Leistungen in eingebürgerten Verbreitungsgebieten gemeinsam durch ihre Wechselwirkungen mit anderen Arten und mit Böden beeinflusst. Trotz der Fortschritte auf diesem Gebiet lässt die

Erforschung des Zusammenspiels dieser drei Bereiche noch Lücken offen, die es zu füllen gilt.

Um diese Fragen anzugehen habe ich in meinem ersten Kapitel die Beziehung zwischen der Leistung gebietsfremder Arten und dem Reichtum einheimischer Gemeinschaften und ihren dynamischen Interaktionen im Laufe der Zeit getestet. Ich habe fünf gebietsfremde und fünf einheimische Arten als Eindringlinge in insgesamt 21 Ansammlungen von einer, zwei oder vier einheimischen Konkurrenzarten verwendet und Pflanzen in allen drei frühen, mittleren und späten Wachstumsstadien geerntet. Insgesamt wirkten sich Invasoren und ortsständige Pflanzengemeinschaft negativ aufeinander aus. Fremdartige Eindringlinge hatten in späteren Wachstumsphasen eine bessere Leistung als Einheimische. Multispezies-Gemeinschaften waren widerstandsfähiger gegen negative Auswirkungen von Eindringlingen.

Basierend auf diesem Ergebnis habe ich in meinem zweiten Kapitel weiter getestet, ob der Erfolg nachfolgender Invasoren von den trainierten Böden abhängt, auf denen sie wachsen (d.h. die von verschiedenen früheren Invasoren und/oder Gemeinschaften unterschiedlicher Vielfalt trainiert wurden). Ich habe trainierte Böden aus der ersten Studie verwendet und einen Folgetest mit fünf gebietsfremden und fünf einheimischen nachfolgenden Pflanzenarten separat durchgeführt. Insgesamt verschlechterten sich nachfolgende Pflanzen auf Böden, die von mehr Pflanzen und Artgenossen konditioniert worden waren. Nachfolgende gebietsfremde Pflanzen können durch Boden-Legacy-Effekte von früheren heterospezifischen gebietsfremden Invasoren profitieren. Dieser Effekt nahm jedoch ab, wenn der Boden auch von Multi-Art-Gemeinschaften trainiert wurde. Diese Ergebnisse deuten darauf hin, dass frühere gebietsfremde Pflanzenarten die Etablierung nachfolgender gebietsfremder Pflanzenarten fördern können, aber die Vielfalt der

Gemeinschaften die Widerstandsfähigkeit gegen nachfolgende Eindringlinge durch Wechselwirkungen mit dem Boden erhöhen kann.

In meinem dritten Kapitel habe ich weiter getestet, ob die nachfolgenden Pflanzen in Konkurrenz stehen, ob die Leistung gebietsfremder Pflanzen immer noch von Boden-Altlasteffekten abhängt und ob sich ihre Leistung in Gegenwart weiterer Konkurrenten (d.h. der dritten gebietsfremden oder einheimischen Art) unterscheidet. Der Boden wurde zunächst mit vier gebietsfremden und vier einheimischen Pflanzenarten konditioniert, die separat oder ohne Pflanzenkonditionierung angebaut wurden. Als nächste Testphase habe ich dann alle 16 paarweise gebietsfremden Pflanzenarten auf unkonditioniertem Boden oder Böden angebaut, die durch die gebietsfremden Arten, die einheimischen Arten oder eine Mischung aus beidem und mit oder ohne eine zusätzliche gebietsfremde oder einheimische Pflanzenart konditioniert sind. Im Durchschnitt reduzierten sowohl das Vorhandensein einer zusätzlichen Pflanzenart als auch Boden-Altlasteffekte das Wachstum der Testart. Fremde Arten wuchsen am schlechtesten auf dem Boden, der von Artgenossen konditioniert wurde. Diese negativen Auswirkungen des Bodens auf gebietsfremde Arten wurden jedoch teilweise gemildert, wenn eine zusätzliche gebietsfremde Art vorhanden war. Das Ergebnis dieses Kapitels deutet darauf hin, dass gebietsfremde Pflanzen von anderen mitwachsenden gebietsfremden Pflanzen profitieren können, was in der Folge zu einem Wettbewerbsvorteil der gebietsfremden Pflanzen gegenüber den einheimischen Pflanzen führen kann.

In meinem vierten Kapitel habe ich außerdem die räumliche Heterogenität des Bodens einbezogen, um zu testen, ob die Leistung gebietsfremder Arten durch die räumliche Heterogenität des Bodenerbes und die Herkunft ihrer Konkurrenten (gebietsfremd oder einheimisch) beeinflusst wird. Ich führte ein kombiniertes Pflanzen-Boden-Feedback- und Wettbewerbsexperiment durch, bei dem ich den Boden zunächst mit fünf gebietsfremden und fünf einheimischen Pflanzenarten konditionierte, die einzeln angebaut wurden. Dann züchtete ich 45 paarweise Pflanzenarten aus gebietsfremd-gebietsfremden, gebietsfremd-heimischen und heimisch-heimischen Kombinationen auf unkonditionierten Böden oder homogen konditionierten Böden oder heterogen konditionierten Böden. Obwohl Boden-Altlasten-Effekte das Wachstum der Testarten reduzierten, dominierten gebietsfremde Arten auf heterogenen Altböden, wenn die Konkurrenten heimisch waren. Dies deutet darauf hin, dass die Leistung gebietsfremder Pflanzen sowohl vom Erbe des Bodens, auf dem sie wachsen, als auch von der Herkunft ihrer Konkurrenten abhängt.

Zusammenfassend zeigt meine Dissertation, dass die Leistungsfähigkeit gebietsfremder Pflanzenarten durch die Diversität der Lebensgemeinschaften der eingebürgerten Verbreitungsgebiete, ihre nachbarschaftlichen Konkurrenten und Interaktionsprozesse zwischen Pflanzen und Boden eingeschränkt wird. Die individuelle Dominanz gebietsfremder Pflanzen sowie ihre positiven Interaktionen, die durch das Erbe des Bodens vermittelt werden, können durch die Anwesenheit vielfältigerer Gemeinschaften reduziert werden. Das gleichzeitige Vorkommen anderer gebietsfremder Arten und die räumliche Heterogenität des Bodens können jedoch die Etablierung nachfolgender gebietsfremder Pflanzen relativ begünstigen. Meine Dissertation unterstreicht die Wichtigkeit, sowohl Pflanzen-Pflanzen- als auch Pflanzen-Boden-Interaktionen für die Koexistenz von exotischen und einheimischen Pflanzenarten zu kombinieren.

General introduction

Biological invasion as a global issue

Since the Industrial Revolution, a variety of technological innovations have facilitated the movement of humans and the intensity of our activities across the globe (Rodrigue *et al.* 2013). During these processes, numerous species have intentionally or through accidental transportation been introduced beyond their native ranges. As these alien species have accumulated over the centuries, the concept of biological invasions has emerged frequently (Hulme 2009; van Kleunen *et al.* 2015; Hulme 2021). Considering the impacts these species can have on native species and ecosystems, biologists have increasingly focused on understanding processes of biological invasions. However, despite continued monitoring and efforts to predict the development of these alien species, their accumulation shows no sign of saturation, and the number of alien species are likely to continue growing in the future (Pysek *et al.* 2020; Seebens *et al.* 2021). Therefore, exploring the dynamics of alien species remains a persistent issue in ecology.

The need for attention on alien species arises from the harm they can cause within their naturalized ranges. After alien species are introduced, they often compete with native organisms from the same trophic level, potentially reducing species diversity (Vilà *et al.* 2011; Linders *et al.* 2019). Furthermore, due to the interconnectedness of ecosystems, alien species can indirectly affect the functions of other trophic levels through cascade effects. When alien species rapidly spread, grow, and reproduce in naturalized ranges, they can become invasive and dominate communities, even disrupting ecosystem structure and functioning (Walsh *et al.* 2016; Feit *et al.* 2020). Many countries worldwide have invested substantial economic resources in preventing and managing alien invasive species (Liu *et al.* 2021; Zenni *et al.* 2021), but new alien species still continue to emerge. Consequently, it is particularly critical to explore how alien species interact with other species within their naturalized ranges.

Species interaction in invaded ecosystems

Species interactions influence their coexistence and are also intrinsic drivers of community composition (Callaway *et al.* 2002; Losapio *et al.* 2021; Lebrija-Trejos *et al.* 2023). When alien species are introduced into a community, they often engage in resource competition with native species (Wang *et al.* 2017). Charles Elton, a pioneer in invasion ecology, suggested in his book, *The Ecology of Invasion by Animals and Plants*, that the success of alien species is likely attributed to their strong competitive abilities (Elton 1958). Many studies have explored the invasion mechanisms of alien species by testing competition outcomes between alien and native species (Golivets & Wallin 2018). However, due to variations among invaded communities and the complexity of species interaction networks, pairwise-species studies may not accurately predict the general invasion patterns (Gibbs *et al.* 2022). Thus, it is relevant to utilize multi-species studies to explore the invasiveness of alien species.

The classical diversity-invasibility hypothesis posed that communities with higher species richness are more resistant to invaders (Elton 1958). Local-scale studies often support this negative relationship (Levine 2000; Kennedy *et al.* 2002; van Ruijven *et al.* 2003). Due to the promotion of biodiversity by spatial heterogeneity (Stein *et al.* 2014), however, some studies at large spatial scales have yielded contrasting results (Jauni & Hyvonen 2012; Peng *et al.* 2019). Temporally, because of the dynamic development of native communities, their interactions with alien species are not static (Diez *et al.* 2010; Dostál *et al.* 2013). In other words, the interaction patterns of alien species with native community diversity may change over time. Therefore, testing at multiple time points can provide a more comprehensive understanding of the dynamic development between alien species and native communities.

Invaded ecosystems often experience subsequent invasions (Kuebbing & Nuñez 2016; Banks *et al.* 2018). While subsequent invaders will interact with the native community, they will also be affected by previous invasions. Futhurmore, when multiple alien species was invaded simultaneously, they can also affect each other, thereby increasing the complexity of species interactions. At the end of last century, Simberloff and Von Holle (Simberloff & Von Holle 1999) formulated the invasional meltdown hypothesis, posing that synergistic interactions between alien invaders can promote the establishment of other alien invaders. Multiple lines of evidence from various ecosystems show that such positive interactions between alien species can occur both between primary producers and also between producers and consumers (Bourgeois *et al.* 2005; Relva *et al.* 2010; Green *et al.* 2011). However, these alien species will not act independently of the constraints from the native community. In this case, exploring the joint effects of native community diversity and these facilitations on invasion success is of practical significance.

Plant-soil interaction on species coexistence

Species interactions are often influenced by their growth substrates. In terrestrial ecosystems with soil as the primary substrate, interactions between aboveground and belowground multi-trophic levels are closely related (Jing *et al.* 2022). With extensive research by ecologists and soil scientists on plant-soil interactions, the meta-analysis based on large number of experimental studies have found that the performance of terrestrial plants can be influenced by how the soil was altered by previous occupants (soil-legacy effects;

Kulmatiski *et al.* 2008; Lekberg *et al.* 2018; Crawford *et al.* 2019). In the temporal dimension, as soils had undergone multiple generations of plant establishment and reproduction, their biotic and abiotic components have been gradually altered, consequently exerting positive or negative effects on subsequent plants and their offspring (Bever *et al.* 1997; van der Putten *et al.* 2013) . Moreover, due to the variation in distribution and growth of plant species on the soil, the composition of soil legacies can also exhibit spatial heterogeneity, which may drive species coexistence within the resident community (Bever 2003; Mangan *et al.* 2010; Revilla *et al.* 2013).

When alien plants established in the community, the dynamics of the belowground component become more complicated. Alien plants, on the one hand, are considered to experience fewer belowground natural enemies in their naturalized ranges (Keane & Crawley 2002; Mitchell & Power 2003), resulting in a competitive advantage over native plants. On the other hand, alien plants are also thought to release novel allelochemicals into the soil (Callaway & Ridenour 2004; Callaway *et al.* 2004), which may further increase growth inequalities between alien and native species. However, soil legacy is often shaped by multiple species in a community and heterogeneously distributed, resulting in the performance of alien plants being constrained by the composition of soil legacy. Additionally, this spatial heterogeneity of soil legacy may also contribute to their dominance due to their plasticity in naturalized ranges (Richards et al. 2006; Davidson et al. 2011; Chen et al. 2019). In this case, the performance of alien plants will somewhat depend on both the composition and distribution of the soil legacy. Therefore, incorporating the interactions among alien and native species and the various soil-legacy conditions in which they grow can provide a more comprehensive understanding of the general patterns of species coexistence.

Approach and contribution of the thesis

To better understand the joint effects of plant-plant and plant-soil interactions on plant invasions and the coexistence of alien and native plants, I conducted four experimental studies (Fig. 1). First, I tested how the diversity of native communities interacted with first-generation invaders (Chapter 1). I then incorporated the interactions between previous invaders, subsequent invaders and diverse native communities under different soil-legacy conditions (Chapter 2). By moving beyond pairwise competitive interactions and introducing additional competitors, I further tested more complex multi-species interactions and their responses to various soil legacies and soil-legacy spatial heterogeneity (Chapter 3 and 4).

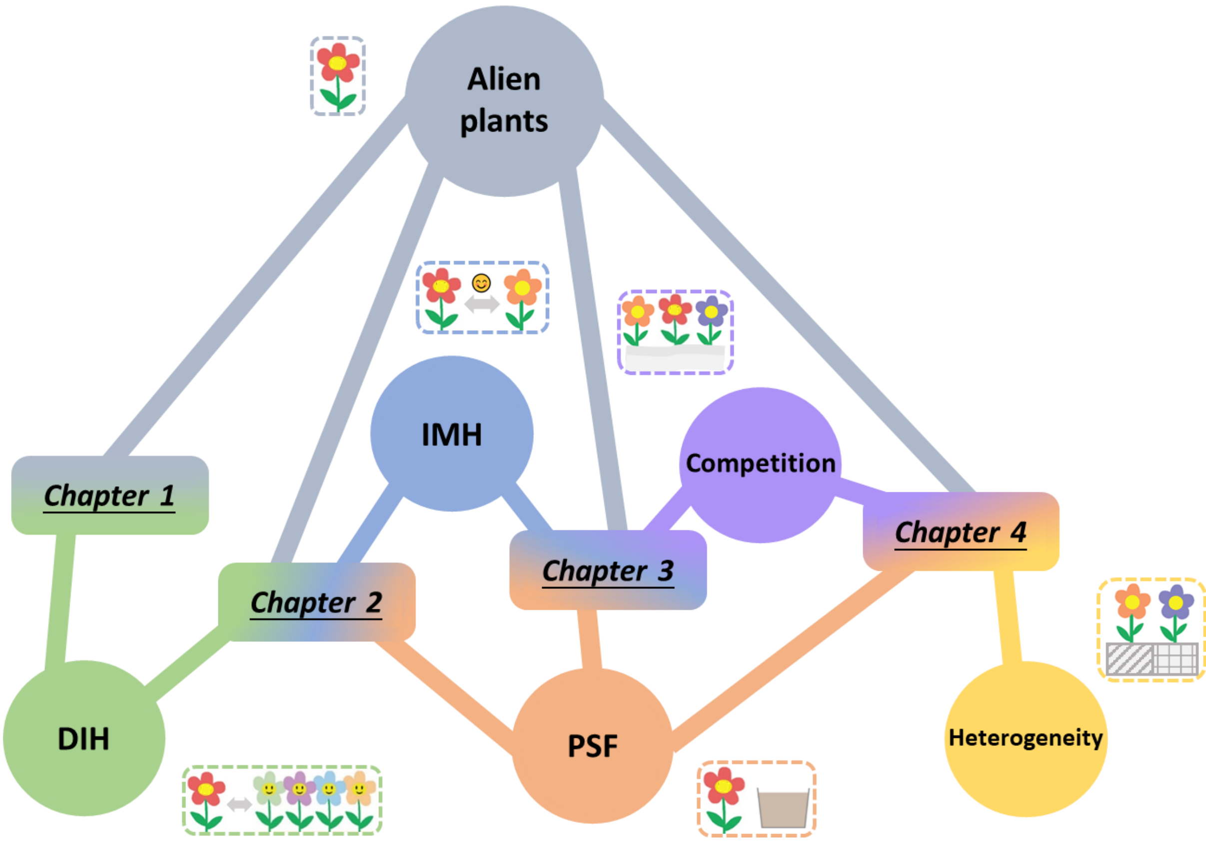

Figure 1 The overview of the thesis structure and the links between chapters. The four chapters overall focused on the performance of alien and native plant species under different plant-plant and plant-soil conditions. The first two chapters were related to diversity-

invasibility hypothesis (DIH). Chapter 2 and 3 were related to the invasional meltdown hypothesis (IMH). The last three chapters tested soil-legacy effects on plants through plant-soil feedback (PSF) processes, and the last two chapters focused on multi-species competitions under various soil-legacy and soil spatial heterogeneity conditions.

In **Chapter 1**, to test the relationship between community diversity and invasibility, I conducted an experiment through growing five alien species and five native species (as control) with or without 21 native plant assemblages of three diversity levels (1, 2 or 4 native species). To test if competitive effects and responses of invaders changed over time, I also harvested them in the start, middle and late growth stages, respectively. I asked: (1) how does the presence of a native competitor assemblage (i.e. a community) affect growth of the invader, and what is the impact of species richness of the competitor assemblage? (2) How do invaders affect the productivity of the plants they compete with, and does it depend on the origin of the invader and species richness of the competitor assemblage? (3) Do the competitive effects and responses of the invader change over time?

In **Chapter 2**, to test how various soil legacies from previous invaders and communities affect subsequent invasion, I performed a plant-soil feedback experiment based on the diversity-invasibility hypothesis and the invasional meltdown hypothesis. I grew five alien and five native focal species again on the soils previously trained by invaders and/or communities. I asked: (1) do the effects of soil conditioned by invaders and/or communities differ between alien and native test species (i.e. subsequent invaders)? If so, (2) how does the alien-native origin of the previous invader affect the growth of the subsequent alien and native test species, and (3) how does this effect change with the presence and diversity of a co-occurring community?

In **Chapter 3**, I conducted a two-phase plant-soil-feedback experiment by including multi-species competition to test if the invasional meltdown could happen between co-competing alien species. I first grew each of four alien and four native herbaceous species separately to condition the soil. In the subsequent test phase, I then grew all 16 pairwise alien-native species combinations on unconditioned soil or soils conditioned by the respective alien species, the respective native species or a mixture of both, and with or without an additional alien or native species. I asked: (1) how do the different soil legacies affect the growth of and competition between the alien and native species? (2) How does the presence of an additional alien or native species affect the growth of and competition between the alien and native species? (3) Do the soil-legacies and the additional species interact in their effects on growth and competition between the alien and native species, and does it matter whether the additional species is alien or native?

In **Chapter 4**, I continued to conduct a plant-soil feedback experiment with species competition under heterogeneous soil-legacy conditions. I first conditioned soil by five alien and five native herbaceous plants separately. Thereafter, I grew all 45 pairwise species combinations (25 alien-native, 10 alien-alien and 10 native-native pairs) under four homogeneous and two heterogeneous soil-legacy conditions. I aimed to test (1) whether soil-legacy effects on plant biomass production are negative, but weaker for alien species than for native species, (2) whether soil-legacy effects reduce growth inequalities of the competing species when grown on soil conditioned by only the larger of the two species, and increase growth inequalities when grown on soil conditioned by only the smaller of the two species, (3) whether the effects of soil legacies on size inequalities are strongest when both competitors are natives, and (4) in alien-native species pairs, whether the proportional biomass of the alien species (relative to the native species) decrease on soil conditioned by the alien species and increase on soil conditioned by the native species, with intermediate values in soils conditioned by both species.

CHAPTER 1 Competitive effects of plant invaders on and their responses to native species assemblages change over time

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Abstract

Alien plant invaders are often considered to be more competitive than natives, and species-rich plant communities are often considered to be more resistant to invaders than species-poor communities. However, the competitive interactions between invaders and assemblages of different species richness are unlikely to be static over time (e.g. during a growth season). To test this, we grew five alien and five native species as invaders in a total of 21 artificial assemblages of one, two or four native competitor species. To test for temporal changes in the reciprocal effects of invaders and the competitor assemblages on each other, and how these depend on the species richness of the assemblages, we harvested plants at three growth stages (weeks 4, 8 and 12). We found that the invaders and competitor assemblages had negative effects on each other. Aboveground biomass of invaders was reduced by the presence of a competitor assemblage, irrespective of its species richness, and this difference gradually increased over time. Alien invaders accumulated more aboveground biomass than the native invaders, but only after 12 weeks of growth. Meanwhile, the invaders also negatively affected the biomass of the competitor assemblages. For multi-species assemblages, the increase in the negative effect of the presence of the invader occurred mainly between weeks 4 and 8, whereas it happened mainly between weeks 8 and 12 for the one-species assemblages. Our results suggest that although alien invaders are more competitive than native invaders, the competitive effects of the invaders on and their responses to native competitor assemblages changed over time, irrespective of the origin of the invaders.

Keywords: coexistence, community assembly, diversity-invasibility, exotic, native invader, plant invasion, resistance, species richness

Introduction

Biological invasions, as one of the major components of global change (Hobbs & Mooney 2005; Vilà *et al.* 2011; Essl *et al.* 2020), have become of high concern in recent decades. More and more alien species have established populations outside their native ranges (Strayer 2010; Dawson *et al.* 2017), resulting in biotic homogenization (Yang *et al.* 2021). The accumulation of such naturalized alien species is still increasing globally and forecasted to continue increasing (Seebens *et al*. 2017; Seebens *et al*. 2021). Some naturalized alien plant species have become widespread and dominant, and are considered invasive as they seriously threaten native biodiversity and ecosystem functioning (Richardson *et al.* 2000a; Vilà *et al.* 2011). What allows alien species to establish, what are the impacts on the native species they compete with, and what allows communities to resist invaders have therefore become major research questions in ecology.

Although invasion biology focuses on alien species, the process of invasion is not restricted to alien species, as native species can also invade communities (Valéry *et al.* 2008; Carey *et al.* 2012). Actually, invasion (or colonization) by native species is an inherent part of community assembly and metacommunity dynamics, and invading native species can also impact the other species (Holyoak *et al.* 2005). However, as alien and native species differ in their eco-evolutionary experience with the other community members, their invasion dynamics might differ (Saul *et al.* 2013). Therefore, a major question is whether the establishment and impacts of alien species differ from those of native species.

The relationships between invaders and the species they interact with has been of high interest to biologists for a long time (Darwin 1859; Elton 1958; Fridley *et al.* 2007; Howeth 2017; Li *et al.* 2022). For example, Elton (1958) proposed that more diverse communities should be more resistant to invaders. This now classic diversity-invasibility hypothesis is based on the idea that more niches are already occupied in species-rich communities than in species-poor ones. This reduces the available resources in species-rich communities and thereby creates a more competitive environment. Consequently, it will be more difficult for invaders to establish when they have to compete with multiple species (Knops *et al.* 1999; Levine *et al.* 2004). While theoretical studies generally support the diversity-invasibility hypothesis, empirical studies have provided inconsistent results (Levine & D'Antonio 1999). In particular, studies at large spatial scales frequently find positive instead of negative relationships (Levine 2000; Shea & Chesson 2002). However, even in studies at small spatial scales, the diversity-invasibility relationship is not always negative, as it can depend on the environmental conditions (Naeem *et al.* 2000; Zeiter & Stampfli 2012) and community productivity (Davies *et al.* 2007). In more diverse communities, the growth of plants can be limited by resource availability (e.g. light and nutrients), and this may also affect the possibility of invasion (Mata *et al.* 2013; Kelso *et al.* 2020).

While invaders may impact the native community, and the latter might affect the establishment success of the invader, these competitive effects and responses are not static over time (Dostál *et al.* 2013; Yelenik & D'Antonio 2013). Some studies have shown that the negative effects of invaders on their competitors are more pronounced at the early stages, that is, the superior competitiveness of invaders is more likely to provide advantages in the early stages of growth (Goldberg 1990; Golivets & Wallin 2018). Meanwhile, the change in reciprocal effects may also be related to the species richness of the community (i.e. the competitive environment, Clark & Johnston 2011; Clark *et al.* 2013). Studies have shown that species-richness effects can become important during later stages of establishment (Nitschke *et al.* 2010; Roscher *et al.* 2013). After a period of growth, a species-rich community can establish a more stable community structure (Cavieres & Badano 2009), so that invaders are more strongly suppressed than when competing in a species-poor community. However, it could also be that the often high competitive ability of alien invaders may be sufficient to overcome the competitive pressures (Ridenour *et al.* 2008; Golivets & Wallin 2018). In that case, the biomass of the competitors could decrease without obvious suppression of the invaders. Therefore, the competitive effects and responses of invaders need to be assessed at different time points during the growth period.

To test how alien and native plant invaders and native competitor assemblages of different species richness affect each other over time, we conducted a mesocosm experiment using five alien and five native invader species and 21 competitor assemblages of three species-richness levels (1, 2 or 4 native species). To test if competitive effects and responses of invaders changed over time, we had three harvesting times (4, 8 and 12 weeks after the start of the experiment). We addressed the following specific questions: (1) How does the presence of a native competitor assemblage (i.e. a community) affect growth of the invader, and does it depend on the origin of the invader and species richness of the competitor assemblage? (2) How do invaders affect the productivity of the plants they compete with, and does it depend on the origin of the invader and species richness of the competitor assemblage? (3) Do the competitive effects and responses of the invader change over time?

Materials and Methods

Study species

To test the effects of alien and native invaders on competitor assemblages of different diversities, we selected five pairs of taxonomically related species to be used as invaders. Each pair consisted of one species that is a naturalized alien and one species that is native to Germany. The five pairs of species are from four families, as we chose two pairs of Poaceae so that the numbers of grasses and forbs were relatively balanced (Table S1). To test the effects of competitor assemblages of different diversities on the invaders, we chose a native species pool of seven species (five grasses and two forbs) that frequently coexist in German grasslands (Table S2a). The alien-native classification of all species used in the experiment was based on the FloraWeb database (www.floraweb.de). Seeds of six species were from the Botanical Garden of the University of Konstanz, and seeds of the other 11 species were ordered from Rieger-Hofmann GmbH (Tables S1, S2a).

Experimental set up

From 10 to 17 February 2020, we sowed the invader and competitor species in trays (18 cm × 14 cm × 5 cm) filled with potting soil (Einheitserde, Gebr. Patzer GmbH & Co. KG, Sinntal, Germany). This was done on different dates (Tables S1, S2a), based on prior knowledge about the time required for germination, so that the seedlings would be in a similar growth stage at transplanting. Seedling cultivation was done in a greenhouse of the Botanical Garden of the University of Konstanz (47°41’33” N, 9°10’35” E) with a temperature maintained between 18°C and 25°C.

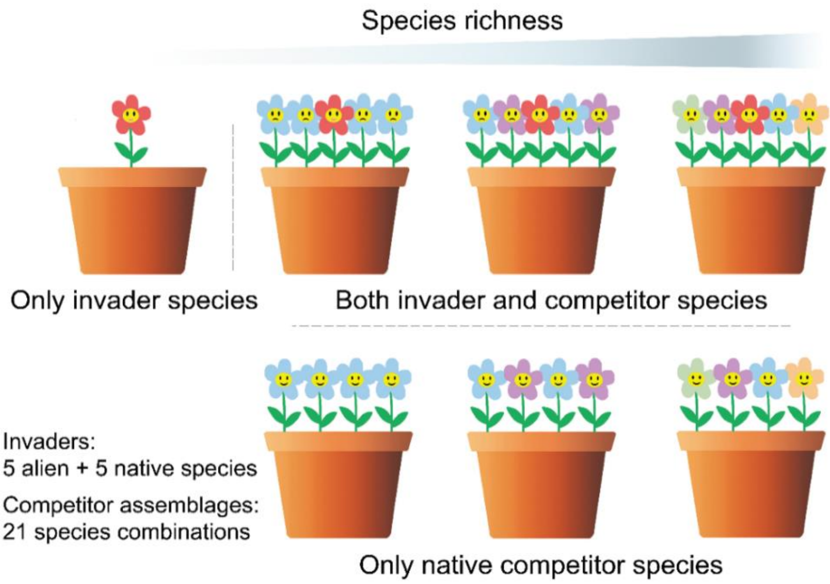

Figure 1 Overview of the experimental design. For the treatments with both an invader and competitors, five seedlings (one invader and four competitor seedlings) were planted into each pot. There was one control treatment with only plants of the native competitor assemblage (four seedlings per pot). The competitor assemblages were created with three species-richness levels: one, two and four native species. Another control treatment had only the invader species (without the competitors; one seedling per pot). All treatment combinations were replicated three times, and one replicate was harvested at each of the three time points (weeks 4, 8 and 12).

For the experiment, we filled 3L pots (Ø = 16 cm, H = 12 cm) with a soil substrate consisting of a mixture of field soil, sand and vermiculite (v:v:v = 1:1.5:1.5). The field soil, which served as inoculum of a natural soil microbiome, was dug up from a native grassland patch in the Botanical Garden of the University of Konstanz and was sieved using a 1-cm metal mesh to remove large plant fragments and pebbles. On 3 and 4 March 2020, we transplanted the seedlings into the pots. We used the pool of seven native species to create a total of 21 competitor assemblages that had different species-richness levels (Table S2b): one, two and four species. For each of the three species-richness levels, we had seven different assemblages. We first transplanted into each pot four individuals of the competitor assemblage. Specifically, we planted four seedlings of the same species in the one-species pots, two seedlings from each of the two species in the two-species pots, and one seedling from each of the four species in the four-species pots. After planting the competitors, we transplanted one of the ten invaders in the center of each pot (Fig. 1). To assess the effect of the presence of a competitor assemblage on the invader, we also had pots in which we only planted a single invader individual (i.e. without competitors; zero-species assemblage). Furthermore, to assess the effect of the invader on the competitors, we also had pots for each competitor assemblage in which we only planted the four individuals of the competitors (i.e. without an invader). Seedlings that died within two weeks after transplanting were replaced. For the two control treatments (i.e. only with invader, only with competitors), we replicated each invader species or competitor assemblage three times, resulting in 909 pots in total ([21 assemblages × 10 invaders + 21 assemblages × 3 replicates + 10 invaders × 3 replicates] × 3 harvest times). All pots were placed on plastic dishes (Ø = 20 cm) and randomly allocated to positions in three greenhouse compartments (24°C/18°C day/night temperature, 16h/8h day/night light). We watered the plants every 1-2 days, and fertilized them six times (1, 3, 5, 7, 9 and 11 weeks after the start of the experiment) with a water-soluble fertilizer (1‰ m/v, Universol Blue).

Measurements

At the start of the experiment, we counted on each invader seedling the number of leaves, and measured the length and width of the largest leaf. From these measurements, we calculated the initial leaf area as number of leaves × length of largest leaf × width of largest leaf. In order to test the reciprocal effects of invaders and competitors over time, we selected three time points for harvesting: week 4, week 8 and week 12. These time points were chosen to represent the early, mid and late growth stages of the species during a season. On 15 April, 13 May and 10 June 2020, we harvested one third of the plants in each treatment combination. After each harvest, the remaining pots were re-randomized to reduce potential effects of environmental heterogeneity in the greenhouse compartments. We separately harvested the aboveground biomass of each individual plant. The belowground biomass, we only harvested at week 4, because it was impossible to separate the roots of the different species at weeks 8 and 12. The biomass of each individual was dried to constant weight at 70°C, and then weighed with an accuracy of 0.001g. To compare differences in biomass between treatments, we calculated the percentage of change in biomass, using the raw data, as (Mean of biomass in the focal treatment - Mean of biomass in reference treatment)/Mean of biomass in reference treatment.

Statistical analysis

To test the effects of origin of the invader and species richness of the competitor assemblage on invader performance over time, we fitted a linear mixed model with the *lme* function in the R package '*nlme*' (Pinheiro *et al.* 2019). This was done for the subset of pots with invaders, and aboveground biomass of the invaders was the main response variable. In addition, to test whether the dominance of the invader relative to the competitors depended on origin of the invader, species richness of the competitor assemblage and time, we also analysed the proportional invader biomass (i.e. aboveground biomass of the invader / [aboveground biomass of the invader + aboveground biomass of the competitors]) as the response variable. The latter was done for the subset of pots with both invader and competitor plants. Invader origin (alien or native), species richness of the competitor assemblage (0, 1, 2 and 4 species or 1, 2 and 4 species when pots without competitors were excluded), harvesting time (weeks 4, 8 and 12) and their interactions were included as fixed effects in the models. For species richness of the competitor assemblage, we also ran orthogonal hierarchical contrasts to test the effect of the presence of the competitors (i.e. without competitors vs. the average of one-, two- and four-species competitor assemblages; this contrast was not included for proportional aboveground biomass), the effect of having multiple species as competitors (i.e. one-species assemblages vs. the average of two- and four-species assemblages), and the effect of having more species in the multi-species competitor assemblages (i.e. two-species assemblages vs. four-species assemblages). To account for variation in initial size of the invaders, we included the initial leaf area of the invaders as a covariate.

To test the effects of the presence of the invader and its origin, and of the species richness of the competitor assemblages on performance of the assemblages over time, we fitted again a linear mixed model. This was done for the subset of pots with competitors, and aboveground biomass of the competitor assemblage and total aboveground biomass per pot (i.e. cumulative biomass of the invader and competitors) were used as response variables. Invader treatment (without invader, with alien invader or with native invader), species richness of the competitor assemblage (1, 2 and 4 species), harvesting time (weeks 4, 8 and 12) and their interactions were included as fixed effects. For the invader treatment, we generated two orthogonal contrasts: without vs. with invader, and alien vs. native invader. We also generated two orthogonal contrasts for species richness of the competitor assemblage: one-species assemblages vs. the average of two- and four-species assemblages, and two-species assemblages vs. four-species assemblages.

To test whether the belowground parts of plants show a similar response as the aboveground parts, we also fitted two linear mixed effects models to analyse the belowground biomass of the invaders and competitor assemblages, respectively, at the first harvest time (i.e. week 4). For the invaders, this was done for the subset of pots with invaders in week 4, and belowground biomass and root weight ratio of the invaders were the response variables. Invader origin (alien or native), species richness of the competitor assemblages (included as three orthogonal contrasts: without competitors vs. the average of one-, two- and four-species assemblages, one-species assemblages vs. the average of two- and four-species assemblages, and two-species assemblages vs. four-species assemblages) and their interactions were included as fixed effects. We also included initial leaf area of the invaders as a covariate in the model. For the competitor assemblages, belowground biomass and root weight ratio (i.e. belowground biomass allocation) for the subset of pots with competitors in week 4 were used as response variables. Invader treatment (two orthogonal contrasts: without vs. with invader, and alien vs. native invader), species richness of the competitor assemblage (included as two orthogonal contrasts: one-species assemblages vs. the average of two- and four-species assemblages, and two-species assemblages vs. four species assemblages) and their interactions were included as fixed effects.

In all models, to account for phylogenetic non-independence of species, and non-independence of plants belonging to the same species, we included species identity and family of the invader plants as random effects. To account for non-independence of measurements in pots with the same competitor assemblage, we also included assemblage identity as a random effect. To meet the assumption of normality, aboveground biomasses of invaders and competitor assemblages were cubic-root-transformed. To improve homoscedasticity of residuals of the models, we allowed the variance to vary among invader species and/or the assemblage identity (Table S6) by using the *varComb* and/or *varIdent* functions of '*nlme'* package. For all models, we used log-likelihood ratios, which are approximately χ^2 distributed, to assess the significances of the fixed effects by comparing models with and without the effect of interest (Zuur *et al.* 2009). All analyses were conducted with R 3.6.2 (R Core Team 2019). An effect was considered significant if $P < 0.05$.

Results

Effects of competitor presence and species richness on invaders at different times

Across all competitor-assemblage treatments, the aboveground biomass of alien and native invaders did not differ significantly after four and eight weeks of growth (Table 1, Fig. 2b). However, after 12 weeks of growth, the alien invaders had produced significantly more aboveground biomass than the native ones (+16.3%; Table 1, Fig. 2a). Compared to the treatment without competitors (i.e. 0-species assemblage), aboveground biomass of invaders was significantly lower in the presence of competitors, and this difference gradually increased over time (-35.5% in week 4, -53.1% in week 8, and -55.5% in week 12; Table 1, Fig. 2b). Belowground biomass of the invader, which was only measured at week 4, was also significantly reduced by the presence of competitors (-45.3%), while the root weight ratio was not significantly affected (Table S4, Fig. S1).

Among the pots with competitors, aboveground biomass of the invader was not significantly affected by the species richness of the competitor assemblage (one-species vs. multi-species assemblages, and two-species vs. four-species assemblages; Table 1, Fig. 2b). The proportional biomass of the invaders relative to the competitors was not significantly affected by the origin of the invader, species richness of the competitor assemblage and time (Table S3, Fig. 3c).

Table 1 Effects of invader origins (alien or native), presence and species richness of the native competitor assemblage (0, 1, 2 or 4 species), harvesting time (week 4, week 8 or week 12) and their interactions on aboveground biomass of invader plants. For the factor Species richness, we created three orthogonal contrasts ($R_{Without/With}$: without competitors vs. average of one-, two- and four species assemblages, $R_{One-/Multi-species}$: one-species assemblages vs. average of two- and four-species assemblages, $R_{Two-/Four-species}$: two-species assemblages vs. four-species assemblages).

		Aboveground biomass	
	df	χ^2	*P*
Fixed effects			
Initial leaf area of invader	1	15.542	**<0.001**
Origin of invader (O)	1	0.339	0.560
Species richness of assemblage (R)	3	18.319	**<0.001**
$R_{Without/With}$	1	17.643	**<0.001**
$R_{One-/Multi-species}$	1	0.750	0.387
$R_{Two-/Four-species}$	1	0.868	0.352
Time of harvest (T)	2	479.275	**<0.001**
O × R	3	0.950	0.813
O × $R_{Without/With}$	1	0.179	0.672
O × $R_{One-/Multi-species}$	1	0.780	0.377
O × $R_{Two-/Four-species}$	1	0.006	0.936
O × T	2	8.655	**0.013**
R × T	6	83.415	**<0.001**
$R_{Without/With}$ × T	2	79.256	**<0.001**
$R_{One-/Multi-species}$ × T	2	4.435	0.109
$R_{Two-/Four-species}$ × T	2	0.614	0.736
O × R × T	6	1.921	0.927
O × $R_{Without/With}$ × T	2	0.492	0.782
O × $R_{One-/Multi-species}$ × T	2	1.034	0.596
O × $R_{Two-/Four-species}$ × T	2	0.380	0.827
Random effects		SD	
Invader family		0.206	
Invader species*		0.314	
Assemblage identity		0.068	
Residual		0.166	

Values are in bold when $P < 0.05$. * Shown is the standard deviation (SD) of *Lepidium virginicum*. The SDs of all invader species are shown in Table S6.

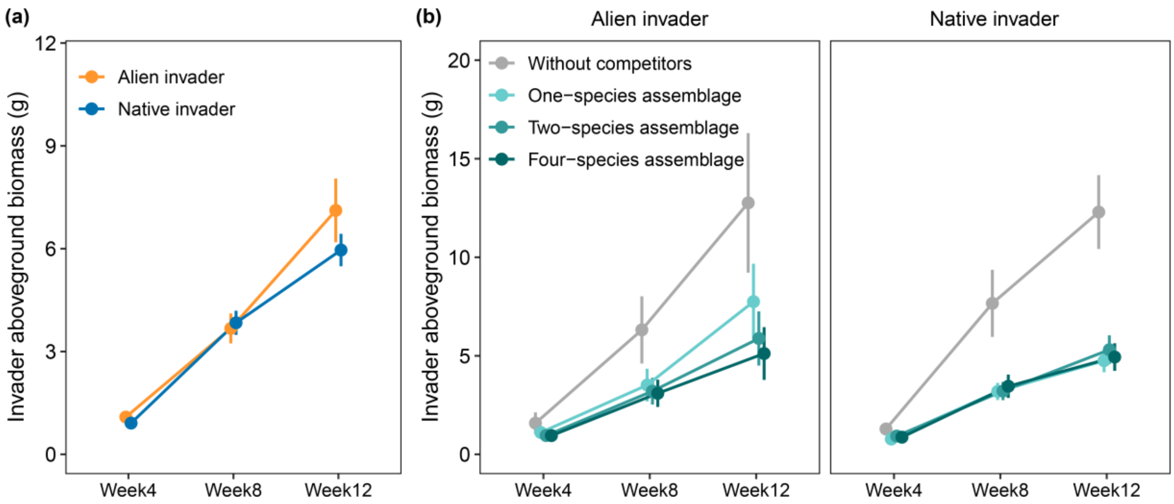

Figure 2 a, Aboveground biomass of alien and native invaders at each of the three harvests; **b**, Aboveground biomass of alien and native invaders in the absence or presence of native competitor assemblages of different species richness at each of the three harvests. Shown are means (±SEs) of the raw data.

Effects of invaders on competitors and overall productivity at different times

After four weeks of growth, the aboveground biomass of the competitor assemblage, irrespective of its species richness, was not affected by the presence of the invader. The same was true for belowground biomass and the root weight ratio of the competitor assemblages (Table S5, Fig. S2). After 8 and 12 weeks of growth, however, the presence of the invader had a significant negative effect on the aboveground biomass of the competitors (Table 2, Fig. 3a). Moreover, while the increase in the negative effect of the presence of the invader happened mainly between weeks 4 and 8 for the multi-species competitor assemblages (two-species assemblages: -8.4% in week 4, -28.7% in week 8 and -15.7% in week 12; four-species assemblages: -0.2% in week 4, -20.6% in week 8 and -11.0% in week 12), it happened mainly between weeks 8 and 12 for the one-species assemblages (-2.7% in week 4, -12.5% in week 8 and -27.1% in week 12; Table 2, Fig. 3a). The origin of the invader did not have a significant effect on the aboveground biomass of the competitor assemblage (Table 2, Fig. 3a).

The total aboveground biomass per pot was not significantly affected by the species richness of the competitor assemblage (Table 2, Fig. 3b). However, having an invader plant, in addition to the competitors, increased biomass for the one-species assemblages after week 4, whereas, for the multi-species assemblages, it only increased biomass at week 12 (significant IWithout/With × ROne-/Multi-species × T in Table 2, Fig. 3b). There was no significant effect of the origin of the invader (Table 2, Fig. 3b).

Table 2 Effects of invader treatment (without invader, with alien or native invader), species richness of competitor assemblage (1, 2 or 4 species), harvesting time (week 4, week 8 or week 12) and their interactions on aboveground biomass of the competitor assemblage and the total aboveground biomass per pot. For the factor Invader, we created two orthogonal contrasts ($I_{Without/With}$: without vs. with invader, $I_{Alien/Native}$: with alien vs. with native invader). For the factor Species richness, we created two orthogonal contrasts ($R_{One\text{-}/Multi\text{-}species}$: one-species assemblages vs. average of two- and four species assemblages, $R_{Two\text{-}/Four\text{-}species}$: two-species assemblages vs. four-species assemblages).

		Aboveground biomass of competitors		Total aboveground biomass per pot	
	df	χ^2	*P*	χ^2	*P*
Fixed effects					
Invader treatment (I)	2	2.870	0.238	0.505	0.777
$I_{Without/With}$	1	2.559	0.110	0.505	0.477
$I_{Alien/Native}$	1	0.368	0.544	0.000	0.997
Species richness of assemblage (R)	2	1.507	0.471	1.351	0.509
$R_{One\text{-}/Multi\text{-}species}$	1	1.506	0.220	1.011	0.315
$R_{Two\text{-}/Four\text{-}species}$	1	0.001	0.978	0.368	0.544
Time of harvest (T)	2	1877.817	**<0.001**	1888.372	**<0.001**
I × R	4	7.421	0.115	1.860	0.761
$I_{Without/With} \times R_{One\text{-}/Multi\text{-}species}$	1	0.003	0.954	0.020	0.889
$I_{Without/With} \times R_{Two\text{-}/Four\text{-}species}$	1	5.053	**0.025**	0.842	0.359
$I_{Alien/Native} \times R_{One\text{-}/Multi\text{-}species}$	1	0.006	0.938	1.041	0.308
$I_{Alien/Native} \times R_{Two\text{-}/Four\text{-}species}$	1	2.375	0.123	0.032	0.858
I × T	4	29.829	**<0.001**	5.454	0.244
$I_{Without/With} \times T$	2	29.675	**<0.001**	4.026	0.134
$I_{Alien/Native} \times T$	2	0.197	0.906	1.534	0.464
R × T	4	5.760	0.218	0.874	0.928
$R_{One\text{-}/Multi\text{-}species} \times T$	2	0.068	0.967	0.103	0.950
$R_{Two\text{-}/Four\text{-}species} \times T$	2	5.593	0.061	0.795	0.672
I × R × T	8	14.863	0.062	11.242	0.188
$I_{Without/With} \times R_{One\text{-}/Multi\text{-}species} \times T$	2	11.933	**0.003**	8.667	**0.013**
$I_{Without/With} \times R_{Two\text{-}/Four\text{-}species} \times T$	2	0.885	0.643	0.491	0.782
$I_{Alien/Native} \times R_{One\text{-}/Multi\text{-}species} \times T$	2	0.049	0.976	0.650	0.723
$I_{Alien/Native} \times R_{Two\text{-}/Four\text{-}species} \times T$	2	2.115	0.347	1.529	0.466
Random effects		SD		SD	
Invader family		0.001		0.073	
Invader species		0.056		0.141	
Assemblage identity*		0.083		0.048	
Residual		0.099		0.105	

Values are in bold when $P < 0.05$. * Shown is the standard deviation (SD) of the one-species assemblage of *Lolium perenne*. The SDs of all assemblage identities are shown in Table S6.

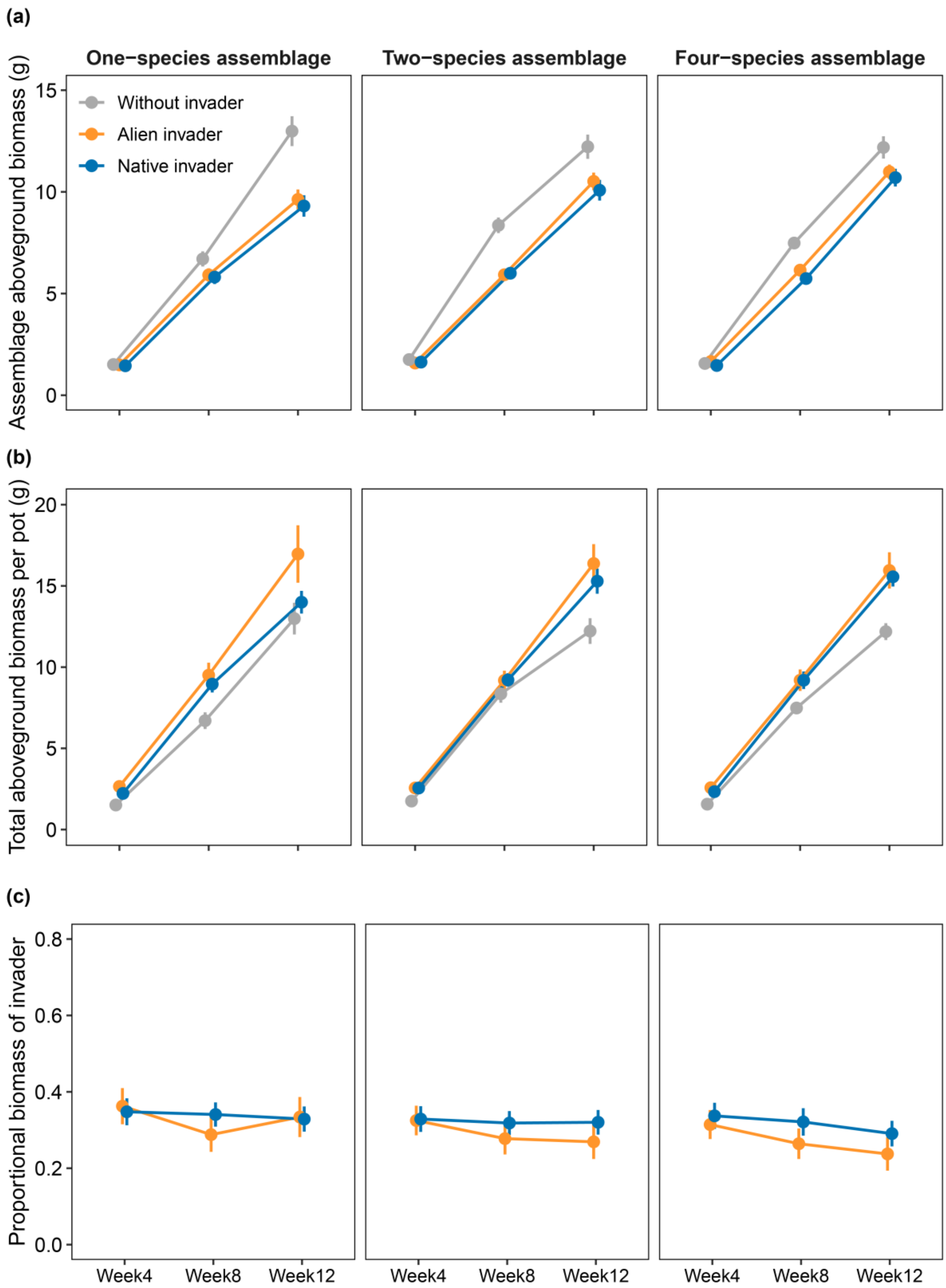

Figure 3 Aboveground biomass of native competitor assemblages (**a**) and total aboveground biomass per pot (**b**) for different competitor assemblages of different species richness and in the absence or presence of alien and native invaders at each of the three harvests; **c**, proportional aboveground biomass per pot of alien and native invaders in competitor assemblages of different species richness at each of the three harvests. Shown are means (±SEs) of the raw data.

Discussion

In our experiment on competitive effects and responses of native and alien invaders over time, we found that the invaders had strongly reduced biomass in the presence of the competitors. This negative effect of the competitors on the invaders strongly increased during the growth period, but did not significantly depend on the species richness of the competitor assemblage. The alien and native invaders produced similar amounts of biomass during the first eight weeks, but after 12 weeks, the alien invaders had produced more biomass than the native ones. Similarly, addition of single invader plants also suppressed the biomass production of the competitor assemblage, and this effect also increased over time. In the multi-species competitor assemblages (two- and four-species assemblages), this effect was already pronounced after eight weeks, whereas in the one-species assemblages, it became most obvious after twelve weeks and then even more pronounced than in the multi-species assemblages. So, although our results did not indicate major roles of the origin of the invader and the species richness of the competitor assemblage, we found that the invader and competitors reciprocally suppressed one another, and that these interactions became more intense over time.

The alien invaders only produced more biomass than the native invaders by week 12. As invasive alien plants are frequently characterized by fast early growth (Grotkopp *et al.* 2010; Dawson *et al.* 2011), and frequently produce more biomass than native species (van Kleunen *et al.* 2010), it is surprising that the difference in biomass did not appear earlier. It could be that the delay is due to the resistance provided by the competitors, which slowed down the overall growth rates of the invaders. Meanwhile, all the seedlings per pot were transplanted at the beginning of the experiment, which may eliminate the effect of differences in the phenological niche between the competitor species, and between the competitor assemblage and the invader in the growth stages (Wolkovich & Cleland 2011; Godoy & Levine 2014). Furthermore, it could reflect that not all naturalized alien species in our study are highly invasive and that the native invaders themselves are also very common. The fact that the alien invaders nevertheless produced more biomass than the native invaders at the end of the experiment may reflect that many naturalized alien species are more competitive than natives (Vilà & Weiner 2004; Kuebbing & Nuñez 2016; Golivets & Wallin 2018). Another reason why alien invaders ultimately performed better than native invaders may be that they have escaped from the co-evolved enemies in their native ranges (Keane & Crawley 2002). As we did our experiment in a greenhouse environment, it is likely that both the aliens and natives were released from aboveground herbivores. However, as we provided all pots with an inoculum of field soil, it is likely that the soil contained root herbivores and pathogens that might have preferentially attacked the native plants. The differences in competitive ability among the native plants can have been equalized by the native soil pathogens (Albornoz *et al.* 2017). Despite the difference in biomass between the alien and native invaders at the end of the experiment, they did not have different effects on the biomass production of the competitor assemblages. Moreover, the proportional biomass of the alien and native invaders in the presence of competitors was not significantly different (Fig. 3c). In other words, the slight superiority in biomass production of the alien invaders did not result in a larger dominance, at least not during the 12 weeks of our experiment.

While the presence of competitors significantly reduced the biomass of the invader, the effect of species richness of the competitor assemblage was not significant. In other words, we did not find support for Elton's diversity-invasibility hypothesis (1958). Other studies found that invader species could be strongly limited by nutrient and light availability when they competed with more diverse species assemblages (Mata *et al.* 2013; Roscher *et* *al.* 2013), whereas other studies found also no significant relationship (Smith & Côté 2019) or actually found a positive one (Jiang & Morin 2004; Zeiter & Stampfli 2012). It has, however, also been reported that the diversity-invasibility relationship can change with time (Clark & Johnston 2011). Actually, at week 12 (i.e. at the end of our experiment), there was a slight, though non-significant, trend that the invader aboveground biomass was lowest in four-species competitor assemblages and highest in one-species assemblages (Fig. 2b). So, possibly if the experiment would have lasted longer, the effect might have become significant. This would be particularly likely, if complementarity effects increase with time (Fargione *et al.* 2007). It could also be that diversity effects on resistance against invaders require higher species-richness levels than we used. Therefore, we recommend that future invasion experiments use species assemblages that are older and have higher maximum species-richness values than the ones we used.

A potential limitation of our study is that the species pool that we used to create the competitor assemblages was relatively small (n = 7). As a consequence, some of the two-species assemblages shared species with one another, and this sharing was even stronger for the four-species assemblages (each species was present in 4 of the 7 four-species assemblages). In other words, with increasing species richness, the assemblages became more similar to each other. In our study this confounding most likely had no major consequences as there were no significant effects of species richness. Visual inspection of the biomass development of each of the competitor assemblages (Fig. S3) also did not reveal clear indications that the presence of a particular species drove the differences in biomass among the assemblages. Nevertheless, we recommend that future experimental studies on the diversity-invasibility hypothesis use larger species pools to avoid such effects.

Like the presence of competitors reduced the biomass of the invader, so did reciprocally the presence of the invader reduce the biomass of the competitors. This most likely reflects that the addition of the invader increased the density of plants per pot, and that this resulted in more intense competition among the plants (Callaway & Walker 1997; Zhang & Tielbörger 2020). Moreover, the effect of the invader on the biomass of the competitor assemblage increased over time, but the pattern of this increase depended on whether the assemblage consisted of one or multiple species (Fig. 3a). Surprisingly, although we had expected that already early on in the experiment the one-species competitor assemblages would suffer more from the invader than the multi-species assemblages, the one-species assemblages only showed a clear effect of the invader at week 12, whereas the multi-species assemblages showed it already at week 8. Possibly, early on, at week 4, the plants were still so small that they hardly interacted with one another. At week 8, the plants in the one-species assemblages without invaders were still relatively small, while in the multi-species assemblages the plants were larger, as intraspecific competition is usually more intense than interspecific competition (Adler *et al.* 2018). Consequently, in the presence of the invader, the one-species competitor assemblages still hardly interacted with the invader, whereas the multi-species assemblages had to share their resources with the invader, and therefore produced less biomass. Indeed, the joint invader and competitor biomass at week 8 differed less between the invaded and non-invaded multi-species assemblages than between the invaded and non-invaded one-species assemblages (Fig. 3b). Whatever the exact reason is for these different patterns over time, at the last census the negative effect of the invader was, as expected, larger for the one-species competitor assemblages than for the multi-species ones. Again this effect might have become even stronger if the experiment would have lasted longer.

In conclusion, we found reciprocal effects of invaders and competitors, and that these effects became stronger over time. Although the alien invaders produced more biomass than the native invaders by the end of the experiment, they were not yet differently affected by the presence and species richness of the competitor assemblages. The effects of the invader on the competitors also did not yet depend on whether the invader was an alien or a native. However, at the end of the experiment, the one-species competitor assemblages were more strongly affected by the invader than the multi-species ones. So, even though our results did not support the diversity-invasibility hypothesis, if the effects that we found continue to increase over time the hypothesis might hold.

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Author contributions

DC and MvK designed the experiment, DC conducted the experiment and analysed the data. DC wrote the first draft of the manuscript with further input from MvK.

Supporting information

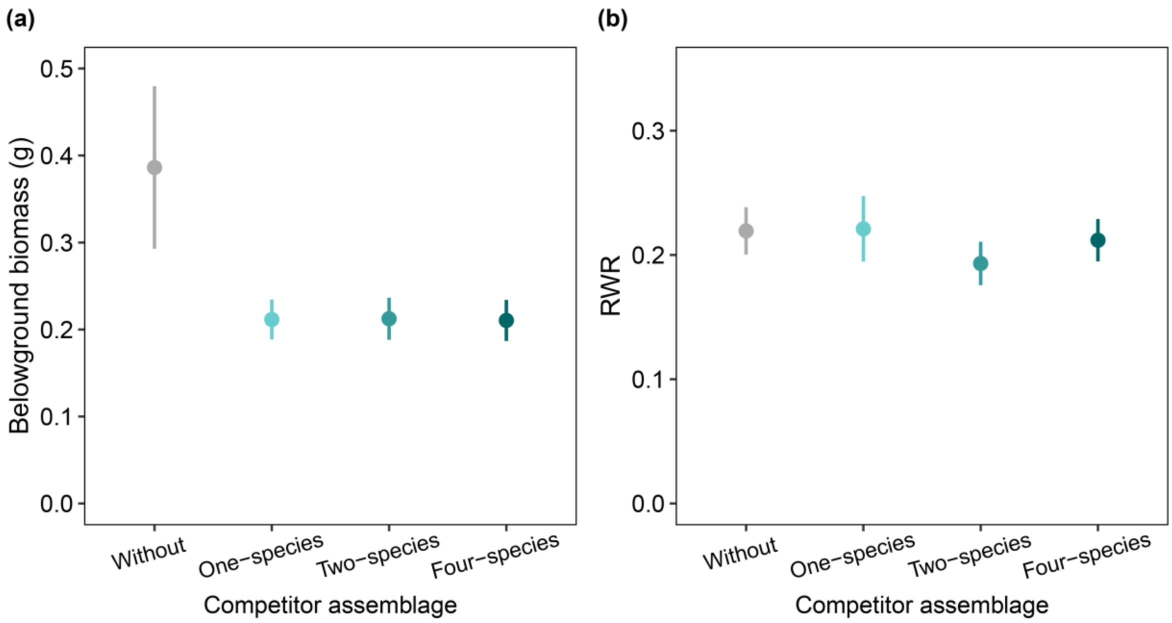

Figure S1 Belowground biomass (**a**) and root weight ratio (RWR, **b**) of invaders in the absence or presence of native competitor assemblages of different species richness at the first harvest (week 4). Shown are means (± SEs) of the raw data.

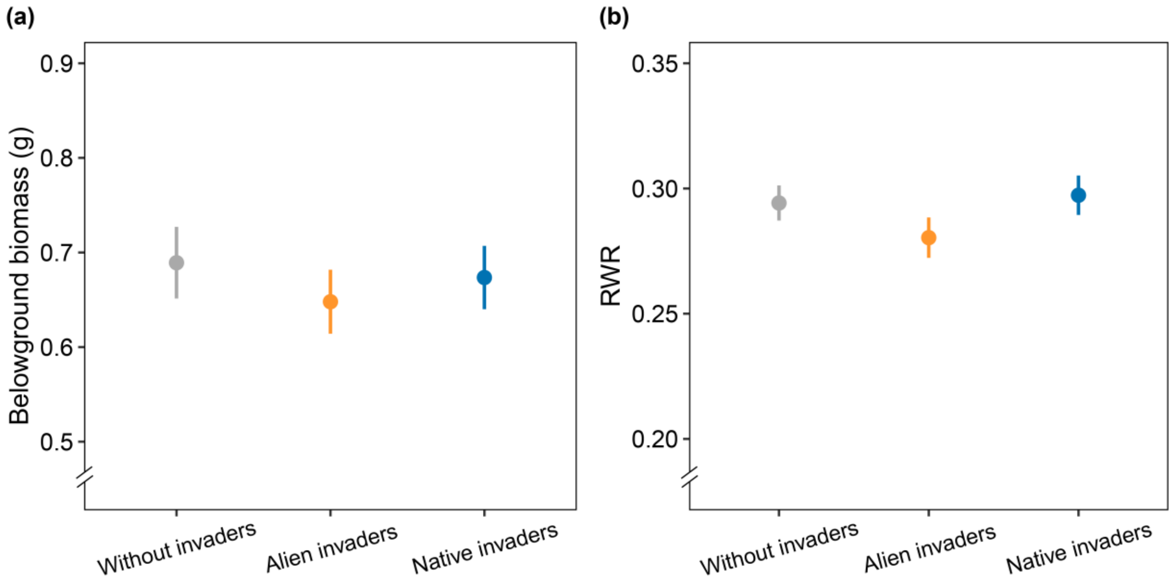

Figure S2 Belowground biomass (**a**) and root weight ratio (RWR, **b**) of the competitor assemblage in the absence or presence of alien and native invader species at the first harvest (week 4). Shown are means (± SEs) of the raw data.

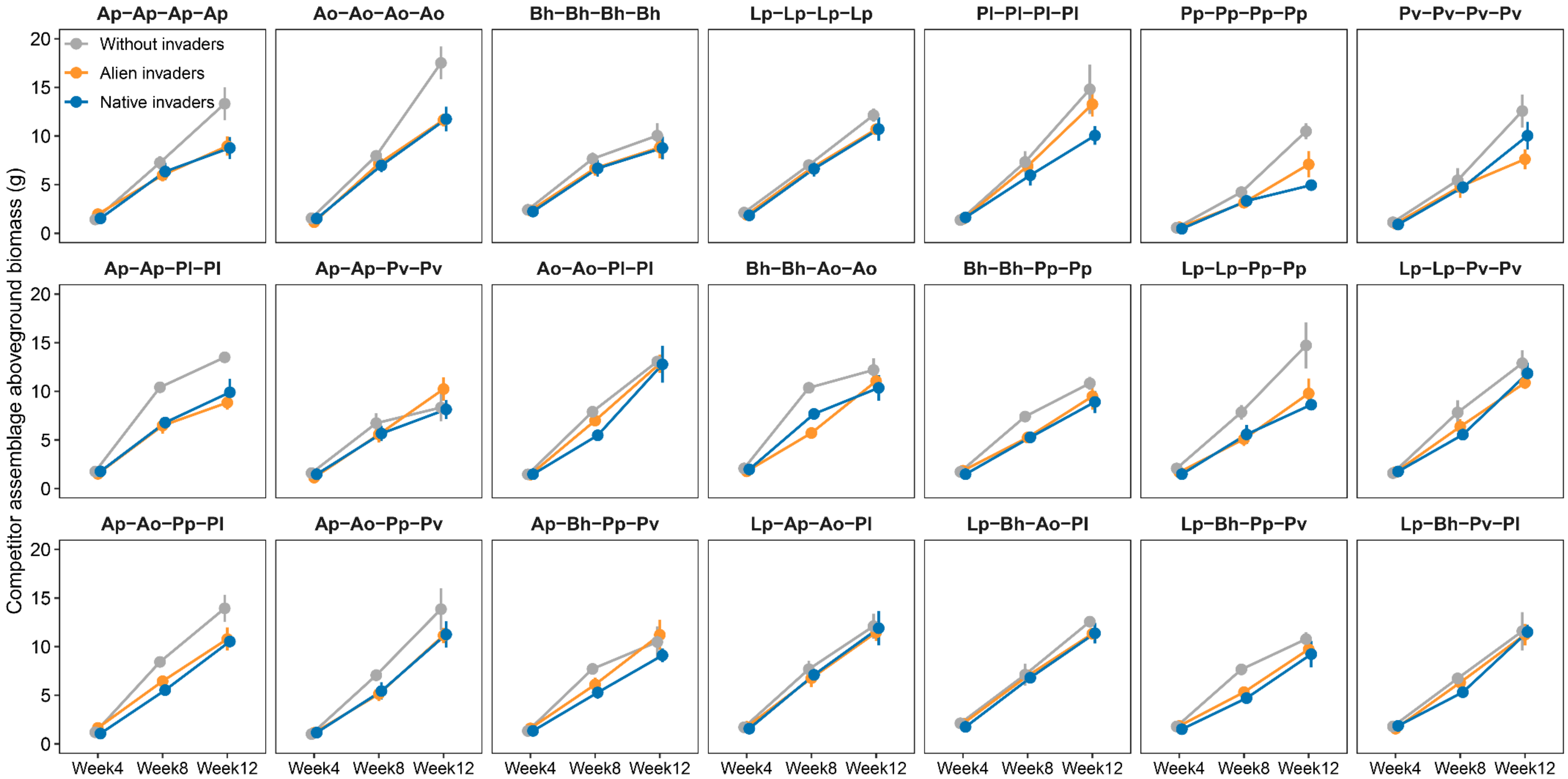

Figure S3 Aboveground biomass of each of 21 native competitor-assemblage combinations in the absence or presence of alien and native invaders at each of the three harvests. Shown are means (± SEs) of the raw data.

Table S1 Alien and native invader species used in the experiment.

Pair	Species	Family	Origin	Native range	Sowing date	Seed source
1	*Lepidium virginicum* L.	Brassicaceae	alien	North America	17 Feb, 2020	University of Konstanz, Germany
	Lepidium campestre (L.) R. Br.	Brassicaceae	native	-	17 Feb, 2020	University of Konstanz, Germany
2	*Setaria faberi* RAW Herrm.	Poaceae	alien	Asia	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
	Setaria viridis (L.) P. Beauv.	Poaceae	native	-	17 Feb, 2020	University of Konstanz, Germany
3	*Lupinus polyphyllus* Lindl.	Fabaceae	alien	North America	17 Feb, 2020	University of Konstanz, Germany
	Trifolium pratense L.	Fabaceae	native	-	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
4	*Bromus carinatus* Hook. & Arn.	Poaceae	alien	North America	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
	Bromus sterilis L.	Poaceae	native	-	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
5	*Solidago gigantea* Aiton	Asteraceae	alien	North America	10 Feb, 2020	University of Konstanz, Germany
	Senecio jacobaea L.	Asteraceae	native	-	17 Feb, 2020	University of Konstanz, Germany

Table S2 a, Competitor species used in the experiment; **b**, Combinations of species to produce seven native competitor assemblages for each of the three species-richness levels. The species names are abbreviated as the first three letters of the genus name and the first three letters of the species epithet.

(a)	Species	Family	Sowing date	Seed source
	Alopecurus pratensis L.	Poaceae	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
	Anthoxanthum odoratum L.	Poaceae	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
	Bromus hordeaceus L.	Poaceae	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
	Lolium perenne L.	Poaceae	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
	Poa pratensis L.	Poaceae	17 Feb, 2020	Rieger-Hofmann GmbH, Germany
	Prunella vulgaris L.	Lamiaceae	10 Feb, 2020	Rieger-Hofmann GmbH, Germany
	Plantago lanceolata L.	Plantaginaceae	17 Feb, 2020	Rieger-Hofmann GmbH, Germany

(b)	One-species assemblage							Two-species assemblage							Four-species assemblage						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Alo.pra	■							■						■	■				■	■	■
Ant.odo		■						■	■						■	■				■	■
Bro.hor			■						■	■					■	■	■				■
Lol.per				■						■	■				■	■	■	■			
Poa.pr					■						■	■				■	■	■	■		
Pru.vul						■						■	■				■	■	■	■	
Pla.lan							■						■	■				■	■	■	■

Table S3 Effects of invader origins (alien or native), species richness of the competitor assemblage (1, 2 or 4 species), harvesting time (week 4, week 8 or week 12) and their interactions on proportional aboveground biomass of invader plants. For the factor Species richness, we created two orthogonal contrasts ($R_{One\text{-}/Multi\text{-}species}$: one-species assemblage vs. average of two- and four-species assemblages, $R_{Two\text{-}/Four\text{-}species}$: two-species assemblages vs. four-species assemblages).

	df	χ^2	*P*
Fixed effects			
Initial leaf area of invader	1	17.446	**<0.001**
Origin of invader (O)	1	1.236	0.266
Species richness of assemblage (R)	2	0.799	0.671
$R_{One\text{-}/Multi\text{-}species}$	1	0.663	0.416
$R_{Two\text{-}/Four\text{-}species}$	1	0.145	0.704
Time of harvest (T)	2	1.618	0.445
O × R	2	2.643	0.267
O × $R_{One\text{-}/Multi\text{-}species}$	1	1.706	0.192
O × $R_{Two\text{-}/Four\text{-}species}$	1	0.965	0.326
O × T	2	1.851	0.396
R × T	4	5.276	0.260
$R_{One\text{-}/Multi\text{-}species}$ × T	2	2.893	0.235
$R_{Two\text{-}/Four\text{-}species}$ × T	2	2.369	0.306
O × R × T	4	0.906	0.924
O × $R_{One\text{-}/Multi\text{-}species}$ × T	2	0.129	0.938
O × $R_{Two\text{-}/Four\text{-}species}$ × T	2	0.782	0.676
Random effects		SD	
Invader family		0.090	
Invader species*		0.095	
Assemblage identity*		0.040	
Residual		0.073	

Values are in bold when $P < 0.05$. * Shown are the standard deviation (SD) of *Lepidium virginicum* and the SD of the one-species assemblage of *Lolium perenne*. The SDs of all invader species and assemblage identities are shown in Table S6.

Table S4 Effects of invader origins (alien or native), presence and species richness of the competitor assemblage (0, 1, 2 or 4 species) and their interactions on belowground biomass and root weight ratio (RWR) of invader plants at the first harvest time (week 4). For the factor Species richness, we created three orthogonal contrasts ($R_{Without/With}$: without competitors vs. average of one-, two- and four-species assemblages, $R_{One-/Multi-species}$: one-species assemblages vs. average of two- and four-species assemblages, $R_{Two-/Four-species}$: two-species assemblages vs. four-species assemblages).

		Belowground biomass		RWR	
	df	χ^2	*P*	χ^2	*P*
Fixed effects					
Initial leaf size of invader	1	11.541	**0.001**	0.850	0.357
Origin of invader (O)	1	0.283	0.595	0.239	0.625
Species richness of assemblage (R)	3	6.349	0.096	3.898	0.273
$R_{Without/With}$	1	6.267	**0.012**	0.872	0.350
$R_{One-/Multi-species}$	1	0.059	0.808	0.165	0.685
$R_{Two-/Four-species}$	1	0.007	0.932	2.863	0.091
O × R	3	1.481	0.687	2.029	0.566
O × $R_{Without/With}$	1	0.001	0.972	1.381	0.240
O × $R_{One-/Multi-species}$	1	1.436	0.231	0.101	0.750
O × $R_{Two-/Four-species}$	1	0.061	0.805	0.560	0.454
Random effects		SD		SD	
Invader family		0.106		0.034	
Invader species*		0.085		0.036	
Assemblage identity		0.015		0.000	
Residual		0.080		0.048	

Values are in bold when $P < 0.05$. * Shown is the standard deviation (SD) of *Lepidium virginicum*. The SDs of all invader species are shown in Table S6.

Table S5 Effects of invader types treatment (without invader, alien or native invader), species richness of competitor assemblage (1, 2 or 4 species) and their interactions on belowground biomass and root weight ratio (RWR) of the native competitor assemblage at the first harvest time (week 4). For the factor Invader, we created two orthogonal contrasts ($I_{Without/With}$: without vs. with invader, $I_{Alien/Native}$: with alien vs. with native invader). For the factor Species richness, we created two orthogonal contrasts ($R_{One-/Multi-species}$: one-species assemblages vs. average of two- and four-species assemblages, $R_{Two-/Four-species}$: two-species assemblages vs. four-species assemblages).

		Belowground biomass		RWR	
	df	χ^2	*P*	χ^2	*P*
Fixed effects					
Invader treatment (I)	2	0.468	0.791	3.527	0.171
$I_{Without/With}$	1	0.345	0.557	0.053	0.818
$I_{Alien/Native}$	1	0.127	0.721	3.497	0.061
Species richness of assemblage (R)	2	0.639	0.726	0.169	0.919
$R_{One-/Multi-species}$	1	0.609	0.435	0.028	0.868
$R_{Two-/Four-species}$	1	0.033	0.857	0.140	0.708
I × D	4	2.491	0.646	4.822	0.306
$I_{Without/With}$ × $R_{One-/Multi-species}$	1	0.258	0.612	1.956	0.162
$I_{Without/With}$ × $R_{Two-/Four-species}$	1	0.095	0.758	0.475	0.491
$I_{Alien/Native}$ × $R_{One-/Multi-species}$	1	0.085	0.770	0.278	0.598
$I_{Alien/Native}$ × $R_{Two-/Four-species}$	1	2.143	0.143	2.128	0.145
Random effects		SD		SD	
Invader family		0.000		0.004	
Invader species		0.030		0.012	
Assemblage identity*		0.102		0.021	
Residual		0.055		0.015	

* Shown is the standard deviation (SD) of the one-species assemblage of *Lolium perenne*. The SDs of all assemblage identities are shown in Table S6.

Table S6 The SDs of the ten invader species and/or 21 assemblage identities from the models shown in Tables 1, 2, S3, S4 and S5.

			Table 1†	Table 2‡		Table S3§	Table S4†		Table S5‡	
Invader species		*Lepidium virginicum*	0.314	-	-	0.095	0.085	0.036	-	-
		Lepidium campestre	0.303	-	-	0.083	0.057	0.026	-	-
		Setaria faberi	0.809	-	-	0.048	0.174	0.036	-	-
		Setaria viridis	0.618	-	-	0.057	0.136	0.044	-	-
		Lupinus polyphyllus	0.279	-	-	0.088	0.112	0.086	-	-
		Trifolium pratense	0.565	-	-	0.096	0.069	0.022	-	-
		Bromus carinatus	0.442	-	-	0.093	0.116	0.030	-	-
		Bromus sterilis	0.471	-	-	0.106	0.102	0.030	-	-
		Solidago gigantea	0.518	-	-	0.140	0.089	0.045	-	-
		Senecio jacobae	0.328	-	-	0.079	0.068	0.040	-	-
	One-species	*Lolium perenne* (*Lp*)	-	0.083	0.048	0.040	-	-	0.102	0.021
		Alopecurus pratensis (*Ap*)	-	0.124	0.061	0.063	-	-	0.229	0.056
		Bromus hordeaceus (*Bh*)	-	0.154	0.085	0.052	-	-	0.251	0.040
		Anthoxanthum odoratum (*Ao*)	-	0.121	0.055	0.060	-	-	0.179	0.029
		Poa pratensis (*Pp*)	-	0.126	0.088	0.080	-	-	0.121	0.051
		Prunella vulgaris (*Pv*)	-	0.147	0.094	0.053	-	-	0.172	0.067

Competitor-assemblage identity		*Plantago lanceolata* (*Pl*)	-	0.140	0.077	0.052	-	-	0.231	0.026
	Two-species assemblages	*Lp-Pp*	-	0.115	0.063	0.056	-	-	0.248	0.069
		Ap-Pl	-	0.096	0.055	0.050	-	-	0.136	0.034
		Bh-Ao	-	0.097	0.078	0.059	-	-	0.205	0.047
		Ap-Pv	-	0.123	0.071	0.042	-	-	0.143	0.041
		Bh-Pp	-	0.091	0.060	0.055	-	-	0.256	0.071
		Ao-Pl	-	0.126	0.061	0.056	-	-	0.175	0.044
		Lp-Pv	-	0.113	0.061	0.073	-	-	0.148	0.049
	Four-species assemblages	*Lp-Bh-Ao-Pl*	-	0.079	0.052	0.043	-	-	0.149	0.033
		Ap-Bh-Pp-Pv	-	0.111	0.063	0.076	-	-	0.141	0.050
		Lp-Bh-Pp-Pv	-	0.108	0.051	0.059	-	-	0.133	0.037
		Lp-Ap-Ao-Pl	-	0.109	0.058	0.044	-	-	0.172	0.035
		Ap-Ao-Pp-Pl	-	0.093	0.045	0.054	-	-	0.126	0.056
		Lp-Bh-Pv-Pl	-	0.097	0.058	0.049	-	-	0.235	0.053
		Ap-Ao-Pp-Pv	-	0.122	0.065	0.068	-	-	0.193	0.056

Variance structure: † invader species were allowed to have different variances by using the *varIdent* function; ‡ assemblage identities were allowed to have different variances by using the *varIdent* function; § invader species and assemblage identities were allowed to have different variances by using the *varComb* and *varIdent* functions.

CHAPTER 2 Invasional meltdown mediated by plant-soil feedbacks may depend on community diversity

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Abstract

It has been suggested that establishment of one alien invader might promote further invasions. Such a so-called invasional meltdown could be mediated by differences in soil-legacy effects between alien and native plants. Whether such legacy effects might depend on the diversity of the invaded community has not been explored yet. Here, we conducted a two-phase plant-soil-feedback experiment. In a soil-conditioning phase, we grew five alien and five native species as invaders in 21 communities of one, two or four species. In the subsequent test phase, we grew five alien and five native species on the conditioned soils. We found that growth of these test species was negatively affected by soils conditioned by both a community and an invader, and particularly if the previous invader was a conspecific (i.e. negative plant-soil feedback). Alien test species suffered less from soil-legacy effects of previous allospecific alien invaders than from legacy effects of previous native invaders. However, this effect decreased when the soil had been co-conditioned by a multi-species community. Our findings suggest that plant-soil-feedback-mediated invasional meltdown may depend on community diversity, and thus provide some evidence that diverse communities could increase resistance against subsequent alien invasions.

Keywords: alien species, belowground, coexistence, diversity-invasibility, native invader, soil legacy, subsequent invasion

Introduction

Coexistence of alien and native plants has become a common phenomenon in many ecosystems (Gross *et al.* 2015; Grainger *et al.* 2019). Some alien plants can, due to characteristics such as fast growth and a high reproductive rate (van Kleunen *et al.* 2010), even become dominant, thereby reducing native biodiversity and ecosystem functioning (Richardson *et al.* 2000b; Vilà *et al.* 2011). When alien invaders have achieved a dominant position in the communities, more invasions often follow (Kuebbing & Nuñez 2016; Banks *et al.* 2018). The phenomenon that one alien species facilitates the establishment of another one is known as invasional meltdown (Simberloff & Von Holle 1999). Indeed, previous invasions can even accelerate subsequent invasions in the invaded ecosystems (Green *et al.* 2011). Therefore, an important question in ecology is how the presence of one invader affects subsequent invaders.

It is now well established that belowground processes play important roles in plant invasion (Callaway *et al.* 2004; Wolfe & Klironomos 2005; Nuñez & Dickie 2014; Fahey *et al.* 2020). The phenomenon that plants affect subsequent plants by altering the biotic and abiotic qualities of the soil they grow on is called plant-soil feedback (Bever *et al.* 1997; van der Putten *et al.* 2013). In particular, when a species grows on soil previously occupied by conspecifics, its performance is usually reduced, indicating a negative plant-soil feedback (Bonanomi *et al.* 2005; Kardol *et al.* 2006; Mangan *et al.* 2010). However, the performance of a species might also be affected by the legacy of other species that previously grew on the soil (van der Putten *et al.* 2013; Bennett & Klironomos 2019). Such soil-legacy effects can be negative or positive, and they may depend on whether the species are native or alien. For example, a recent study showed that when alien species grew on soil that had previously been occupied by other alien species, they were more competitive than native species (Zhang *et al.* 2020b). However, in invaded ecosystems, soil-legacy effects of invaders may be modified by the presence of other community members, which will have their own soil-legacy effects. How the soil legacy of alien and native invaders and communities separately and jointly affect subsequent invaders has to the best of our knowledge not been assessed yet.

Charles Elton proposed that species-rich communities should be more resistant to invaders than species-poor communities (Elton 1958). Some studies, particularly those that focus on small spatial scales, support Elton's hypothesis that in more diverse communities, the community members occupy more niches resulting in a stable community that reduces the possibility for establishment of invaders. The effect of community diversity on invaders could also be mediated by soil-legacy effects. Plant communities of high diversity can accumulate more types of pathogens (De Deyn *et al.* 2004; Eisenhauer *et al.* 2011), which may reduce the growth of invaders. Indeed, Zhang et al. (2020a) recently found that alien species produced 11.7% less aboveground biomass when grown on soils trained by four instead of two native species. Most studies, however, focused on the relationship between community diversity and co-competing current invaders, and consequently still little is known about whether and how community diversity affects subsequent invaders. When the communities are already invaded, the accompanying changes in the availability of soil nutrients and in microbial composition may also depend on the diversity of the community (Liao *et al.* 2015). This could magnify or reduce the soil-legacy effect on subsequent invaders. Therefore, whether and how community diversity and invasions affect subsequent invasions through soil-legacy effects is important for understanding coexistence of invaders and communities.

Here, we conducted a two-phase plant-soil-feedback experiment to test whether and how soil legacies of alien invaders and native control invaders, and of invaded and non-invaded communities of different diversities affect the growth of subsequent alien and native plants. In the soil-conditioning phase, we grew five alien and five native species as invaders in 21 native communities of three diversity levels to train the soil. In the test phase, we used five alien and five native species to test the effects of the conditioned soils. We addressed the following main questions: (1) Do the effects of soil conditioned by invaders and/or communities differ between alien and native test species (i.e. subsequent invaders)? If so, (2) how does the alien-native origin of the previous invader affect the growth of the subsequent alien and native test species, and (3) how does this effect change with the presence and diversity of the community?

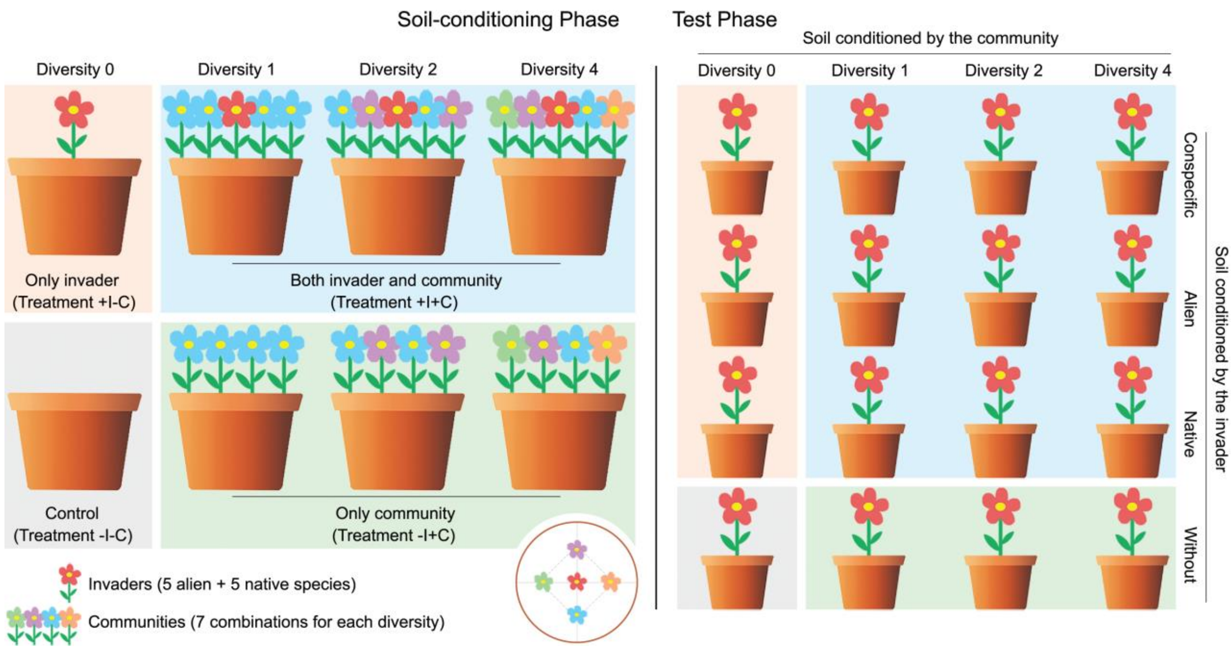

Figure 1 Overview of the experimental design. In the soil-conditioning phase, four soil treatments were created by the presence of an invader and/or a community. To test the effects of community diversity on the test species, four diversity levels were created (Diversity 0 [no community], 1, 2, and 4 species). The top view of the pot showed where

the seedlings were planted in the pot. In the test phase, each of the test species (red plants) were separately tested on the unconditioned and different conditioned soils. The soil conditioned by invaders was divided into four subsets of soil treatment (Without, Conspecific, and allospecific Alien and Native) according to the status of the invader species.

Materials and Methods

Study species

For the soil-conditioning phase of the experiment, we used mesocosms with invaded and non-invaded plant communities. As invaders, we selected five alien species from the Asteraceae, Brassicaceae, Fabaceae and Poaceae (i.e. alien invaders; Table S1). To test whether the alien and native species conditioned the soil differently, we also selected as controls five native species from the same family as the alien invaders, which we refer to as native invaders (Table S1). Moreover, to create 21 native communities, varying in species richness, we used seven species that are native to Germany and frequently co-occur (Table S2). For the test phase, we initially wanted to use the same ten invader species as in the conditioning phase. However, as two of the ten species - the alien *Lepidium virginicum* L. and the native *Senecio jacobaea* L.- had not enough seedlings, we replaced those two species with another alien (*Ambrosia artemisiifolia* L.) and another native species (*Hypericum perforatum* L.; Table S1). The classification of the species as alien or native to Germany was based on information from the FloraWeb database (www.floraweb.de, accessed January 2020), and all alien study species are considered naturalized in Germany. Seeds of the species were either from the seed collection of the Botanical Garden of the University of Konstanz or ordered from Rieger-Hofmann GmbH (Table S1, S2).

Experimental set up

The experiment was conducted in the greenhouse of the Botanical Garden of the University of Konstanz, Germany (47°41’32” N, 9°10’41” E).

Soil-conditioning phase

From 10 to 17 February 2020, we sowed seeds of the invader and community species for the soil-conditioning phase into trays (18 cm × 14 cm × 5 cm) filled with potting soil (Topferde, Einheitserde Co.). According to prior knowledge about the time required for germination of each species, the species were sown on different dates (Table S1, S2), so that all seedlings would be in similar growth stages at transplanting. The trays were placed in a greenhouse with a temperature maintained between 18°C and 25°C.

To make sure that the pot substrate would contain live soil organisms, we dug out field soil from a grassland site in the Botanical Garden of the University of Konstanz on 24 February 2020. We sieved the soil using a 1-cm mesh to remove pebbles and plant material. We then filled 333 pots (3L, Ø = 16 cm, height = 12 cm) with a soil substrate consisting of the field soil (25%, v/v), and a 1:1 mixture of sand and vermiculite (75%, v/v). On 3 and 4 March 2020, we transplanted the seedlings into the pots. The 21 native communities were created from a pool of seven native species, and had three diversity levels (Table S2): one species (7 monocultures), two species (7 combinations) and four species (7 combinations). We had each of the 21 communities without invaders, and with one individual of each of the five alien or five native invader species. In addition, we had each of the ten invaders without communities, and, as a control for the effect of soil conditioning by plants, we also had pots without any plants. So, the four main soil treatments were: 1) soil conditioned by both an invader and a community (+I+C), 2) soil only conditioned by an invader (+I-C), 3) soil only conditioned by a community (-I+C), and 4) unconditioned soil (control, -I-C). For the pots with communities, we transplanted four seedlings of the community at 4-cm from the center in square formation. For the pots with invaders, we planted one invader seedling in the center of the pot (Fig. 1). For each invader by community combination of treatment

+I+C, we had one replicate (10 invaders × 21 communities = 210 pots). For each invader in treatment +I-C and each community in treatment -I+C, we had three replicates ([10 invaders + 21 communities] × 3 replicates = 93 pots). For treatment -I-C (i.e. unconditioned soil), we had 30 replicates (30 pots). So, there were 333 pots in total in the conditioning phase.

On 4 March 2020, all pots were individually placed on plastic dishes (Ø = 20 cm) and randomly assigned to positions in three greenhouse compartments (24°C/18°C day/night temperature, 16h/8h day/night light). Seedlings that died within two weeks after transplanting were replaced. We watered all pots, including the pots without plants, every 1-2 days, and fertilized the pots four times with a water-soluble fertilizer (1‰ m/v, Universol Blue). Positions of the pots were re-randomized six weeks after start of the soil-conditioning phase. From 13 to 29 May 2020, ten weeks after the start of the soil-conditioning phase, we harvested the plants and soil. For each pot, we first cut the aboveground parts of each plant, and then sieved the soil through a 5-mm mesh to remove the roots. The soil of the pots without any plants was also sieved. The mesh was sterilized by using 70% ethanol between pots. The soil was immediately stored at 4°C. The aboveground biomass of each species was dried at 70°C to constant weight and weighed with an accuracy of 0.001 g.

Test phase

We sowed the seeds of the five alien and five native species to be used as subsequent species on 15 or 22 May 2020 (Table S1). Eight of those species were the same as the invaders in the soil-conditioning phase (see the section ‘Study species’ above). As we did not have enough soil from the conditioning phase to fill all 2100 pots (0.5L; length × width × height = 9 cm × 9 cm × 8 cm) with conditioned soil only, we filled the lower half of each pot with a 1:1 sand-vermiculite mixture. Then we filled up the pots with the soil from the conditioning phase. This way, the seedlings would first experience the conditioned soil, and the microbes in the conditioned soil would have time to spread into the fresh sand-vermiculite mixture in the bottom half of the pots. From 6 to 8 June 2020, we transplanted one seedling into each pot. For soil treatments -I+C and -I-C (i.e. soil conditioned by native communities only, and unconditioned soil), the soil of each pot was separately filled into ten pots of the test phase. In other words, we grew each of the ten test species on each of the soils from treatments -I+C and -I-C, respectively. For soil treatments +I+C and +I-C (i.e. soil conditioned by invaders), the soil of each pot was separately filled into six pots of the test phase. To avoid that the experiment would become too large to handle, we grew for those soil-conditioning treatments, each test species only on soils of six of the ten previous invaders (Table 1), similar to a partial-diallel design used in quantitative genetics (Kempthorne & Curnow, 1961). Specifically, these six soils included three invader treatments (Table 1): i) soil conditioned by a conspecific plant, ii) soil conditioned by a non-conspecific alien invader, and iii) soil conditioned by a non-conspecific native plant. For the one native and one alien invader species without soil conditioned by conspecifics (i.e. *H. perforatum* and *A. artemisiifolia*), we instead used a soil conditioned by another native and alien species, respectively (Table 1). So, each species was grown on soils conditioned by three alien and three native invaders. For each of the ten test species, the soils of the six chosen previous invaders and each of the 21 communities (treatment +I+C), were replicated only once because each of the seven 1-species, 2-species and 4-species communities provides replication of the diversity levels (totaling 1260 pots). The corresponding soils conditioned by invaders only (i.e. treatment +I-C), the 21 soils conditioned by native communities only (i.e. treatment -I+C) and the non-conditioned soil (i.e. treatment -I-C) were replicated three times (totaling 630 [treatment +I-C] + 180 [treatment -I+C] + 30 [treatment -I-C] = 840 pots). Ideally, we would thus have had 2100 pots in total. However, because we did not have enough seedlings for *Solidago gigantea* and *Bromus carinatus* in the test phase, and the previous invader had died in eight pots of the conditioning phase, we had 1852 pots.

All pots were individually placed on plastic dishes (Ø = 15 cm) and randomly allocated to positions in two greenhouse compartments (24°C/18°C day/night temperature, 16h/8h day/night light). All pots were watered every 1-2 days, and re-randomized once (13 July 2020, i.e. five weeks after start of the soil-conditioning phase). To reduce potential effects of nutrient depletion that might have happened during the conditioning phase, we fertilized all pots in the test phase two times with 80 ml of a water-soluble fertilizer (1‰ m/v, Universol Blue). From 10 to 14 August 2020, nine weeks after the start of the test phase, we harvested the above- and belowground biomass of each plant separately. We cut the aboveground biomass at the surface of the soil, and washed the roots of each plant free from substrate. The biomass of each plant was dried at 70°C to constant weight and weighed with an accuracy of 0.001 g.

Statistical analysis

To test our different research questions, we fitted different linear mixed models to different subsets of the data. All models included total biomass of the subsequent plants (i.e. the test species) as the response variable. All analyses were conducted with R 3.6.2 (R Core Team 2019), and the *lme* function in the R package '*nlme*' (Pinheiro *et al.* 2019).

First, using the entire dataset (n = 1852), we tested whether the effects of soil conditioned by invaders and/or communities differed between alien and native test species (Table S3). The origin of the test species (alien or native), soil treatment (unconditioned, only conditioned by an invader, only conditioned by a community, conditioned by both an invader and a community) and their interactions were included as the fixed effects. For soil treatment, we ran orthogonal hierarchical contrasts to test the effect of conditioned soil ($Soil_{Unconditioned/Conditioned}$, i.e. unconditioned soil vs. the average of all three conditioned soils), the effect of the presence of the invader or the community alone or both together ($Soil_{Both/Single}$, i.e. soil conditioned by both invader and community vs. the average of soil only conditioned by either the invader or the community), and the effect of conditioning by the invader or community ($Soil_{Invader/Community}$, i.e. soil only conditioned by the invader vs. soil only conditioned by the community). To account for phylogenetic non-independence of the test species, and non-independence of plants belonging to the same family, we included species identity and family of the test species as random effects. To account for non-independence of plants growing on soils conditioned by the same invaders or communities, we also include identity of invader species and communities in the conditioning phase as random effects. Furthermore, to account for non-independence of soil replicates from the same pot of the conditioning phase, we included pot identity of the conditioning phase as a random effect. In addition, to account for the effect of biomass produced by conditioning plants on the plants of the test phase, which could be an indicator of nutrient depletion, we also ran this model by adding the square-root-transformed aboveground biomass of the soil-conditioning plants as a covariate (Table S4).

Second, we tested how the alien-native origin of the previous invader affects the growth of the alien and native test species. For this analysis, we used the subset of pots (n = 162) that had not been conditioned by any of the communities (i.e. we only used the plants of treatments +I-C and -I-C), and we excluded the two invader species that had not been used in the test phase. The origin of the test species, invader treatment during the conditioning phase and their interactions were included as fixed effects. Here, we included four levels of the invader treatment according to the presence and origin of the previous invaders that grew on the soil: i) without an invader, ii) with an invader belonging to the same species as the test species (i.e. conspecific), iii) with an invader of another species (i.e. allospecific) that is alien or iv) with an invader of another species that is native. Then we generated three orthogonal contrasts for the invader treatment: without vs. with invader ($\text{Invader}_{\text{Without/With}}$), con- vs. allospecific invader ($\text{Invader}_{\text{Con/Allo}}$, and alien vs. native allospecific invader ($\text{Invader}_{\text{Alien/Native}}$). Identity of the previous invader species, identity and family of the test species and pot identity of the conditioning phase were used as random effects.

Third, we tested how the effects of previous invaders on the test species changed with the presence and diversity of the community during the conditioning phase. For this analysis, we used the subset of pots (n = 980) with previous invaders (i.e. we only used the plants of treatments +I+C and +I-C), and we excluded the two invader species that had not been used in the conditioning phase. The origin of the test species (alien or native), previous invader treatment (conspecies, allospecific alien or allospecific native), community diversity (0, 1, 2 or 4 species) and their interactions were included as fixed effects. In addition to the two orthogonal contrasts of invader treatment ($\text{Invader}_{\text{Con/Allo}}$ and $\text{Invader}_{\text{Alien/Native}}$), we also generated three orthogonal contrasts for community diversity: $\text{Community}_{\text{Without/With}}$ (i.e. diversity 0 vs. the average of diversities 1, 2 and 4), which effectively tests the effect of plant density per pot; $\text{Community}_{\text{Mono/Multi}}$ (i.e. diversity 1 vs. the average of diversities 2 and 4); $\text{Community}_{\text{Div2/Div4}}$ (i.e. diversity 2 vs. diversity 4). Identities of the previous invader species and communities, identity and family of the test species and pot identity of the conditioning phase were used as random effects. In addition, to account for the effect of biomass of the soil-conditioning plants on the results of test phase, we also ran this model by adding the square-root-transformed aboveground biomass of the soil-conditioning plants as a covariate (Table S5).

Fourth, to test whether the presence and diversity of the community without invaders affected the test species differently, we additionally ran a model for the subset of pots (n = 599) that did not have invaders during the conditioning phase (i.e. treatments -I+C and -I-C). In contrast to the analysis above, this one also included the two test species that had not been used in the conditioning phase. The origin of the test species, community diversity (included as three orthogonal contrasts: $\text{Community}_{\text{Without/With}}$, $\text{Community}_{\text{Mono/Multi}}$ and $\text{Community}_{\text{Div2/Div4}}$) and their interactions were included as fixed effects. Community identity, identity and family of the test species and pot identity of the conditioning phase were used as random effects (Table S6).

In all models, we accounted for differences in initial sizes of the test species by including initial height as a covariate. To meet the assumption of normality, total biomass of the test species was square-root-transformed. To improve homoscedasticity of residuals of the models, we allowed the variance to vary among the test species by using the *varIdent* function (Table S7). For all models, we used log-likelihood ratio tests to assess the significance of the fixed effect by comparing models with and without the effect of interest (Zuur *et al.* 2009). An effect was considered significant if $P < 0.05$ and marginally significant if $0.05 \leq P < 0.1$.

Table 1 Overview of the combinations of the test species in the test phase and the previous invaders used to condition the soils. The species names are abbreviated as the first three letters of the genus name and the first three letters of the specific epithet. For the soil conditioned by invaders (i.e. conditioned by invaders only or by both invaders and communities; treatments +I+C and +I-C), Each subsequent invader was tested on six of the ten soils conditioned by invaders only and on the six corresponding soils conditioned by both invaders and the community (purple and blue cells). For all test species, we had three soils conditioned by an alien invader (purple cells) and three cells condition by a native invader (blue cells). For the eight invader species that were used in both the conditioning and test phase, one of the soils had been conditioned by a conspecific plant (hatched cells). For the soil only conditioned by the communities and for the unconditioned soil, each of the ten subsequent invader species was tested on all soils (green cells). The numbers of replicates for each treatment combination are shown separately in the cells for each community-diversity level.

Test species		Conditioned soil											Unconditioned soil (Control)
		Soil conditioned by the invader and community (including diversities 0/1/2/4)										Soil only conditioned by the community (including diversities 1/2/4)	
		Soil conditioned by alien invaders					Soil conditioned by native invaders						
		Lep.vir	*Set.fab*	*Lup.pol*	*Bro.car*	*Sol.gig*	*Lep.cam*	*Set.vir*	*Tri.pra*	*Bro.ste*	*Sen.jac*		
Alien	*Amb.art*	3/7/7/7			3/7/7/7	3/7/7/7	3/7/7/7			3/7/7/7	3/7/7/7	3/3/3	3
	Set.fab	3/7/7/7	3/7/7/7		3/7/7/7		3/7/7/7	3/7/7/7		3/7/7/7		3/3/3	3
	Lup.pol		3/7/7/7	3/7/7/7		3/7/7/7		3/7/7/7	3/7/7/7		3/7/7/7	3/3/3	3
	Bro.car	3/7/7/7		3/7/7/7	3/7/7/7		3/7/7/7		3/7/7/7	3/7/7/7		3/3/3	3
	Sol.gig		3/7/7/7	3/7/7/7		3/7/7/7		3/7/7/7	3/7/7/7		3/7/7/7	3/3/3	3
Native	*Lep.cam*	3/7/7/7			3/7/7/7	3/7/7/7	3/7/7/7			3/7/7/7	3/7/7/7	3/3/3	3
	Set.vir	3/7/7/7	3/7/7/7		3/7/7/7		3/7/7/7	3/7/7/7		3/7/7/7		3/3/3	3
	Tri.pra		3/7/7/7	3/7/7/7		3/7/7/7		3/7/7/7	3/7/7/7		3/7/7/7	3/3/3	3
	Bro.ste	3/7/7/7		3/7/7/7	3/7/7/7		3/7/7/7		3/7/7/7	3/7/7/7		3/3/3	3
	Hyp.per		3/7/7/7	3/7/7/7		3/7/7/7		3/7/7/7	3/7/7/7		3/7/7/7	3/3/3	3

Results

Overall effects of soil-conditioning treatments on alien and native test species

Overall, the test species, irrespective of whether they were alien or native, produced significantly less biomass (-25.7%) when growing on soil that had been conditioned by previous invaders, communities or both than on unconditioned control soil (Table S3, Fig. 2). Moreover, among the conditioned soils, the biomass of the test species was significantly lower (-7.7%) when the soil had been conditioned by both an invader and community than by just one of them (Table S3, Fig. 2). Furthermore, alien test species produced significantly more biomass than native test species on soil conditioned by an invader only (+14.5%), whereas this was not true on soil conditioned by a community only (significant Origin × $\text{Soil}_{\text{Invader/Community}}$ interaction in Table S3, Fig. 2).

Effects of previous invaders on the test species

For the subset of test plants that grew on the soil not conditioned by the community (i.e. treatments +I-C and -I-C), the biomass was lower on the invader-conditioned soil than on the unconditioned soil (-29.2%; Table 2, Fig. 3a), and also on soil conditioned by conspecific invaders than on soil conditioned by allospecific alien or native invaders (-14.1%; Table 2, Fig. 3a). The biomass of the test plant did, on average, not differ between soil conditioned by allospecific alien and native invaders (Table 2, Fig. 3a). However, alien test species produced more biomass on soil conditioned by allospecific alien invaders than on soil conditioned by allospecific native invaders (+10.5%), whereas this was not the case for native test species (marginally significant Origin × $\text{Invader}_{\text{Alien/Native}}$ in Table 2, Fig. 3a).

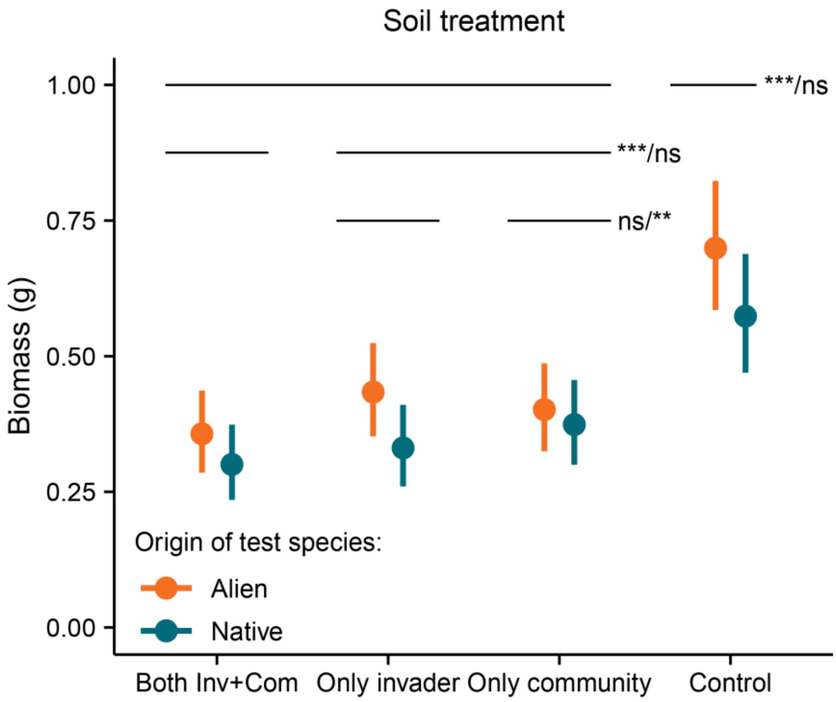

Figure 2 Total biomass of alien and native test species in the four main soil-conditioning treatments. Shown are modelled means (±SEs) after back-transformation (n = 1852). The hierarchical contrasts between treatments are indicated by the horizontal lines, and the significance of the main effect of the contrast is indicated next to the respective line before the slash, and the significance of the interaction of the contrast with origin of the test species is indicated after the slash. Log-likelihood ratio tests were used to assess the significance. ***: $P < 0.001$, **: $0.001 \leq P < 0.01$, ns: not significant.

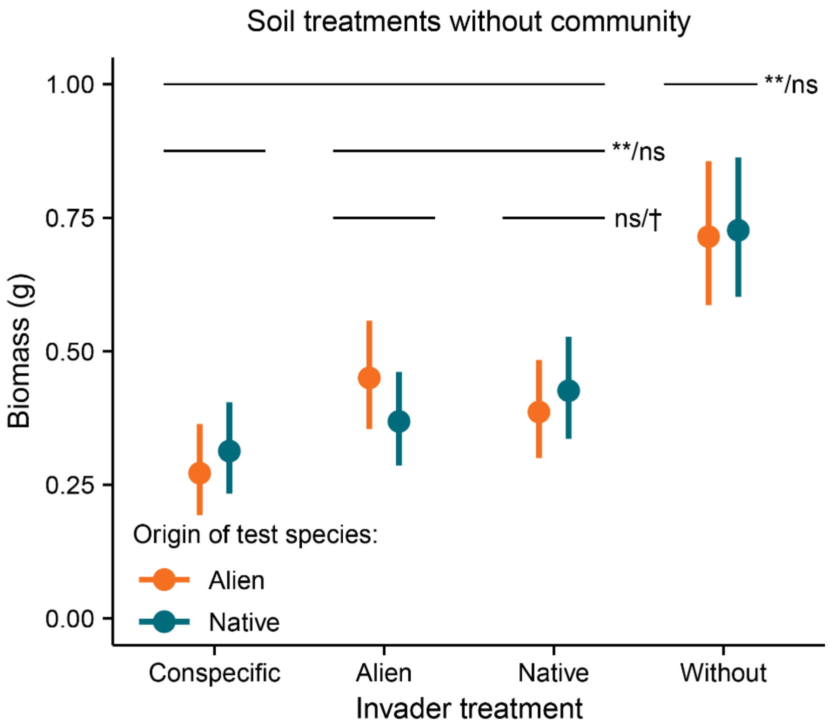

Figure 3 Total biomass of alien and native test species in the four soil-conditioning invader-type treatments (without, conspecific, allospecific alien, allospecific native) of the pots that were not conditioned by native communities (i.e. treatments +I-C and -I-C; n = 162). Shown are modelled means (±SEs) after back-transformation. The hierarchical contrasts between treatments are indicated by the horizontal lines, and the significance of the main effect of the contrast is indicated next to the respective line before the slash, and the significance of the interaction of the contrast with origin of the test species is indicated after the slash. Log-likelihood ratio tests were used to assess the significance. **: $0.001 \leq P < 0.01$, †: $0.05 \leq P < 0.1$, ns: not significant.

Table 2 Effects of the origin of the test species (alien or native), invader treatment of the soil-conditioning phase (without, conspecific and allospecific alien or native) and their interactions on total biomass of the test species in the subset of pots that did not experience conditioning with the communities (treatments +I-C and -I-C).

	df	χ^2	*P*
Fixed effects			
Initial plant height of test species	1	5.339	**0.021**
Origin of test species (O)	1	0.001	0.976
Invader-conditioning treatment (I)	3	19.944	**<0.001**
$I_{Without/With}$	1	10.476	**0.001**
$I_{Con/Allo}$	1	8.180	**0.004**
$I_{Alien/Native}$	1	0.014	0.904
O × I	3	4.532	0.209
O × $I_{Without/With}$	1	0.005	0.946
O × $I_{Con/Allo}$	1	0.744	0.388
O × $I_{Alien/Native}$	1	3.450	*0.063*
Random effects		SD	
Test species family		0.001	
Test species species*		0.137	
Conditioning invader species		<0.001	
Conditioning pot		<0.001	
Residual		0.080	

Shown are results of linear mixed models. Values are in bold when $P < 0.05$ and in italic when $0.05 \leq P < 0.1$. * Standard deviations for all the test species are shown in Table S7.

Effects of previous invaders and community diversity on the test species

For the subset of test species that grew on soil conditioned by invaders and communities of different diversities (i.e. treatments +I+C and +I-C, thus including diversities 0 [i.e. invader only], 1, 2 and 4), the biomass was significantly lower on soil conditioned by conspecific invaders than on soil conditioned by allospecific alien and native invaders (-8.2%; Table 3, Fig. 4c). Compared to the soil treatment without community (i.e. diversity 0), this con- vs. allospecific conditioning effect was smaller in the presence of a community (-16.5% vs. 6.7%; significant $\text{Invader}_{\text{Con/Allo}}$ × $\text{Community}_{\text{Without/With}}$ in Table 3, Fig. 4c). Moreover, among the soil treatments with communities, the test plants produced less biomass on soil conditioned by allospecific alien invaders and multi-species communities than on soil conditioned by allospecific alien invaders and single-species communities (-5.6%), whereas this was not the case on soils conditioned by allospecific native invaders (marginally significant $\text{Invader}_{\text{Alien/Native}}$ × $\text{Community}_{\text{Mono/Multi}}$ in Table 3, Fig. 4c).

The biomass of the test plants did not differ between soil conditioned by allospecific alien and native invaders. However, alien test species, irrespective of the diversity of the community that trained the soils, produced more biomass on soil conditioned by alien invaders than on soil conditioned by native invaders (+7.2%), whereas this was not the case for native test species (significant Origin × $\text{Invader}_{\text{Alien/Native}}$ in Table 3, Fig. 4a). For the soil treatment with the community and the invader, irrespective of whether the invader was alien or native, the biomass of alien test species was lower on soil conditioned by multi-species communities than on soil conditioned by single-species communities (-5.5%), whereas this was not the case for native test species (significant Origin × $\text{Community}_{\text{Mono/Multi}}$ in Table 3, Fig. 4b). When the test plants grew on soil conditioned by the community only, no significant difference in biomass was found between community diversities (Table S6, Fig. S1).

Table 3 Effects of the origin of the test species (alien or native), invader treatment of the soil-conditioning phase (conspecific, and allospecific alien or native), the diversity of the native community (0 [no community], 1, 2 or 4 species) and their interactions on total biomass of the test species in the subset of pots that experienced conditioning with an invader (treatments +I+C and +I-C).

	df	χ^2	*P*
Fixed effects			
Initial plant height of test species	1	40.104	**<0.001**
Origin of test species (O)	1	0.032	0.859
Invader-conditioning treatment (I)	2	20.040	**<0.001**
$I_{Con/Allo}$	1	20.034	**<0.001**
$I_{Alien/Native}$	1	0.076	0.783
Conditioning community diversity (CD)	3	5.359	0.147
$CD_{Without/With}$	1	4.672	**0.031**
$CD_{Mono/Multi}$	1	0.913	0.339
$CD_{Div2/Div4}$	1	0.026	0.872
O × I	2	11.944	**0.003**
O × $I_{Con/Allo}$	1	0.005	0.943
O × $I_{Alien/Native}$	1	11.928	**0.001**
O × CD	3	8.598	**0.035**
O × $CD_{Without/With}$	1	2.098	0.147
O × $CD_{Mono/Multi}$	1	5.685	**0.017**
O × $CD_{Div2/Div4}$	1	0.661	0.416
I × CD	6	13.020	**0.043**
$I_{Con/Allo}$ × $CD_{Without/With}$	1	5.552	**0.018**
$I_{Con/Allo}$ × $CD_{Mono/Multi}$	1	1.010	0.315
$I_{Con/Allo}$ × $CD_{Div2/Div4}$	1	1.698	0.193

$I_{Alien/Native} \times CD_{Without/With}$	1	0.281	0.596
$I_{Alien/Native} \times CD_{Mono/Multi}$	1	3.785	*0.052*
$I_{Alien/Native} \times CD_{Div2/Div4}$	1	0.722	0.396
O × I × CD	6	1.453	0.963
$O \times I_{Con/Allo} \times CD_{Without/With}$	1	1.051	0.305
$O \times I_{Con/Allo} \times CD_{Mono/Multi}$	1	0.100	0.752
$O \times I_{Con/Allo} \times CD_{Div2/Div4}$	1	0.011	0.916
$O \times I_{Alien/Native} \times CD_{Without/With}$	1	0.170	0.680
$O \times I_{Alien/Native} \times CD_{Mono/Multi}$	1	0.002	0.969
$O \times I_{Alien/Native} \times CD_{Div2/Div4}$	1	0.119	0.730
Random effects		SD	
Test species family		<0.001	
Test species species*		0.134	
Conditioning invader species		0.012	
Conditioning community		0.016	
Conditioning pot		0.027	
Residual		0.098	

Shown are results of linear mixed models. Values are in bold when $P < 0.05$ and in italic when $0.05 \leq P < 0.1$. * Standard deviations for all the test species are shown in Table S7.

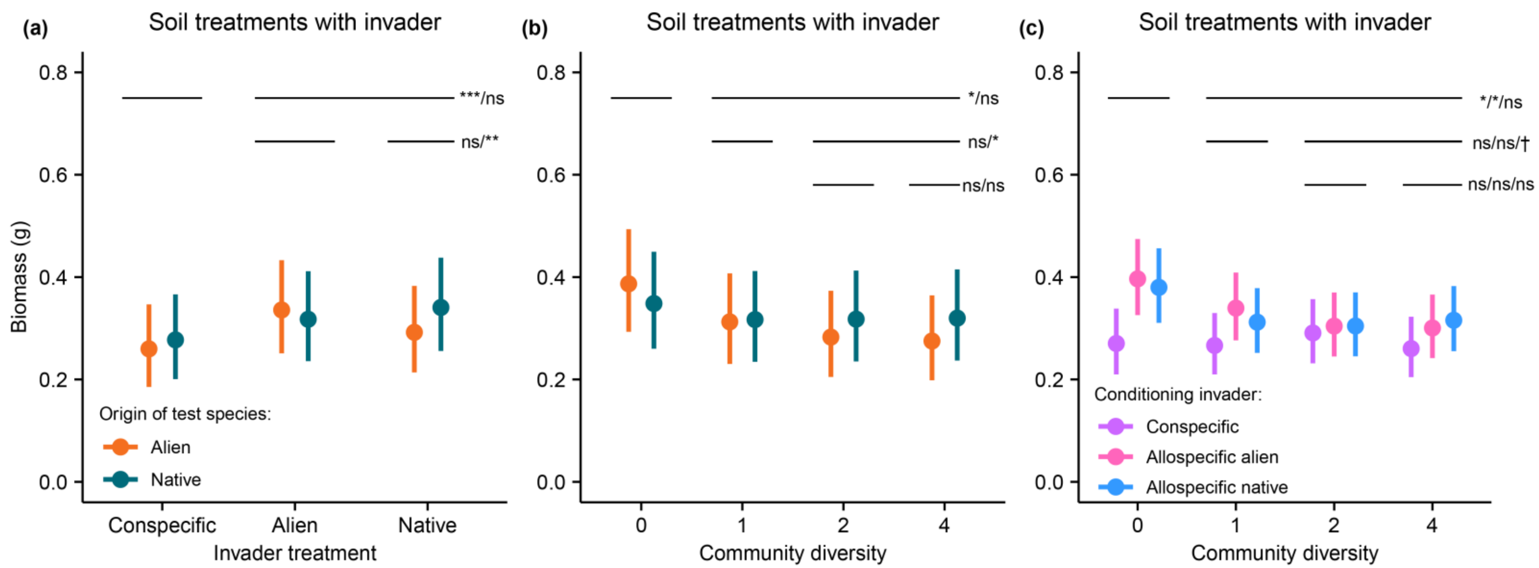

Figure 4 Effect of soil treatments with conditioning invaders on the test species. **a**, Total biomass of alien and native test species in the three soil-conditioning invader-type treatments (conspecific, allospecific alien, allospecific native) of the pots conditioned by both invaders and communities (i.e. treatments +I+C and +I-C; n = 980). **b**, Total biomass of alien and native test species in the four soil-conditioning community-diversity treatments (0 [no community], 1, 2, 4 species) of the pots conditioned by both invaders and communities (i.e. treatments +I+C and +I-C; n = 980). **c**, Total biomass of the test species in the three soil-conditioning invader treatments (conspecific, allospecific alien, allospecific native) with native community of different diversities (0, 1, 2, 4 species; treatments +I+C and +I-C; n = 980). Shown are modelled means (±SEs) after back-transformation. The hierarchical contrasts between treatments are indicated by the horizontal lines. For **a** and **b**, the significance of the main effect of the contrast is indicated next to the respective line before the slash, and the significance of the interaction of the contrast with origin of the test species is indicated after the slash. For **c**, the significance of the main effect of the community contrast is indicated under the respective line before the first slash, the significance of the interaction of the community contrast with conspecific or allospecific test species is indicated before the second slash, and the significance of the interaction of the community contrast with allospecific alien or native test species is indicated after the second slash. Log-likelihood ratio tests were used to assess the significance. ***: $P < 0.001$, **: $0.001 \leq P < 0.01$, *: $0.01 \leq P < 0.05$, †: $0.05 \leq P < 0.1$, ns: not significant.

Discussion

Our experiment revealed relatively strong soil-legacy effects of previous alien and native invaders and native communities on alien and native test species. The test plants produced the most biomass on unconditioned soils, less biomass on soils conditioned by either an invader only or a community only, and the least biomass on soils conditioned by both a community and an invader. So, the higher the density of plants that conditioned the soil, the stronger the negative effect was on the test species. This was true irrespective of the origin of the test species. However, while biomass of the native test species was similar on soils conditioned by an invader only and soils conditioned by a community only, the alien test species performed better on soils conditioned by an invader only, particularly when this was an alien too. Plants performed worse when the soil had been conditioned by a conspecific invader, indicating negative plant-soil feedback, for both the native and alien test species. This negative plant-soil feedback was reduced by the presence of a native community in the conditioning phase, but irrespective of the diversity of the native community. However, the test species tended to produce more biomass when a single-species community had previously been invaded by an alien species than when it had been invaded by a native species, whereas this was not the case for multi-species communities. This indicates that the performance of the test species depends on both the origin of the preceding invader and the diversity of the native community.

Effects of soil conditioning

The test plants produced less biomass on any of the conditioned soils than on the unconditioned soil (Fig. 2). This negative effect was the strongest when soil had been conditioned by both an invader and a native community. These findings are not surprising, and are consistent with the results of two meta-analyses (Lekberg *et al.* 2018; Crawford *et al.* 2019), which found that conditioned soil generally has negative effects on subsequent plants. These negative soil-legacy effects could indicate depletion of soil nutrients by the conditioning plants or accumulation of soil pathogens. We fertilized the plants in the test phase to reduce effects of nutrient depletion, but we cannot exclude that nutrient depletion played a role. The biomass of the test plants slightly decreased with the aboveground biomass of the previous plants that had conditioned the soil (Fig. S2). However, inclusion of the latter as a covariate in the analysis did not affect the significance of the other effects (Table S4). This suggests that the observed soil-legacy effects are not solely driven by nutrient depletion, and are at least partly due to plant-induced changes in the soil-microbial community.

Effects of presence and origin of previous invaders on subsequent invaders

Averaged over all soil-conditioning treatments, biomass of the alien and native test species did not significantly differ. However, there was a significant interaction between the origin of the test species and the main soil-conditioning treatments (Table S3). In particular, alien test species produced more biomass than native test species when they grew on soil that had been conditioned by invaders only (Fig. 2). More detailed analysis of the subset of pots with unconditioned soil or soil conditioned by invaders only, showed that this difference is mainly due to the weaker negative effect of alien invaders on other alien test species (Fig. 3). In other words, compared to native plants, alien plants tended to have less negative soil-legacy effects on subsequent alien test species. This indicates that alien species, in contrast to native species, can help subsequent alien species by reducing the negative feedbacks through their soil legacy, and thereby promote the establishment and growth of subsequent alien species on the conditioned soils. Whether the subsequent alien species then will become dominant remains to be seen as the strong negative plant-soil feedback of the alien species on itself might contain its population growth. Simulation studies could provide insights into such dynamics. Nevertheless, irrespective of whether the aliens will become dominant or not, our findings support the “invasional meltdown” hypothesis, which poses that synergistic interactions between alien invaders can promote the establishment of other alien invaders (Simberloff & Von Holle 1999).

A recent study, also found evidence that such invasional meltdown effects might be mediated by soil microorganisms (Zhang *et al.* 2020b). One potential mechanism could be that the alien plants have been released from many of their pathogens (Mitchell & Power 2003; van Kleunen & Fischer 2009), as predicted by the enemy-release hypothesis (Keane & Crawley 2002), and therefore accumulated fewer pathogens than the native plants did during soil-conditioning. Although pathogen release might play a role, it could not explain why native plants have stronger negative soil-legacy effects on the alien plants. Zhang et al. (2020b), however, found that soil-legacy effects became less negative when the root fungal endophyte communities of the conditioning and test species were less similar, and that these endophyte communities were less similar between two alien species than between an alien and a native species. They also found that the aliens were more competitive than natives on soil conditioned by other aliens, supporting the invasional meltdown hypothesis. So, irrespective of the exact underlying mechanism, our study and others (e.g. Adams *et al.* 2003; O'Dowd *et al.* 2003; Bourgeois *et al.* 2005; Green *et al.* 2011; Hohenadler *et al.* 2018) show that alien invaders might facilitate establishment of subsequent alien invaders.

Effects of presence and diversity of native communities on subsequent invaders

When an alien or native invader and a native community co-conditioned the soil, alien test species were still slightly less negatively affected if the previous invader was an alien instead of a native (Fig. 4a). The corresponding difference in biomass, however, decreased from +10.5% when the soil had been conditioned by an invader only to +7.2% when the soil had been conditioned by both an invader and a community. Similarly, compared to the soil conditioned by an invader only, when an invader and a community co-conditioned the soil, the negative effect of conspecific invaders on the biomass of the test species changed from -16.5% to -6.7%. These findings indicate that the presence of a native community reduces but does not completely remove negative conspecific plant-soil feedback and neither removes the facilitative effect of one alien invader on the next one.

We had separated the community diversity effect using three orthogonal contrasts. Here, the first contrast, comparing the effect of soil conditioned without a community vs the effect of soils conditioned with a community, effectively tested a density effect and not a diversity effect. The contrast showed that plant density during conditioning had a significant negative effect on biomass of the test plants. The two other contrasts tested the true diversity effect of the conditioning communities. When soil had been conditioned by a community only, the biomass of the test plants did not depend on the diversity of the conditioning community (Fig. S1). However, when the soil had been co-conditioned by an invader, the alien test species produced less biomass when the community had multiple species instead of just one (Fig. 4b). This was not true for the native test species, which indicates that alien invasions might be reduced by the diversity of the community that previously grew there. Also, the biomass produced by plants in the soil-conditioning phase had no significant effect on biomass of the test species (Table S5). Therefore, a more likely explanation is that diverse communities in the conditioning phase accumulated a wider variety of soil pathogens, as has been found for aboveground pathogens (Rottstock *et al.* 2014). This should be particularly true for generalist soil pathogens (Crawford *et al.* 2019), which are also more likely to attack novel alien plant species. This would be in line with the recent finding of Zhang et al. (2020a) that soil-microbes-mediated apparent competition could be a mechanism underlying the frequently observed negative relationship between diversity and invasibility.

Conclusions

In conclusion, subsequent alien and native species grew worse on soil that had been conditioned by plants. These soil-legacy effects were particularly strong if the soil had been conditioned by both a community and an invader (i.e. by more plants) and if the previous invader was a conspecific of the subsequent plant (i.e. negative plant-soil feedback). Moreover, we found that subsequent alien species can benefit from previous allospecific alien invaders through soil-legacy effects, irrespective of the presence and absence of a community, supporting the invasional meltdown hypothesis. Importantly, multi-species communities can decrease the performance of subsequent alien plants when they co-conditioned the soil with alien or native invaders, thereby providing some evidence that diversity might increase resistance against alien invaders.

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Author contributions

DC conceived the idea. DC and MvK designed the experiment, DC conducted the experiment and analyzed the data. DC wrote the first draft of the manuscript with further input from MvK.

Supporting information

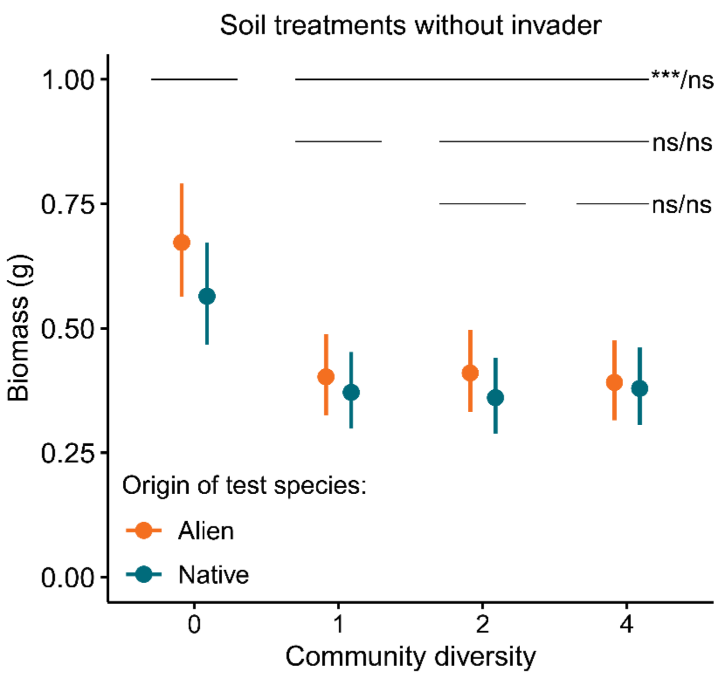

Figure S1 Total biomass of alien and native the test species in the four soil-conditioning community-diversity treatments (0 [no community], 1, 2, 4 species) of the pots that were not conditioned by invaders (i.e. treatments -I+C and -I-C; n = 599). Shown are modelled means (±SEs) after back-transformation. The hierarchical contrasts between treatments are indicated by the horizontal lines, and the significance of the main effect of the contrast is indicated next to the respective line before the slash, and the significance of the interaction of the contrast with origin of the test species is indicated after the slash. Log-likelihood ratio tests were used to assess the significance. ***: $P < 0.001$, ns: not significant.

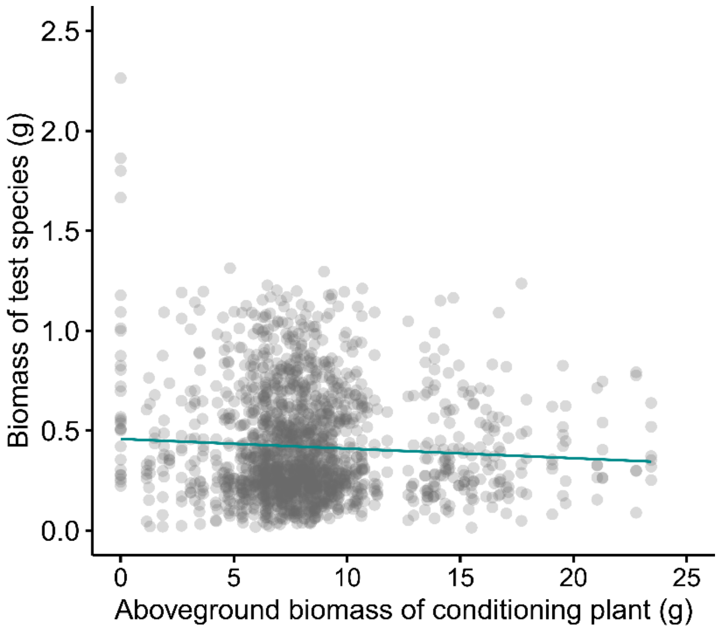

Figure S2 Effect of aboveground biomass produced by plants in the soil-conditioning phase on the biomass of the test plants. The data points are the biomass of the test plants. The line shows the fitted non-significant regression line.

Table S1 Alien and native invader species used in the soil-conditioning and test phases of the experiment.

Species	Family	Origin	Native range	Sowing date (Year 2020)		Seed source
				Conditioning phase	Test phase	
Ambrosia artemisiifolia L.*	Asteraceae	alien	North America	-	22 May	University of Konstanz, Germany
Bromus carinatus Hook. & Arn.	Poaceae	alien	North America	17 February	22 May	Rieger-Hofmann GmbH, Germany
Lepidium virginicum L.†	Brassicaceae	alien	North America	17 February	-	University of Konstanz, Germany
Lupinus polyphyllus Lindl.	Fabaceae	alien	North America	17 February	22 May	University of Konstanz, Germany
Setaria faberi RAW Herrm.	Poaceae	alien	Asia	17 February	22 May	Rieger-Hofmann GmbH, Germany
Solidago gigantea Aiton	Asteraceae	alien	North America	10 February	15 May	University of Konstanz, Germany
Bromus sterilis L.	Poaceae	native	-	17 February	22 May	Rieger-Hofmann GmbH, Germany
Hypericum perforatum L.*	Hypericaeae	native	-	-	15 May	Rieger-Hofmann GmbH, Germany
Lepidium campestre (L.) R. Br.	Brassicaceae	native	-	17 February	22 May	University of Konstanz, Germany
Senecio jacobaea L.†	Asteraceae	native	-	17 February	-	University of Konstanz, Germany
Setaria viridis (L.) P. Beauv.	Poaceae	native	-	17 February	22 May	University of Konstanz, Germany
Trifolium pratense L.	Fabaceae	native	-	17 February	22 May	Rieger-Hofmann GmbH, Germany

* The species was only used in the test phase.

† The species was only used in the soil-conditioning phase.

Table S2 Native community species used in the soil-conditioning phase of the experiment, and combinations of species to produce seven native communities for each of the three diversity levels. A grey filling of the cell in a column indicates that the species was used for the specific combination. The seeds of all seven species were ordered from Rieger-Hofmann GmbH, Germany.

Species	Family	Sowing date	Diversity 1							Diversity 2							Diversity 4						
Alopecurus pratensis L.	Poaceae	17 Feb, 2020	■							■						■	■				■	■	■
Anthoxanthum odoratum L.	Poaceae	17 Feb, 2020		■						■	■						■	■				■	■
Bromus hordeaceus L.	Poaceae	17 Feb, 2020			■						■	■					■	■	■				■
Lolium perenne L.	Poaceae	17 Feb, 2020				■						■	■				■	■	■	■			
Poa pratensis L.	Poaceae	17 Feb, 2020					■						■	■				■	■	■	■		
Prunella vulgaris L.	Lamiaceae	10 Feb, 2020						■						■	■				■	■	■	■	
Plantago lanceolata L.	Plantaginaceae	17 Feb, 2020							■						■	■				■	■	■	■

Table S3 Effects of the origin of the test species (alien or native), soil treatment of the soil-conditioning phase (unconditioned [control], only conditioned by the invader, only conditioned by the community or conditioned by the invader and the community) and their interactions on total biomass of the test species.

	df	χ^2	*P*
Fixed effects			
Initial plant height of test species	1	56.652	**<0.001**
Origin of test species (O)	1	0.247	0.619
Soil treatment (S)	3	44.98	**<0.001**
$S_{Unconditioned/Conditioned}$	1	34.655	**<0.001**
$S_{Both/Single}$	1	14.448	**<0.001**
$S_{Invader/Community}$	1	0.179	0.672
O × S	3	10.922	**0.012**
O × $S_{Unconditioned/conditioned}$	1	0.371	0.542
O × $S_{Both/Single}$	1	0.081	0.776
O × $S_{Invader/Community}$	1	9.028	**0.003**
Random effects		SD	
Test species family		<0.001	
Test species species*		0.141	
Conditioning invader species		0.008	
Conditioning community		0.011	
Conditioning pot		0.024	
Residual		0.100	

Shown are results of linear mixed models. Values are in bold when *P* < 0.05.

* Standard deviations for all the test species are shown in Table S7.

Table S4 Effects of the origin of the test species (alien or native), soil treatment of the soil-conditioning phase (unconditioned [control], only conditioned by the invader, only conditioned by the community or conditioned by the invader and the community) and their interactions on total biomass of the test species (including square-root-transformed total aboveground biomass of conditioning plants as a covariate).

	df	χ^2	*P*
Fixed effects			
Aboveground biomass$_{\text{Conditioning phase}}$	1	13.782	**<0.001**
Initial plant height of test species	1	53.925	**<0.001**
Origin of test species (O)	1	0.247	0.619
Soil treatment (S)	3	31.541	**<0.001**
$S_{\text{Unconditioned/Conditioned}}$	1	31.243	**<0.001**
$S_{\text{Both/Single}}$	1	14.051	**<0.001**
$S_{\text{Invader/Community}}$	1	0.249	0.618
O × S	3	10.933	**0.012**
O × $S_{\text{Unconditioned/conditioned}}$	1	0.384	0.535
O × $S_{\text{Both/Single}}$	1	0.081	0.776
O × $S_{\text{Invader/Community}}$	1	9.020	**0.003**
Random effects		SD	
Test species family		<0.001	
Test species species*		0.141	
Conditioning invader species		0.007	
Conditioning community		0.011	
Conditioning pot		0.024	
Residual		0.100	

Shown are results of linear mixed models. Values are in bold when $P < 0.05$.

* Standard deviations for all the test species are shown in Table S7.

Table S5 Effects of the origin of the test species (alien or native), invader treatment of the soil-conditioning phase (conspecific, and allospecific alien or native), the diversity of native community (0 [no community], 1, 2 or 4 species) and their interactions on total biomass of the test species in the subset of pots that experienced conditioning with an invader (treatments +I+C and +I-C; including square-root-transformed total aboveground biomass of conditioning plants as a covariate.

	df	χ^2	*P*
Fixed effects			
Aboveground biomass$_{\text{Conditioning phase}}$	1	0.949	0.330
Initial plant height of test species	1	39.887	**<0.001**
Origin of test species (O)	1	0.031	0.859
Invader-conditioning treatment (I)	2	19.674	**<0.001**
$I_{\text{Con/Allo}}$	1	19.665	**<0.001**
$I_{\text{Alien/Native}}$	1	0.078	0.780
Conditioning community diversity (CD)	3	5.021	0.170
$CD_{\text{Without/With}}$	1	4.311	**0.038**
$CD_{\text{Mono/Multi}}$	1	0.913	0.339
$CD_{\text{Div2/Div4}}$	1	0.027	0.869
O × I	2	11.956	**0.003**
O × $I_{\text{Con/Allo}}$	1	0.003	0.954
O × $I_{\text{Alien/Native}}$	1	11.944	**0.001**
O × CD	3	8.646	**0.034**
O × $CD_{\text{Without/With}}$	1	2.115	0.146
O × $CD_{\text{Mono/Multi}}$	1	5.728	**0.017**
O × $CD_{\text{Div2/Div4}}$	1	0.653	0.419
I × CD	6	12.964	**0.044**
$I_{\text{Con/Allo}}$ × $CD_{\text{Without/With}}$	1	5.503	**0.019**
$I_{\text{Con/Allo}}$ × $CD_{\text{Mono/Multi}}$	1	1.008	0.315
$I_{\text{Con/Allo}}$ × $CD_{\text{Div2/Div4}}$	1	1.696	0.193
$I_{\text{Alien/Native}}$ × $CD_{\text{Without/With}}$	1	0.259	0.611

$I_{Alien/Native} \times CD_{Mono/Multi}$	1	3.806	*0.051*
$I_{Alien/Native} \times CD_{Div2/Div4}$	1	0.722	0.395
O × I × CD	6	1.485	0.961
$O \times I_{Con/Allo} \times CD_{Without/With}$	1	1.100	0.294
$O \times I_{Con/Allo} \times CD_{Mono/Multi}$	1	0.096	0.757
$O \times I_{Con/Allo} \times CD_{Div2/Div4}$	1	0.010	0.918
$O \times I_{Alien/Native} \times CD_{Without/With}$	1	0.159	0.690
$O \times I_{Alien/Native} \times CD_{Mono/Multi}$	1	0.001	0.970
$O \times I_{Alien/Native} \times CD_{Div2/Div4}$	1	0.114	0.735
Random effects		SD	
Test species family		<0.001	
Test species species*		0.134	
Conditioning invader species		0.012	
Conditioning community		0.016	
Conditioning pot		0.027	
Residual		0.098	

Shown are results of linear mixed models. Values are in bold when $P < 0.05$ and in italic when $0.05 \leq P < 0.1$.

* Standard deviations for all the test species are shown in Table S7.

Table S6 Effects of the origin of the test species (alien or native), the diversity of the native community (0 [no community], 1, 2 or 4 species) and their interactions on total biomass of the test species in the subset of pots that did not experience conditioning with the invaders (treatments -I+C and -I-C).

	df	χ^2	*P*
Fixed effects			
Initial plant height of test species	1	25.949	**<0.001**
Origin of test species (O)	1	0.096	0.757
Conditioning community diversity (CD)	3	24.063	**<0.001**
$CD_{Without/With}$	1	24.025	**<0.001**
$CD_{Mono/Multi}$	1	0.015	0.903
$CD_{Div2/Div4}$	1	0.165	0.685
O × CD	3	2.889	0.409
O × $CD_{Without/With}$	1	1.025	0.311
O × $CD_{Mono/Multi}$	1	<0.001	0.988
O × $CD_{Div2/Div4}$	1	1.797	0.180
Random effects		SD	
Test species family		<0.001	
Test species species*		0.139	
Conditioning community		0.007	
Conditioning pot		0.018	
Residual		0.104	

Shown are results of linear mixed models. Values are in bold when $P < 0.05$.

* Standard deviations for all the test species are shown in Table S7.

Table S7 The standard deviations for the ten test species in the test phase from the models shown in Tables 2, 3, S3, S4, S5 and S6.

Test species	Table 2	Table 3	Table S3	Table S4	Table S5	Table S6
Ambrosia artemisiifolia	-	-	0.141	0.141	-	0.139
Lepidium campestre	0.137	0.134	0.135	0.135	0.134	0.110
Setaria faberi	0.224	0.136	0.161	0.160	0.136	0.161
Setaria viridis	0.140	0.156	0.161	0.161	0.157	0.122
Bromus sterilis	0.283	0.147	0.151	0.151	0.148	0.105
Lupinus polyphyllus	0.193	0.131	0.145	0.145	0.132	0.150
Trifolium pratense	0.353	0.257	0.253	0.253	0.257	0.216
Hypericum perforatum	-	-	0.153	0.153	-	0.129
Solidago gigantea	0.274	0.148	0.189	0.189	0.149	0.187
Bromus carinatus	0.229	0.117	0.121	0.121	0.117	0.091

CHAPTER 3 Negative conspecific plant-soil feedback on alien plants competing with natives is partly mitigated by another alien

Duo Chen & Mark van Kleunen

Abstract

Alien plant species that have established in the wild can impact native species directly through competition and indirectly through soil legacies. Through these same mechanisms, alien plants can also affect other alien plant species directly or indirectly. However, it is still unclear how pairwise competition between alien and native species is affected by their soil legacies and by the presence of an additional alien or native species. To test this, we conducted a combined plant-soil-feedback and competition experiment. We first grew each of four alien and four native plant species separately in pots to condition the soil. Thereafter, we grew all 16 pairwise alien-native species combinations on unconditioned soil or soils conditioned by the alien species, the native species or a mixture of both, and with or without an additional alien or native species. We found that both the presence of an additional species and soil-legacy effects reduced the growth of our alien and native test species. Among the soil-conditioning treatments, alien and native test species grew the worst on soils conditioned by conspecifics. Interestingly, we found that the negative conspecific soil-legacy effects on alien test species were partly alleviated when an additional alien species was present. Our results thus show that alien plants subjected to conspecific plant-soil feedbacks can benefit from the presence of other alien plants, as they partly mitigate the

negative conspecific plant-soil feedback effect. The presence of another alien species may thus give the alien test species an advantage over the native plants they compete with.

Keywords: biological invasion; coexistence; competition; exotic; invasional meltdown; soil-legacy effect; species interaction

Introduction

The numbers of alien species that have established in the wild (i.e. have become naturalized) have steadily increased over the past centuries (Dawson *et al.* 2017, Seebens *et al.* 2017). For plants, already more than 13,000 species have become naturalized (van Kleunen *et al.* 2015), and this has resulted in a global homogenization of regional floras (Yang *et al.* 2021). As the number of invasions are likely to continue in the next decades (Seebens *et al.* 2021), many studies have focused on how alien and native species compete and whether they can coexist. Many invasive alien plant species show superior competitive abilities (Vilà & Weiner 2004; Kuebbing & Nuñez 2016; Golivets & Wallin 2018), which may be partly due to characteristics such as fast growth and reproduction (van Kleunen *et al.* 2010). Consequently, some alien species can outcompete the native species and dominate the invaded communities (Simberloff 2013; Divíšek *et al.* 2018). This also means that alien species are increasingly likely to interact with other alien species. Therefore, an important question is how alien plant species are affected by the presence of other alien plant species.

At the end of the last century, Simberloff and Von Holle (1999) formulated the invasional meltdown hypothesis, posing that an alien species can facilitate the establishment of another alien species. Multiple lines of evidence suggest that some alien species can promote invasion by other aliens directly (Ricciardi 2001; Braga *et al.* 2018). For example, a study in France showed that when the alien aquatic plant *Ludwigia grandiflora* grows at low densities, it can promote root production and growth of another alien aquatic species, *Myriophyllum aquaticum* (Thouvenot *et al.* 2013). Similarly, a study in Argentina found positive interactions between two alien woody species (Tecco *et al.* 2006). Interactions between alien species, however, can also be indirect (Ricciardi 2001). For example, it has been shown that alien plants can positively affect one another through soil-microbial legacies (Zhang *et al.* 2020b) and by reducing the pressure from consumers (Nuñez *et al.* 2008). However, it has also been found that some naturalized alien species might, just like native species or even more strongly, provide resistance against invasion by other alien species (Haeuser et al. 2019). So, it is still unclear how different alien species interact and how this affects competition with native species.

In recent decades, it has become clear that plant-soil interactions play a major role in determining plant growth and species coexistence (Callaway *et al.* 2004, Wolfe & Klironomos 2005, Fahey *et al.* 2020). The phenomenon that previous plants affect subsequent plants through altering the biotic and abiotic properties of the soil is known as plant-soil feedback (Bever *et al.* 1997, van der Putten *et al.* 2013). As soil-legacy effects of conspecifics are usually more negative than those of heterospecifics, plant-soil feedbacks can drive coexistence and thus maintain species diversity (Mangan *et al.* 2010). However, alien species are, due to their novelty (Callaway & Ridenour 2004; Callaway *et al.* 2008) or due to the release from species-specific natural enemies and pathogens (Keane & Crawley 2002; Mitchell & Power 2003; Blumenthal *et al.* 2009), likely to be less negatively affected by soil legacies than native species. Moreover, alien plant species can positively affect the growth of later ones through their soil legacies (Chen & van Kleunen 2022), and differences in soil-legacy effects of previous alien species on subsequent alien and native species can drive the outcome of competition (Zhang *et al.* 2020b). So, when an additional alien plant species is introduced into a native community that has already been invaded by another alien, growth of the co-competing alien species may either be promoted, as predicted by the invasional meltdown hypothesis, or be reduced due to increased competition. Therefore, it remains to be explored how additional competitors and soil legacies jointly affect competition between alien and native species.

Here, we conducted a two-phase plant-soil-feedback experiment to test whether and how the soil legacies of alien and native plants, and the presence of an additional species affect the growth of and competition between alien and native species. We first grew each of four alien and four native species separately in pots to condition the soil. In the subsequent test phase, we then grew all 16 pairwise alien-native species combinations on unconditioned soil or soils conditioned by the respective alien species, the respective native species or a mixture of both, and with or without an additional alien or native species. We addressed the following questions: (1) How do the different soil legacies affect the growth of and competition between the alien and native species? (2) How does the presence of an additional alien or native species affect the growth of and competition between the alien and native species? (3) Do the soil-legacies and the additional species interact in their effects on growth and competition between the alien and native species, and does it matter whether the additional species is alien or native?

Materials and Methods

Study species and pre-cultivation

As study species, we selected four herbaceous plant species that are native (*Bromus sterilis* L., *Centaurea jacea* L., *Daucus carota* L., *Plantago lanceolata* L.) and four that are naturalized aliens (*Bidens frondosa* L., *Lepidium virginicum* L., *Lolium multiflorum* Lam., *Solidago canadensis* L.) in Germany (Table S1). The eight species are typically found in ruderal or grassland habitats (FloraWeb database; www.floraweb.de, accessed March 2021), and are from five families: the Asteraceae, Apiaceae, Brassicaceae, Plantaginaceae and Poaceae (Table S1). The classification of the species as alien or native to Germany was also based on information from the FloraWeb database. Seeds of three of the eight species were ordered from Rieger-Hofmann GmbH, and seeds of the other five species came from the seed collection of the Botanical Garden of the University of Konstanz (Tables S1).

From 12 to 21 April 2021, we sowed each of the eight species for the soil-conditioning phase separately into trays (18 cm × 14 cm × 5 cm) filled with potting soil (Topferde; Einheitserde Co., Sinntal-Altengronau, Germany). To make sure that all seedlings would be in similar developmental stages at transplanting, seeds were sown on different dates (Table S1) based on prior knowledge about the time required for germination. The pre-cultivation was done in a greenhouse of the Botanical Garden of the University of Konstanz, Germany (47°41′32″N, 9°10′41″E), and the temperature was maintained between 18 and 25 °C.

Experimental set up

Soil-conditioning phase

To make sure that the substrate used in the soil-conditioning phase contained live soil organisms, we collected field soil from a native grassland site near the Botanical Garden of the University of Konstanz on 26 April 2021. All four native study species occur in this site, and none of the alien study species. To remove large pebbles and plant fragments, we sieved the field soil using a metal grid with a mesh width of 1 cm. We then filled 2-L pots (14 cm × 14 cm × 14.5 cm) with a substrate consisting of a mixture of 25% of the field soil, 37.5% sand and 37.5% vermiculite (by volume). On 3 May 2021, we transplanted one seedling into the center of each pot. To obtain sufficient amounts of conditioned soil, we had 96 replicate pots for each of the eight species (i.e. 768 pots). In addition, we had 256 pots of substrate without any plant as a control treatment, resulting in a total of 1024 pots in the soil-conditioning phase. Seedlings that died within two weeks after transplanting were replaced. All pots were individually placed on plastic dishes (Ø = 17 cm) and randomly allocated to positions in three greenhouse compartments (24°C/18°C day/night temperature, 16 h/8 h day/night). We watered all pots every 2-3 days, and fertilized them four times (13 May, 30 May, 18 June and 7 July 2021, respectively) with a water-soluble fertilizer (1‰ m/v, Universol Blue with an NPK ratio of 3:2:3). To reduce potential effects of environmental heterogeneity within and among the greenhouse compartments, we re-randomized the positions of the pots five weeks after the start of the soil-conditioning phase.

On 19 July 2021, 11 weeks after the start of the soil-conditioning phase, we harvested the plant and soil of each pot. The aboveground plant parts were dried at 70°C to a constant weight, and then weighed. The soil of each pot, including the pots without plants, was sieved through a 5-mm mesh to remove the roots. In between the harvesting of different pots, the mesh was sterilized with 70% ethanol. The sieved soil was stored at 4°C until use in the test phase.

Test phase

From 12 to 19 July 2021, we sowed the eight species to produce seedlings for the test phase (Table S1). The pre-cultivation conditions were the same as for the soil-conditioning phase. We filled 0.5-L pots (9 cm × 9 cm × 8 cm) with soil from the conditioning phase. To test the effects of the various conditioned soils on each of the 16 possible alien-native species pairs, we used four soil-conditioning treatments in the test phase (Fig. 1): (1) soil conditioned without a plant (Control), (2) soil conditioned by the alien species of the respective species pair (Alien), (3) soil conditioned by the native species of the respective species pair (Native), and (4) a 1:1 mixture of soil conditioned by the alien species and the native species of the respective species pair (Mixed). For each pot in the Mixed treatment, we took soil from one soil-conditioning-phase pot of the respective alien species and one soil-conditioning-phase pot of the respective native species. To avoid possible differences between soil-conditioning treatments due to combining soils from different numbers of pots, we also mixed soils from two soil-conditioning-phase pots for the Control, Alien and Native treatments. For example, for a test-phase pot in the Alien treatment of the species pair *Solidago canadensis* - *Bromus sterilis*, we mixed soils from two pots conditioned by *S. canadensis*. In each of the test-phase pots, the two soils were mixed in equal volumes, and each of the soil-conditioning-phase pots, including the controls, was used only once.

On 2 and 3 August 2021, we transplanted seedlings into the pots of the test phase. For each of the 16 pairs of alien and native species, we had 32 pots and in each of those we planted one seedling of the respective alien species and one seedling of the respective native species. Eight of the 32 pots per species pair had unconditioned control soil, eight pots had soil conditioned by the respective alien species, eight pots had soil conditioned by the respective native species, and eight pots had a mixture of soils conditioned by the respective alien and native species. Of the eight pots per soil-conditioning treatment of a species pair, six pots received an additional seedling (i.e. a third plant) of one of the other six species. Each of those other three native and three alien species was used only once per soil-conditioning treatment of a species pair, so that we effectively had three replicates with an additional alien species and three replicates with an additional native species. The two remaining pots per soil-conditioning treatment of a species pair did not receive an additional species and served as controls for the effects of the presence of an additional species. In the pots without additional species, the two seedlings were planted 6 cm apart: 3 cm to the left and 3cm to the right of the center of the pot (Fig. 1). In the pots with an additional species, the three seedlings were also planted 6 cm apart but in a centrosymmetric equilateral triangle (Fig. 1). In total, we had 512 pots in the test phase.

All pots were individually placed on plastic dishes (Ø = 15 cm) and randomly allocated to positions in a greenhouse compartment (24°C/18°C day/night temperature, 16 h/8 h day/night). We watered pots every 2-3 days and fertilized them two times with 80 mL of a water-soluble fertilizer (1‰ m/v, Universol Blue) on 16 August and 13 September 2021, respectively. Positions of all pots were re-randomized four weeks after the start of the test phase.

Measurements

On 4 and 5 August 2021, at the start of the test phase, we measured the length and width of the largest leaf on each seedling, and also counted the number of leaves. The initial leaf area of each seedling was then estimated as length of the largest leaf × width of largest leaf × number of leaves. On 4 October 2021, nine weeks after the start of the test phase, we harvested the aboveground biomass of each plant separately from each pot. Because it was impossible to separate the roots of the different species in each pot, the belowground biomass was not harvested. The plant materials were dried at 70°C to constant weight, and were then weighed.

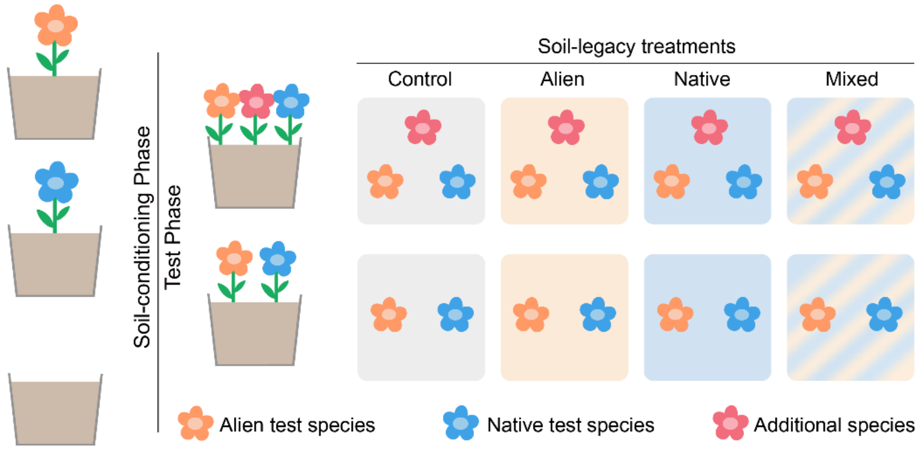

Figure 1 Overview of the experimental design. In the soil-conditioning phase, the soil was conditioned separately by each of the four alien (orange flowers) and four native species (blue flowers), or without any plants. In the test phase, pots were created for each of four soil-legacy treatments by filling them with soil without plant conditioning (Control, grey background), soil conditioned by an alien species (Alien, orange background), soil conditioned by a native species (Native, blue background), or a mixture of soil conditioned by an alien species and soil conditioned by a native species (Mixed, orange-blue striped background). The alien and native test species that were planted corresponded to the species that had been used to condition the soil. For pots in the additional species treatment, another alien or native species (red plants) was also planted.

Statistical analysis

To test whether and how the two experimental factors, soil legacy and additional species, and their interaction affected the performance of the alien and native species in the test phase, we fitted a linear mixed effect model with the *lme* function in the R package 'nlme' (Pinheiro *et al.* 2022). Biomass of the test species was included as the response variable. The origin of the test species (alien or native), soil-legacy treatment (Control, Alien, Native or Mixed), the additional plant treatment (without, with alien or with native) and their interactions were included as fixed effects. We additionally ran three orthogonal hierarchical contrasts for the soil-legacy treatments (SL). First, to test whether the effects of soils not conditioned by a plant differed from those that had been conditioned by a plant, we compared Control vs the average of Alien, Native and Mixed ($SL_{Unconditioned/Conditioned}$). Second, to test whether the effects of soils conditioned by only one of the two species of a species pair differed from those conditioned by both species, we compared Mixed vs the average of Alien and Native ($SL_{Mixed/Single}$). Third, to test whether the effects of soils conditioned by the alien species of a species pair differed from those conditioned by the native species, we compared Alien vs Native ($SL_{Alien/Native}$). Furthermore, we also ran two orthogonal hierarchical contrasts for the additional plant treatment (AP). First, to test the effect of the presence of an additional species, we compared the treatment without an additional species vs the average of the treatments with an additional alien or native species ($AP_{Without/With}$). Second, to test the effect of whether the additional species was an alien or a native species, we compared the treatment with an additional alien species vs the treatment with an additional native species ($AP_{Alien/Native}$). To account for nonindependence of test species belonging to the same family and of plants belonging to the same test species, family and identity of the test species were used as random effects. To account for the identity of the additional species, it was also included as a random effect. Furthermore, to account for nonindependence of species from the same pot of the test phase, identity of the test-phase pot was included as a random effect. Finally, to account for differences in initial sizes of the test species, we included initial leaf area as a fixed covariate.

To test how the soil legacy and the presence of additional species affect the biomass proportion of alien test species, we fitted a model including the proportional biomass of alien test species, calculated as $biomass_{alien}/(biomass_{alien} + biomass_{native})$, as a response variable. We included soil-legacy treatment (Control, Alien, Native or Mixed), the additional species treatment (without, with alien or with native) and their interactions as fixed effects. Identities of the alien, native and additional species were included as random effects, and proportional initial leaf area of alien test species was included as a covariate. In addition, to test how the total aboveground plant biomass per pot was affected by the treatments, we ran a similar model for biomass per pot as a response variable. Here, we used the combined initial leaf area of all plants in a pot as a covariate. Also, to test the effect of biomass of soil-conditioning plants on biomass production of test plants, we also ran such a model using the subset of pots with soil conditioning (i.e. we excluded Control soil pots). In this model, total aboveground biomass per pot was included as a response variable, and the average aboveground biomass of the two corresponding conditioning plants used for soil mixing was added as a covariate. In addition to account for initial size variation of the plants in the test phase, the combined initial leaf area of all plants in a pot was also added as a covariate.

To additionally test whether and how the soil legacies affect the additional species, we also fitted a model using the subset of pots with additional species. In this model, we included proportional biomass of the additional plant, calculated as $biomass_{additional}/(biomass_{additional} + biomass_{alien} + biomass_{native})$, as the response variable. We included the origin of the additional species (alien or native), soil-legacy treatment (included as three orthogonal hierarchical contrasts: $SL_{Unconditioned/Conditioned}$, $SL_{Mixed/Single}$ and $SL_{Alien/Native}$) and their interactions as fixed effects. Identities of the alien, native and additional species were included as random effects, and proportional initial leaf area of the additional species was included as a covariate.

In all models, to improve homoscedasticity of residuals of the models, we allowed the variance to vary among the test species and among the additional species by using the *varIdent* and *varComb* functions (Table S6). To meet the assumption of normality, biomass of the test species, and biomass proportion of alien test species and additional species were square-root transformed. We used log-likelihood ratio tests to assess the significance of fixed effects by comparing models with and without the effect of interest (Zuur *et al.* 2009). All analyses were conducted in R 3.6.2 (R Core Team 2022).

Results

Effects of soil legacies and additional species on test species

On average, the aboveground biomass of alien and native test species, as well as the total biomass per pot (Appendix S1: Table S2, Fig. S1), were reduced when plants were grown on conditioned instead of unconditioned control soil (Table 1, Fig. 2). The test species achieved more biomass in the Mixed soil treatment compared to the average of the Alien and Native soil treatments when there was no additional competitor present (+14.1%), whereas the reverse was true in the presence of an additional competitor (-7.3%; significant $AP_{Without/With} \times SL_{Mixed/Single}$ in Table 1, Fig. 2). Furthermore, conspecific plant-soil feedback was more negative than heterospecific plant-soil feedback, as the alien test species performed worse on soil conditioned by the alien species than on soil conditioned by the native species, whereas the opposite was true for the native species (significant O × $SL_{Alien/Native}$ interaction in Table 1, Fig. 2). As a consequence, although the alien test species produced much more biomass than the native species in most soil-conditioning treatments, this difference almost disappeared when the plants were grown on soils conditioned by the alien species.

The presence of an additional species reduced the biomass of the native and alien test species, irrespective of whether the additional species was alien or native (Table 1, Fig. 2b, c). However, the difference in biomass between alien and native test species in the presence of an additional alien species was larger than in the presence of an additional native species when grown on soil conditioned by the alien species (25.5% vs 11.8%), whereas the opposite pattern was found when plants were grown on soil conditioned by the native species (55.8% vs 64.4%; significant O × $AP_{Alien/Native} \times SL_{Alien/Native}$ interaction in Table 1, Fig. 2b, c).

Table 1 Results of a linear mixed model testing the effects the origin of the test species (alien or native), the presence of an additional species (without, with an alien or with a native), soil-legacy treatment (Control, Alien, Native or Mixed), and their interactions on aboveground biomass of the test species. Values are in bold when $P < 0.05$ and in italic when $0.05 \leq P < 0.1$.

	df	χ^2	*P*
Fixed effects			
Initial leaf area of test species	1	62.807	**<0.001**
Origin of test species (O)	1	1.100	0.294
Additional plant treatment (AP)	2	5.207	*0.074*
$AP_{Without/With}$	1	4.718	**0.030**
$AP_{Alien/Native}$	1	0.878	0.349
Soil-legacy treatment (SL)	3	103.601	**<0.001**
$SL_{Unconditioned/Conditioned}$	1	103.201	**<0.001**
$SL_{Mixed/Single}$	1	0.017	0.897
$SL_{Alien/Native}$	1	0.056	0.814
O × AP	2	2.536	0.281
$O \times AP_{Without/With}$	1	0.675	0.411
$O \times AP_{Alien/Native}$	1	1.792	0.181
O × SL	3	74.440	**<0.001**
$O \times SL_{Unconditioned/Conditioned}$	1	0.659	0.417
$O \times SL_{Mixed/Single}$	1	0.784	0.376
$O \times SL_{Alien/Native}$	1	72.998	**<0.001**
AP × SL	6	8.247	0.221
$AP_{Without/With} \times SL_{Unconditioned/Conditioned}$	1	0.071	0.789
$AP_{Without/With} \times SL_{Mixed/Single}$	1	6.428	**0.011**
$AP_{Without/With} \times SL_{Alien/Native}$	1	0.349	0.555
$AP_{Alien/Native} \times SL_{Unconditioned/Conditioned}$	1	0.080	0.777
$AP_{Alien/Native} \times SL_{Mixed/Single}$	1	0.752	0.386
$AP_{Alien/Native} \times SL_{Alien/Native}$	1	0.331	0.565
O × AP × SL	6	10.404	0.109
$O \times AP_{Without/With} \times SL_{Unconditioned/Conditioned}$	1	1.291	0.256
$O \times AP_{Without/With} \times SL_{Mixed/Single}$	1	0.014	0.906
$O \times AP_{Without/With} \times SL_{Alien/Native}$	1	2.059	0.151
$O \times AP_{Alien/Native} \times SL_{Unconditioned/Conditioned}$	1	0.630	0.427
$O \times AP_{Alien/Native} \times SL_{Mixed/Single}$	1	1.784	0.182
$O \times AP_{Alien/Native} \times SL_{Alien/Native}$	1	4.567	**0.033**
Random effects		SD	
Family of test species		<0.001	
Test species		0.124	
Additional species		0.036	
Pot identity of test phase		<0.001	
Residual		0.164	

Effects of soil legacies and additional species on proportional biomass of alien species

In line with the strongly reduced difference in biomass between the alien and native test species, when grown on soil conditioned by the alien species, the proportional biomass of the alien test species ($biomass_{alien}/[biomass_{alien} + biomass_{native}]$) was significantly lower when grown on soil conditioned by the alien species instead of on soil conditioned by the native species (Table 2, Fig. 3). However, compared to additional native species, the presence of an additional alien species relatively improved the biomass proportion of alien test species on soil conditioned by the alien species (+7.5%), whereas this was not the case on soil conditioned by the native species (marginally significant $AP_{Alien/Native} \times SL_{Alien/Native}$ interaction in Table 2, Fig. 3).

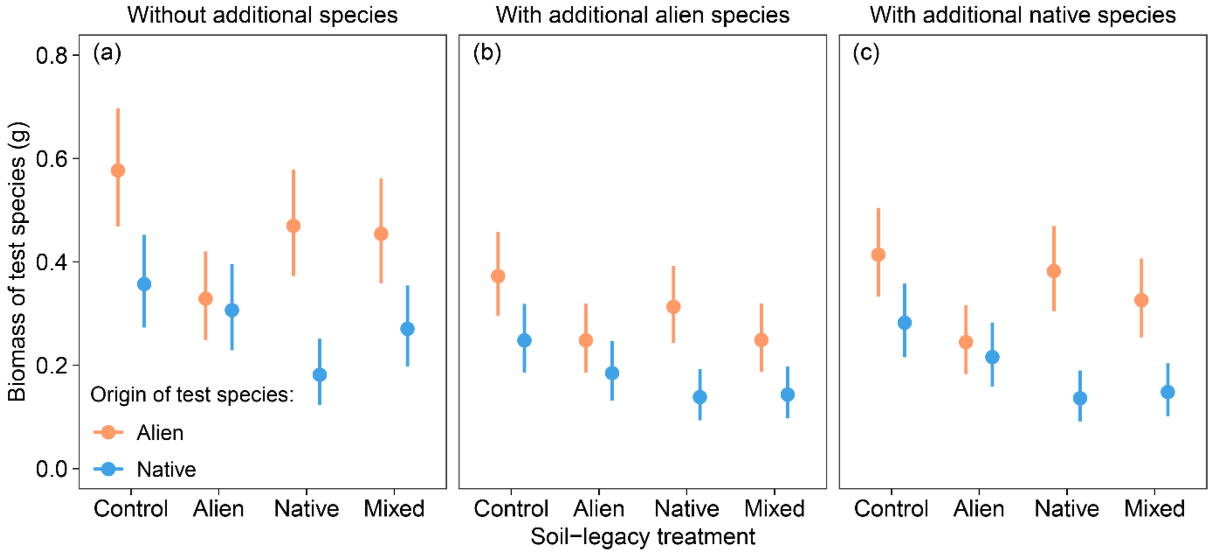

Figure 2 Effects of the presence of an additional species on the aboveground biomass of the alien and native test species in the four soil-legacy treatments. Shown are modelled means (±SEs) after back-transformation.

Table 2 Results of a linear mixed model testing the effects of the presence of an additional species (without, with an alien or with a native), soil-legacy treatment (Control, Alien, Native or Mixed), and their interactions on proportional biomass of the alien test species. Values are in bold when $P < 0.05$ and in italic when $0.05 \leq P < 0.1$.

	df	χ^2	*P*
Fixed effects			
Proportional initial leaf area of alien species	1	35.839	**<0.001**
Additional plant treatment (AP)	2	0.750	0.687
$AP_{Without/With}$	1	0.015	0.903
$AP_{Alien/Native}$	1	0.731	0.393
Soil-legacy treatment (SL)	3	66.715	**<0.001**
$SL_{Unconditioned/Conditioned}$	1	0.156	0.693
$SL_{Mixed/Single}$	1	1.326	0.250
$SL_{Alien/Native}$	1	65.407	**<0.001**
AP × SL	6	10.769	*0.096*
$AP_{Without/With} \times SL_{Unconditioned/Conditioned}$	1	1.012	0.314
$AP_{Without/With} \times SL_{Mixed/Single}$	1	0.917	0.338
$AP_{Without/With} \times SL_{Alien/Native}$	1	2.467	0.116
$AP_{Alien/Native} \times SL_{Unconditioned/Conditioned}$	1	0.558	0.455
$AP_{Alien/Native} \times SL_{Mixed/Single}$	1	2.113	0.146
$AP_{Alien/Native} \times SL_{Alien/Native}$	1	3.657	*0.056*
Random effects		SD	
Alien test species		0.098	
Native test species		0.036	
Additional species		0.014	
Residual		0.114	

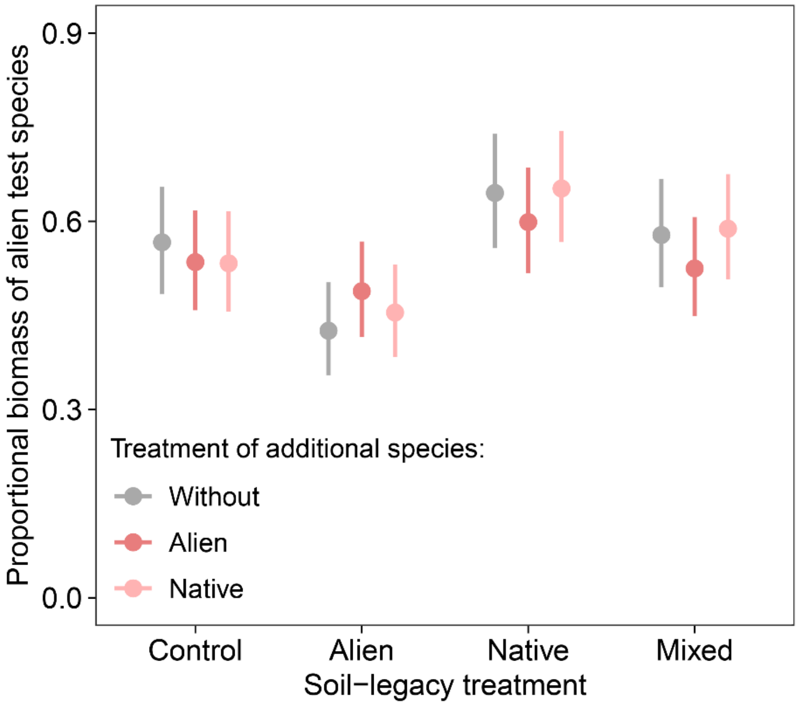

Figure 3 Effects of the presence of an additional species on the biomass proportion of the alien test species in the four soil-legacy treatments. Shown are modelled means (±SEs) after back-transformation.

Discussion

Our combined plant-soil-feedback and competition experiment tested whether and how the soil legacy of previous plants and the presence of an additional species affect the performance of alien and native test species. As expected, we found that the alien and native plants performed worse when the soil had been conditioned by plants, indicating negative plant-soil feedback, and when an additional species was present, indicating competition. The negative plant-soil feedback was stronger when the soil had been conditioned by a conspecific instead of a heterospecific plant. Consequently, although the alien species produced overall more biomass than the native species, this difference was reduced when the soil had been conditioned by the alien species, indicating that alien species also suffered strong conspecific feedback effects. However, proportional biomass of the alien test species on such conspecific soil was relatively increased when there was another alien species present. This suggests that, in our study, the presence of another alien species partly alleviated the negative conspecific plant-soil-feedback effects of alien species.

When the alien and native test species grew on soils that had been conditioned by the alien species, the native species or both, they produced less biomass than when grown on the unconditioned soils. This could partly reflect that the soils that had been conditioned by plants contained less nutrients than the unconditioned soils. However, although the biomass produced during the conditioning phase had a negative effect on the biomass production per pot in the test phase, this effect was relatively weak (Table S3, Fig. S3), most likely because we fertilized the pots during both the conditioning and test phase. Furthermore, if nutrient depletion would explain the effect of soil conditioning, then the test plants and the additional plants should be affected to similar degrees. However, the additional plants had increased their proportional biomass on the conditioned soils (Table

S5, Fig. S2b). Therefore, the negative plant-soil feedback is most likely due to the accumulation of herbivorous and pathogenic soil organisms.

The results of many previous studies also show that the effects of plant-soil feedback are usually negative (Lekberg *et al.* 2018, Crawford *et al.* 2019). Furthermore, most previous studies also found that conspecific plant-soil feedback is usually stronger than heterospecific plant-soil feedback (e.g. MacDougall *et al.* 2011; Rutten *et al.* 2016; Lozano *et al.* 2022). This was also the case in our study, for both the alien and native species, as aliens had the lowest biomass on soils conditioned by the conspecific aliens, and natives had the lowest biomass on soils conditioned by the conspecific natives. However, we found that alien species experienced less conspecific plant-soil feedbacks than native species when we compared their biomass on conspecific soil with that on unconditioned soil (-39.5% vs -48.5%). This supports the hypothesis that alien species have been released from some of their species-specific natural enemies (Keane & Crawley 2002, Klironomos 2002, Mitchell & Power 2003, MacDougall *et al.* 2011).

As the alien species produced on average more biomass than the native species in our study, the strongly negative conspecific plant-soil feedback resulted in a smaller biomass difference between the alien and native species on the soils conditioned by the alien species. As a consequence, the proportional biomass of the alien species was lowest on soils conditioned by the alien species and highest on soils conditioned by the native species. On the soils conditioned by both the alien and native species (i.e. the Mixed treatment), the proportional biomass of the alien species was intermediate, indicating that here the negative conspecific plant-soil-feedback effect was diluted. This was the case both with and without additional species, but, without additional species, the biomass of the test species in the mixed soils exceeded the average of the biomass on each of the separate soils (+14.1%).

This suggests that the test species experienced relatively weak negative plant-soil feedbacks on mixed soils. It has been suggested that the mixing of soils conditioned by different species can increase the diversity of soil legacies and thus increase the probabilities that the test species will interact with them (van der Heijden *et al.* 2008; Thakur *et al.* 2021). However, species-specific pathogens and secondary metabolites in the soils will also be diluted during this process, reducing the negative effect on plant growth. As a consequence, both alien and native test species accumulated relatively more biomass on mixed soils than they did on their conspecific soils.

When an increase in the number of species coincides with an increase in plant density, this should result in more intense competition (Grace & Tilman 1990; Weigelt & Jolliffe 2003). Consistent with this, we found that growth of the alien and native test species was reduced in the presence of the additional competitor. Although the alien species in our study were generally larger than the native ones, the effect of the additional competitor did not significantly depend on its origin. This suggests that in our study, the intensity of competition was more strongly affected by the density of individuals than by the identity of the additional competitor. However, the origin of the additional competitor had an effect on the biomass difference between the alien and native test species. More precisely, the reduction in the biomass difference between the alien and native species on the soils conditioned by the alien species was smaller when the additional species was an alien instead of a native species. Consequently, the proportional biomass of the alien species, when grown on soils conditioned by the alien species, was highest when the additional species was also an alien. Plants usually experience stronger competition from tall than from small plants (Dostal 2011; Feng *et al.* 2016). Therefore, a possible explanation for our finding could be that the native test species, which were overall smaller than the alien species, suffered more from the additional alien competitor than the alien test species did. Irrespective of the exact reason, our finding indicates that the growth of alien test species benefited from the presence of another alien species, which supports the invasional meltdown hypothesis (Simberloff & Von Holle 1999).

Although the alien species, when grown on conspecific soil, benefited from the presence of another alien species, the proportional biomass of this other alien species was unaffected by the origin of the soil-conditioning species. In other words, the additional alien species did not benefit, nor did it incur a cost on the soil conditioned by the alien test species (Table S4-S5, Fig. S2). Indeed, outcomes of invasional meltdown are not necessarily mutually beneficial for both alien species (Simberloff & Von Holle 1999), and unidirectional positive effects for just one of the alien species are common (e.g. Jäger *et al.* 2009; Relva *et al.* 2010; Flory & Bauer 2014; Ma *et al.* 2014). As plant-soil-feedback-mediated invasional meltdown is a temporal one-way process (i.e. from earlier to later in time), it is more likely that only the subsequent alien species benefit from the soil legacies of previous alien species. Indeed, Chen and van Kleunen (2022) found that subsequent alien plants experienced less negative soil-legacy effects on soils conditioned by heterospecific alien plants. Furthermore, Zhang et al. (2020b) found that the soil legacies of alien plants benefited later alien plants when growing in competition with native plants. In our study, although the biomass of the additional alien plants did not benefit from the soil conditioned by other alien plants, it was not at a competitive disadvantage either.

In conclusion, we found that soil legacies and the presence of an additional species negatively affected the growth of plants in the test phase. In the absence of an additional species, test plants had relatively better performance on mixed conditioned soil than on soils conditioned by only the native or the alien species. Moreover, both alien and native test plants grew worse on soil conditioned by conspecifics than on soils conditioned by heterospecifics, and alien test plants were more negatively affected than native test plants. However, this negative conspecific soil-legacy effect experienced by alien test plants was partly alleviated by the presence of another alien species. Our study therefore suggests that alien plants can benefit from other alien plants as the latter mitigate the reduction in growth caused by their conspecific soil legacies. Ultimately this might result in a competitive advantage of the alien over the native plants.

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Author contributions

DC conceived the idea. DC and MvK designed the experiment, DC conducted the experiment and analyzed the data. DC wrote the manuscript with further inputs from MvK.

Supporting information

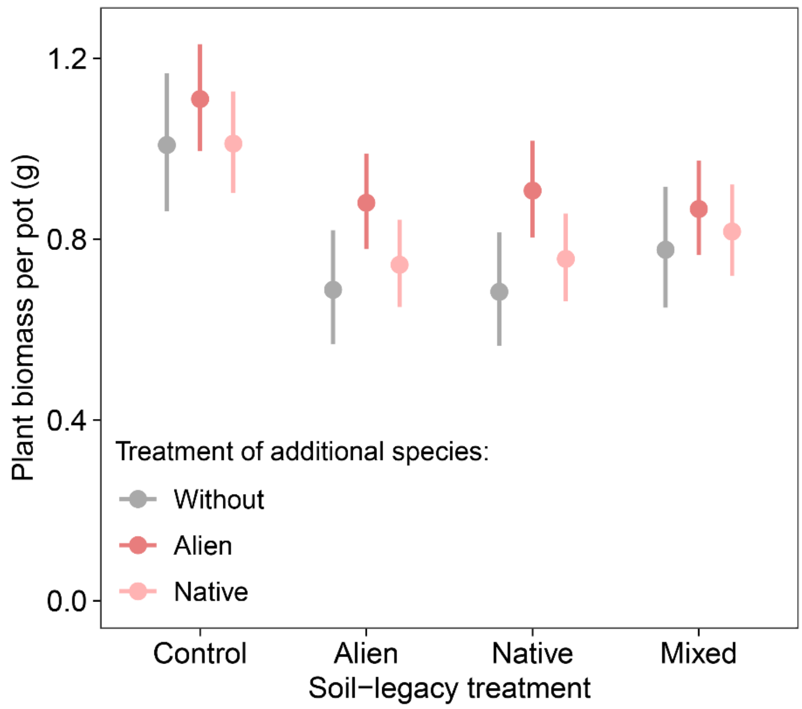

Figure S1 Effects of the presence of an additional alien or native species on the biomass per pot in four soil-legacy treatments. Shown are modelled means (±SEs) after back-transformation.

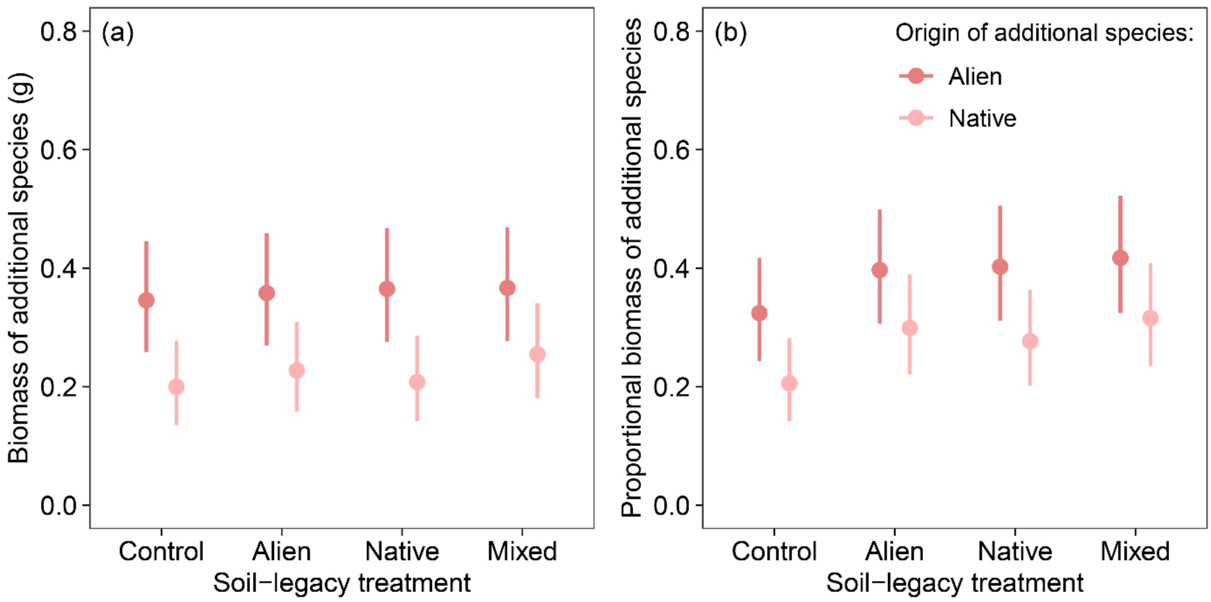

Figure S2 a, Biomass of additional alien and native species in the four soil-legacy treatments. **b**, Proportional biomass of the additional species in the four soil-legacy treatments. Shown are modelled means (±SEs) after back-transformation.

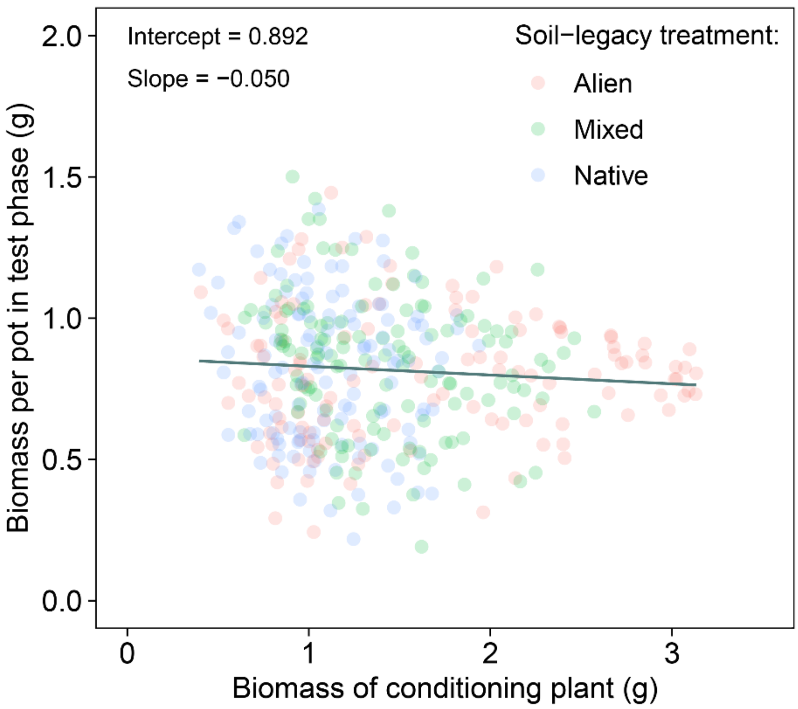

Figure S3 Effects of aboveground biomass of conditioning plants on aboveground biomass per pot in the test phase. Points indicate the average aboveground biomass of the two corresponding conditioning plants from the two pots used for soil mixing, and the line indicates the intercept and slope for the biomass of soil-conditioning plants of the model shown in Table S3. The points are color-coded to indicate the soil-legacy treatments.

Table S1 Plant species used in the experiment.

Species	Family	Origin	Native range	Sowing date		Seed source
				Soil-conditioning phase	Test phase	
Bidens frondosa L.	Asteraceae	Alien	North America	21.04.2021	14.07.2021	University of Konstanz, Germany
Lepidium virginicum L.	Brassicaceae	Alien	North America	21.04.2021	12.07.2021	University of Konstanz, Germany
Lolium multiflorum Lam.*	Poaceae	Alien	Europe	21.04.2021	12.07.2021	Rieger-Hofmann GmbH, Germany
Solidago canadensis L.	Asteraceae	Alien	North America	12.04.2021	19.07.2021	University of Konstanz, Germany
Bromus sterilis L.	Poaceae	Native	-	21.04.2021	12.07.2021	University of Konstanz, Germany
Centaurea jacea L.	Asteraceae	Native	-	12.04.2021	12.07.2021	Rieger-Hofmann GmbH, Germany
Daucus carota L.	Apiaceae	Native	-	19.04.2021	19.07.2021	Rieger-Hofmann GmbH, Germany
Plantago lanceolata L.	Plantaginaceae	Native	-	19.04.2021	12.07.2021	University of Konstanz, Germany

* The species is native in part of Europe but alien in Germany.

Table S2 Results of a linear mixed model testing the effects of the presence of an additional species (without, with an alien or with a native), the soil-legacy treatment (Control, Alien, Native or Mixed), and their interactions on aboveground plant biomass per pot. Values are in bold when $P < 0.05$ and in italic when $0.05 \leq P < 0.1$.

	df	χ^2	*P*
Fixed effects			
Initial leaf area of all plants per pot	1	5.435	**0.020**
Additional plant treatment (AP)	2	2.344	0.310
$AP_{Without/With}$	1	0.739	0.390
$AP_{Alien/Native}$	1	1.740	0.187
Soil-legacy treatment (SL)	3	117.073	**<0.001**
$SL_{Unconditioned/Conditioned}$	1	113.635	**<0.001**
$SL_{Mixed/Single}$	1	3.952	**0.047**
$SL_{Alien/Native}$	1	0.322	0.570
AP × SL	6	9.078	0.169
$AP_{Without/With} \times SL_{Unconditioned/Conditioned}$	1	2.208	0.137
$AP_{Without/With} \times SL_{Mixed/Single}$	1	2.400	0.121
$AP_{Without/With} \times SL_{Alien/Native}$	1	0.194	0.660
$AP_{Alien/Native} \times SL_{Unconditioned/Conditioned}$	1	0.329	0.566
$AP_{Alien/Native} \times SL_{Mixed/Single}$	1	4.042	**0.044**
$AP_{Alien/Native} \times SL_{Alien/Native}$	1	0.048	0.826
Random effects		SD	
Alien test species		0.084	
Native test species		0.037	
Additional species		0.058	
Residual		0.167	

Table S3 Results of a linear mixed model testing the effects the presence of an additional species (without, with an alien or with a native), the soil-legacy treatment (Alien, Native or Mixed), and their interactions on aboveground plant biomass per pot. The results were analyzed for the subset of data without control pots, and included the aboveground biomass of soil-conditioning plants as a covariate. Values are in bold when P < 0.05.

	df	χ^2	*P*
Fixed effects			
Aboveground biomass of conditioning plants	1	56.900	**<0.001**
Initial leaf area of all plants per pot	1	4.507	**0.034**
Additional plant treatment (AP)	2	2.485	0.289
$AP_{Without/With}$	1	0.809	0.368
$AP_{Alien/Native}$	1	1.825	0.177
Soil-legacy treatment (SL)	2	9.407	**0.009**
$SL_{Mixed/Single}$	1	3.989	**0.046**
$SL_{Alien/Native}$	1	5.793	**0.016**
AP × SL	4	7.490	0.112
$AP_{Without/With} \times SL_{Mixed/Single}$	1	5.895	**0.015**
$AP_{Without/With} \times SL_{Alien/Native}$	1	0.406	0.524
$AP_{Alien/Native} \times SL_{Mixed/Single}$	1	0.989	0.320
$AP_{Alien/Native} \times SL_{Alien/Native}$	1	0.181	0.670
Random effects		SD	
Alien test species		0.090	
Native test species		0.045	
Additional species		0.051	
Residual		0.118	

Table S4 Results of a linear mixed model testing the effects the origin of the additional species (alien or native), the soil-legacy treatment (Control, Aien, Native or Mixed), and their interactions on the biomass of the additional species. The results were analyzed for the subset of pots with an additional species. Values are in bold when $P < 0.05$ and in italic when $0.05 \leq P < 0.1$.

	df	χ^2	*P*
Fixed effects			
Initial leaf area of additional species	1	25.835	**<0.001**
Origin of additional species (OA)	1	1.353	0.245
Soil-legacy treatment (SL)	3	6.530	*0.088*
$SL_{Unconditioned/Conditioned}$	1	3.663	*0.056*
$SL_{Mixed/Single}$	1	2.915	*0.088*
$SL_{Alien/Native}$	1	0.320	0.571
OA × SL	3	3.343	0.342
OA × $SL_{Unconditioned/Conditioned}$	1	0.547	0.459
OA × $SL_{Mixed/Single}$	1	1.976	0.160
OA × $SL_{Alien/Native}$	1	0.919	0.338
Random effects		SD	
Alien test species		0.054	
Native test species		0.008	
Additional species		0.146	
Residual		0.105	

Table S5 Results of a linear mixed model testing the effects the origin of the additional species (alien or native), the soil-legacy treatment (Control, Alien, Native or Mixed), and their interactions on proportional biomass of the additional species. The results were analyzed for the subset of pots with an additional species. Values are in bold when $P < 0.05$ and in italic when $0.05 \leq P < 0.1$.

	df	χ^2	*P*
Fixed effects			
Proportional initial leaf area of additional species	1	36.624	**<0.001**
Origin of additional species (OA)	1	1.194	0.274
Soil-legacy treatment (SL)	3	50.398	**<0.001**
$SL_{Unconditioned/Conditioned}$	1	48.130	**<0.001**
$SL_{Mixed/Single}$	1	3.125	*0.077*
$SL_{Alien/Native}$	1	0.213	0.645
OA × SL	3	2.460	0.483
OA × $SL_{Unconditioned/Conditioned}$	1	1.264	0.261
OA × $SL_{Mixed/Single}$	1	0.296	0.587
OA × $SL_{Alien/Native}$	1	0.954	0.329
Random effects		SD	
Alien test species		0.091	
Native test species		0.014	
Additional species		0.120	
Residual		0.136	

Table S6 Standard deviations of the alien and native test species, and the additional species in the test phase from the models shown in Tables 1, 2 and S2-S5.

Species	Table 1	Table 2	Table S2	Table S3	Table S4	Table S5
Alien						
Lolium multiflorum	0.124	0.084	0.098	0.090	0.054	0.091
Lepidium virginicum	0.086	0.057	0.172	0.076	0.051	0.082
Solidago canadensis	0.087	0.060	0.130	0.093	0.061	0.078
Bidens frondosa	0.135	0.064	0.139	0.069	0.047	0.088
Native						
Bromus sterilis	0.119	0.037	0.036	0.045	0.008	0.014
Centaurea jacea	0.094	0.030	0.043	0.035	0.008	0.013
Daucus carota	0.094	0.034	0.046	0.041	0.008	0.014
Plantago lanceolata	0.123	0.033	0.048	0.035	0.008	0.015
Additional						
Without	0.032	0.052	0.008	0.053	-	-
Lepidium virginicum	0.036	0.058	0.014	0.051	0.146	0.120
Solidago canadensis	0.029	0.042	0.006	0.047	0.100	0.077
Daucus carota	0.036	0.050	0.010	0.058	0.148	0.111
Plantago lanceolata	0.032	0.044	0.011	0.041	0.140	0.092
Bidens frondosa	0.024	0.047	0.009	0.033	0.143	0.049
Centaurea jacea	0.026	0.041	0.007	0.042	0.106	0.071
Bromus sterilis	0.031	0.063	0.008	0.063	0.169	0.116
Lolium multiflorum	0.028	0.071	0.007	0.062	0.211	0.086

CHAPTER 4 Size inequality of competing plants depends on soil legacies and species origin

Duo Chen & Mark van Kleunen

Abstract

With the number of naturalized alien plant species continuously increasing, it has become of major interest to understand how they can coexist with or even outcompete native species. Plant-soil interactions and soil spatial heterogeneity are thought to play major roles in coexistence of plant species. Depending on the strengths of conspecific and heterospecific soil feedbacks, size inequalities between competing plants might either decrease (i.e. promote coexistence) or increase. However, how spatial heterogeneity in soil legacies affects the biomass production and size inequalities of competing plants, and how this depends on the origins of the plants has never been tested. Here, we first conditioned soil with each of the five naturalized alien and five native species separately. Then, in the test phase, we grew all 45 pairwise species combinations under four homogeneous and two heterogeneous soil-legacy conditions. We found that soil legacies overall had a negative effect on biomass production, irrespective of the origins of the competing species. Biomass inequality between the competing plants was the smallest when they grew on soil conditioned only by the larger one of the two species. In the subset of alien-native species combinations, alien species surprisingly suffered from strong negative conspecific soil-legacy effects in the homogeneous treatment. However, the alien plants produced more biomass and had a higher biomass proportion relative to native plants when planted in a patch of conspecific soil in the heterogeneous treatment. Our findings show that the performance of competing plants may depend on their origins as well as soil legacies. This may ultimately affect competition and coexistence of alien and native plants in natural environments where soil legacies are likely to be heterogeneous.

Keywords: coexistence, conspecific, exotic, heterogeneity, interspecific competition, plant-soil feedback, species interaction

Introduction

In the last centuries, the numbers of alien species that have established self-sustaining populations, have dramatically increased all over the world (Dawson *et al.* 2017; Pyšek *et al.* 2017). The numbers of naturalized alien species are likely to continue increasing (Seebens *et al.* 2021). Consequently, a major research question is how these alien species can coexist with or even outcompete native species. Moreover, with the increasing number of naturalized alien species, it also becomes more likely that alien species interact with other alien species. Among the few studies on alien-alien interactions in plants, some found that naturalized alien species might provide resistance against establishment of other alien species (Haeuser *et al.* 2019), whereas other studies suggest that alien organisms can have positive interactions (Simberloff & Von Holle 1999), particularly through soil-legacy effects (Bourgeois *et al.* 2005; Zhang *et al.* 2020b). Therefore, more research is needed on how interactions between plant species depend on whether the species are alien or native.

The interaction between plants and soil is considered to play a key role in plant competition and coexistence (Wolfe & Klironomos 2005; Fahey *et al.* 2020). Plants alter the biotic and abiotic properties of the soil, and these soil legacies affect the growth of subsequent plants (Bever *et al.* 1997; Kulmatiski *et al.* 2008; van der Putten *et al.* 2013; Crawford *et al.* 2019). Plant-soil feedback usually becomes stronger and more negative when the former (i.e. the conditioning plant) and latter plants are conspecifics (McCarthy-Neumann & Kobe 2010; Lozano *et al.* 2022). This would imply that when two species compete on soil conditioned by the larger of the two species, the size advantage of the larger species should decrease, thereby diminishing the size inequality of the two species. However, size inequality should increase when the two species compete on soil conditioned by the smaller of the two species, and could theoretically through asymmetric competition result in mortality of the smaller species.

In nature, the presence of multiple species results in a mosaic of soil patches conditioned by different species, and this soil-legacy heterogeneity should also affect species coexistence (Brandt *et al.* 2013). For example, heterogeneity in soil legacies could provide refuges in which the growth of plants is less strongly limited by negative conspecific plant-soil feedback, thereby favoring species coexistence (Hendriks *et al.* 2015; Burns *et al.* 2017). Indeed, spatially heterogeneous plant-soil-feedback effects can reduce growth inequalities between competing species (Xue *et al.* 2018). However, ultimately, the effects of heterogeneous soil legacies on size inequality should depend on whether each species is growing in a patch conditioned by a conspecific or by the other species, or even in a mixture of both. To the best of our knowledge, how different homogeneous and heterogeneous soil-legacy scenarios affect size inequalities of competing plants, and thus species coexistence, has not been tested yet.

Alien plants are due to the release from species-specific natural enemies (Keane & Crawley 2002; Mitchell & Power 2003), often less negatively or sometimes even positively affected by conspecific soil legacies (Klironomos 2002). This should give the alien plants an advantage over the native plants they are competing with, resulting in larger size inequalities, at least if the alien plants are overall larger than the native ones, as is frequently the case for invasive aliens (van Kleunen *et al.* 2010). On the other hand, if the alien plants compete with other alien plants, the lack of strong soil-legacy effects for both species may have little influence on size inequalities. However, whether soil legacies affect size inequalities differently depending on the origins of the competing species has, to the best of our knowledge, never been tested yet.

To test how biomass production of two competing plants depends on the origins of the species, and on different homogeneous and heterogeneous soil legacies, we conducted a two-phase plant-soil feedback experiment. We first conditioned soil by five native and five alien herbaceous plants. Thereafter, we grew all 45 pairwise combinations of those species (25 alien-native, 10 alien-alien and 10 native-native species pairs) under four homogeneous and two heterogeneous soil-legacy conditions (Fig. 1). We then measured biomass production and size inequalities of the two competing plants. We used these data to test the following main hypotheses: (1) Soil-legacy effects on plant biomass production are negative, but weaker for alien species than for native species. (2) Soil-legacy effects reduce size inequalities of the competing species when grown on soil conditioned by only the larger of the two species, and increase size inequalities when grown on soil conditioned by only the smaller of the two species. (3) The effects of soil legacies on size inequalities should be strongest when both competitors are natives. (4) In alien-native species pairs, the proportional biomass of the alien species (relative to the native species) should decrease on soil conditioned by the alien species and increase on soil conditioned by the native species, with intermediate values in soils conditioned by both species.

Materials and Methods

Study species and pre-cultivation

We selected ten species from six families to be used in both the soil-conditioning phase and the test phase (Table S1). Five of the species are naturalized aliens (*Bidens frondosa* L., *Lepidium virginicum* L., *Lolium multiflorum* Lam., *Onobrychis viciifolia* Scop., *Solidago canadensis* L.), and five species are native to Germany (*Bromus sterilis* L., *Centaurea jacea* L., *Daucus carota* L., *Plantago lanceolata* L., *Trifolium pretense* L.). Whether a species is naturalized or native was based on information in the FloraWeb website (www.floraweb.de, accessed March 2021). Seeds of the ten species were either ordered from Rieger-Hofmann GmbH or taken from the seed collection of the Botanical Garden of the University of Konstanz (Tables S1).

To make sure that all seedlings would be in a similar developmental stage at transplanting, we sowed seeds of the different species on 12, 19 or 21 April 2021 (Tables S1). This was done based on prior knowledge about the time each species requires for germination. Each of the ten species was separately sown into trays (18 cm × 14 cm × 5 cm) filled with potting soil (Topferde; Einheitserde Co., Sinntal-Altengronau, Germany), and the trays were placed in a greenhouse with a temperature between 18 and 25°C.

Experimental set up

Soil-conditioning phase

The experiment was conducted in greenhouse compartments of the Botanical Garden of the University of Konstanz, Germany (47°41′32″N, 9°10′41″E). On 26 April 2021, we collected field soil from a native grassland site near the Botanical Garden. All five native study species occurred in this grassland but none of the alien study species. We sieved the field soil by using a 1-cm metal mesh to remove plant fragments and pebbles. The substrate used in the soil-conditioning phase consisted of a mixture of the field soil, sand and vermiculite (v:v:v = 2:3:3), and was then filled into 2-L pots (14 cm × 14 cm × 14.5 cm). On 3 May 2021, we transplanted one seedling into the center of each pot. For each of the ten species, we had 115 pots to make sure that we would obtain sufficient amounts of conditioned soil to be used in the next phase. As a control (i.e. unconditioned soil), we also had 225 pots without any plants, resulting in 1375 pots in total. Seedlings that died within two weeks after transplanting were replaced. All pots were randomly allocated to positions in three greenhouse compartments (24°C/18°C day/night temperature, 16 h/8 h day/night) and each of them was placed on a plastic dish (Ø = 17 cm). To reduce potential effects of environmental heterogeneity within and among greenhouse compartments, positions of all pots were re-randomized five weeks after the start of the soil-conditioning phase. We watered all pots every 2-3 days, and fertilized them four times with 150 mL of a water-soluble fertilizer (1‰ m/v, Universol Blue with a NPK ratio of 3:2:3).

From 19 to 23 July 2021, 11 weeks after the start of the soil-conditioning phase, we collected and sieved the soils from each pot, including pots without any plants, through a 5-mm mesh to remove the roots. The mesh was sterilized with 70% ethanol between different pots. The sieved soil was immediately stored at 4°C until use in the test phase.

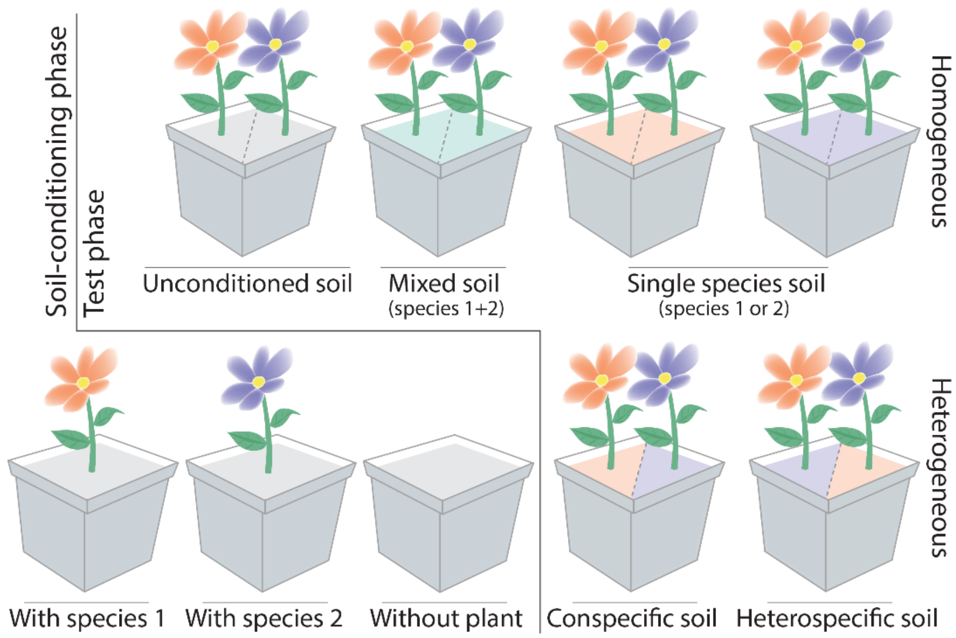

Figure 1 Overview of the experimental design. In the soil-conditioning phase, each of the five alien and five native species was separately planted in pots to condition the soils. The soil without any plant conditioning was used to created unconditioned control soil. In the test phase, the ten species were combined into 45 species pairs (25 alien-native, 10 alien-alien and 10 native-native) and were grown as focal plants and competitors in six soil-legacy treatments created from the soils of the conditioning phase. Pots were divided in half along their diagonals, and these halves were filled separately with soil from the conditioning phase to create various homogeneous and heterogeneous soil-legacy environments in which the two plants of the species pair were planted. This resulted, from the perspective of a focal species (i.e. the orange species 1 in the figure), in four homogeneous and two heterogeneous soil-legacy treatments per species pair.

Test phase

On 5 and 12 July 2021, we sowed the ten species again to produce seedlings for the test phase (Table S1). The pre-cultivation conditions were the same as for the soil-conditioning phase. To test effects of homo- and heterogeneous plant-soil feedback on each of the 45 pairs of species (i.e. 25 alien-native, 10 alien-alien and 10 native-native interspecific combinations), from 24 to 27 July 2021, we divided each 1.3-L pot (12 cm × 12 cm × 13.2 cm) in half along its diagonal using a plastic divider. We then filled the two halves of the pots with the same (homogeneous soil legacies) or different (heterogeneous soil legacies) conditioned soils. The divider was removed once the pot had been filled and was sterilized with 70% ethanol before using it to fill the next pot. For heterogeneous pots, one half was filled with soil conditioned by one species of the species pair that would be planted in the pot and the other half with soil conditioned by the other species of the same pair (Fig. 1). To test whether heterogeneity in soil legacies affects plant growth, we compared them (1) to the two homogeneous treatments in which both halves of the pot were filled with soil conditioned by only one of the two species in a pair, and (2) to a homogeneous treatment with a 1:1 mixture of soils conditioned by the two species. In addition, to test the overall effect of plant-soil feedback, we also had a homogeneous treatment with unconditioned control soil in both halves of the pot. To avoid that effects of the heterogeneous treatments would arise simply because the two halves in a test pot were filled with soils from two conditioning pots, irrespective of whether they are from two different species, the patches in the homogeneous treatments were also filled with soils from two replicate conditioning pots, but of the same species.

On 27 July 2021, we transplanted for each of the 45 species pairs two seedlings per pot, one of each of the two species (Fig. 1). So, each pot half received one seedling. For each species pair, there were four homogeneous soil-legacy treatments with in both pot halves (1) unconditioned soil (homogeneous-unconditioned), (2) soil conditioned by the larger of the two species (homogeneous-larger-species), (3) soil conditioned by the smaller of the two species (homogeneous-smaller-species) and (4) a mixture of soil conditioned by both species (homogeneous-mixed). For homogeneous treatments 2 and 3, the information on which species was larger was based on the growth in the homogeneous-unconditioned treatment. For each species pair, there were furthermore two heterogeneous soil-legacy treatments in which either (1) each species was planted in the pot half filled with soil conditioned by a conspecific plant (heterogeneous-conspecific) or (2) each species was planted in the pot half filled with soil conditioned by the other species (heterogeneous-heterospecific). Each species pair by treatment combination had five replicate pots, so ideally we would have had a total of 1350 pots (45 species pairs × 6 treatments × 5 replicates). However, because we did not have enough seedlings for the alien species *O. viciifolia*, we decreased the number of replicates for all species pairs and treatment combinations including this species. Therefore, the total number of pots in the test phase was 1214 instead of 1350.

All pots were randomly allocated to positions in two greenhouse compartments (24°C/18°C day/night temperature, 16 h/8 h day/night) and each of them was placed on a plastic dish (Ø = 15 cm). Positions of all pots were re-randomized five weeks after the start of the test phase. We watered all pots every 2-3 days, and fertilized them two times with 100 mL of a water-soluble fertilizer (1‰ m/v, Universol Blue).

Measurements

On 28 and 29 July 2021, at the start of the test phase, we measured the length and width of the largest leaf on each seedling, and counted the number of leaves. The initial leaf area of each seedling was then estimated as number of leaves × length of the largest leaf × width of the largest leaf. On 4 and 5 October 2021, ten weeks after the start of the test phase, we harvested the aboveground biomass of each of the two plant per pot separately. The belowground biomass was not harvested because it was not possible to separate the roots of the two plants in each pot. The plant materials were dried at 70°C to constant weight, and were then weighed.

Statistical analysis

To test whether the four homogeneous and two heterogeneous soil-legacy treatments affected the biomass production and size inequality of the two plants in the pots of the test phase, we fitted linear mixed effect models to the combined aboveground biomass of the two plants per pot and to the coefficient of variation (CV) of the biomass values of the two plants per pot with the *lme* function of the '*nlme*' package (Pinheiro *et al.* 2022) in R 4.2.2 (R Core Team 2022). To meet the assumption of normality, plant biomass was square-root transformed and the CV was box-cox transformed (lambda = 0.63). In these models, soil-legacy treatment (homogeneous-unconditioned, homogeneous-larger-species, homogeneous-smaller-species, homogeneous-mixed, heterogeneous-conspecific or heterogeneous-heterospecific), origin combination (alien-native, alien-alien or native-native) and their interaction were included as fixed effects. To account for variation due to the 45 different combinations of test species, we included identity of the species pair as a random effect. Furthermore, because some of the pots in the test phase had been filled with soil from the same two conditioning pots, we included the identity of the two pots from the conditioning phase as a random effect. To account for differences in initial sizes of the plants in the test phase, we included the combined initial leaf area of the two plants per pot as a covariate. To improve homoscedasticity of the model residuals, we allowed the variance to vary among the species pairs using the *varIdent* function.

To test whether the competitive balance between alien and native plants was affected by the various soil-legacy treatments, we fitted two models using subsets of pots from the alien-native competition treatment. In these models, the CV of the biomass values of the two plants per pot, and the proportional biomass of the alien plant, calculated as $biomass_{alien}/(biomass_{alien} + biomass_{native})$, were included as the response variable, respectively. We used soil-legacy treatment as the fixed effect, and used identity of the alien-native species pair and identity of the conditioning-pot combination as random effects. Most of the soil-legacy treatments were the same as in the model that included all origin combinations, but now the two homogeneous soils conditioned by only one species were defined according to whether the soil was conditioned by the alien species (homogeneous-alien-species) or the native species (homogeneous-native-species), instead of by the larger or smaller species. The combined initial leaf area of the two plants per pot was included as a covariate in the first model, while the proportional initial leaf area of the alien species was included in the second one. We also allowed the variance to vary among the species pairs using the *varIdent* function to improve homoscedasticity of the model residuals.

In addition, to assess whether changes in proportional biomass of the alien plant were mainly due to changes in biomass of the alien or native species, we also ran a model on the individual biomass of the plants in each pot of the alien-native origin combination. We used the origin of the focal plant (alien or native), the soil-legacy treatment and their interaction as fixed effects. To account for nonindependence of plants of the same the focal species or competitor, we included identities of the focal species and the competitor species as random effects. As each plant was used both as a focal plant and as a competitor, we accounted for nonindependence of the two plants within the same pot by including pot identity as a random effect. Furthermore, to account for nonindependence of soil patches that had been filled with soil from the same conditioning pot, we included the identity of the conditioning pot as a random effect. We accounted for variation in initial size of the plants by including initial leaf area as a covariate. Homoscedasticity of the model residuals was improved by allowing the variance to vary among the focal species and among the competitor species using the *varIdent* and *varComb* functions.

In all models, we used log-likelihood ratio tests to assess the significance of each fixed effect by comparing models with and without the effect of interest (Zuur *et al.* 2009). Post-hoc multiple comparisons for each model were then separately done with the *emmeans* function of the ‘*emmeans’* package (Lenth *et al.* 2023).

Results

Effects of soil-legacy treatments on biomass production and size inequality per pot

Overall, irrespective of their origins, the competing plants produced the most biomass per pot on unconditioned control soil (Table S2a, Fig. 2a). Among the five soil-legacy treatments with conditioned soil, the combined biomass per pot did not significantly differ (Table S2a, Fig. 2a). Averaged across the soil-legacy treatments, there were no significant differences among the three origin combinations of the species (Table S2a, Fig. 2a). The alien-native and native-native species pairs produced the least biomass per pot on the homogeneous soil conditioned by the larger of the two plant species (i.e. the homogeneous-larger-species treatment; Table S2a, Fig. 2a). However, the alien-alien species pairs produced the least biomass per pot on homogeneous-mixed and heterogeneous-conspecific soils (Table S2a, Fig. 2a).

Across all soil-legacy treatments, the coefficient of variation in biomass (CV) between the two plants in a pot was the smallest when the soil had been conditioned by the largest of the two species only (i.e. homogeneous-largest-species treatment; Table S2b, Fig. 2b). This reduction in size inequality was the most pronounced for alien-alien species pairs, and the least pronounced for native-native species pairs (Table S2b, Fig. 2b).

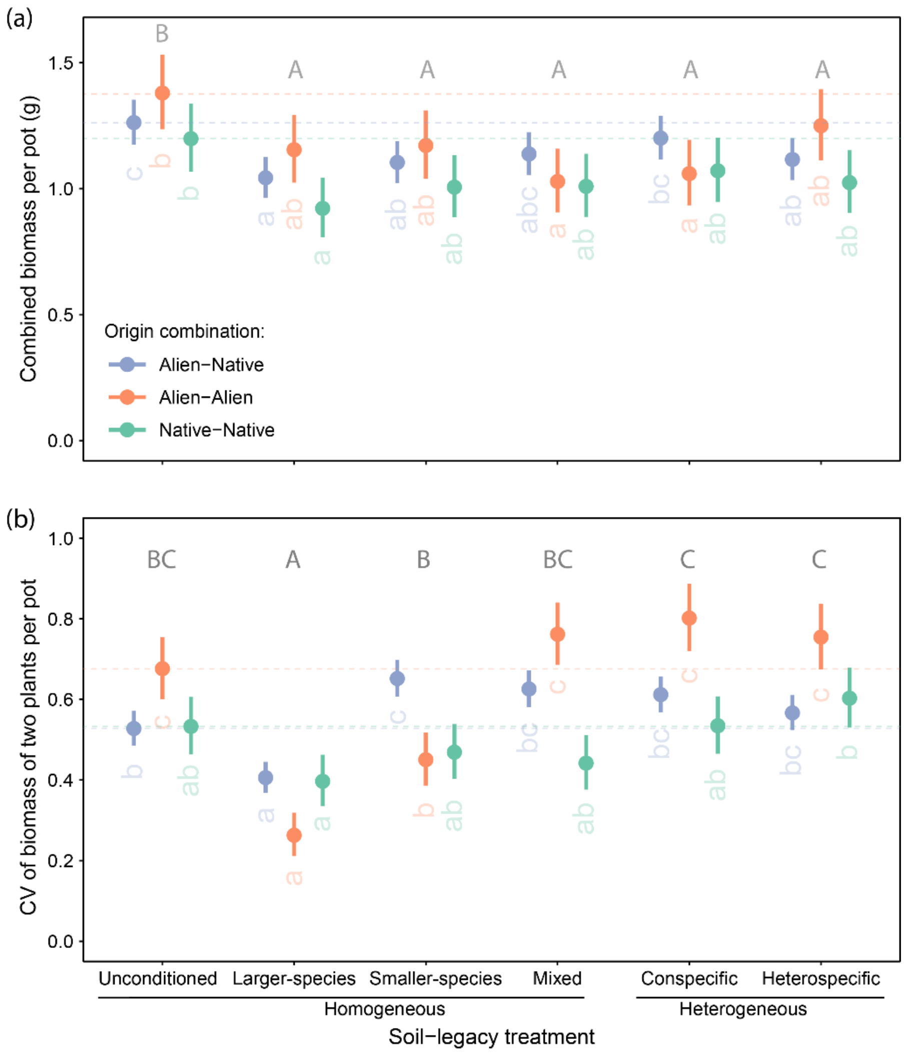

Figure 2 Effects of soil-legacy treatments on (**a**) the combined aboveground biomass per pot and (**b**) the coefficient of variation (CV) of biomass of the two plants per pot for alien-native, alien-alien and native-native species pairs. Shown are modelled means (±SEs) after back-transformation. Horizontal dashed lines indicate mean values of the control. The capital letters indicate the significance ($P < 0.05$) of pairwise multiple comparisons among the soil-legacy treatments (irrespective of the origin combination), and the lowercase letters indicate significances ($P < 0.05$) of pairwise multiple comparisons among the soil-legacy treatments for each of the three origin combinations separately.

Effects of soil-legacy treatments on competitive balance between alien and native plants

For the subset of pots with alien-native species pairs, the conditioned soils overall decreased the individual biomass of the individual plants (Table S4, Fig. S1). Among all conditioned soils, native species produced the lowest biomass on homogeneous-native-species soil, and alien species produced the lowest biomass on homogeneous-alien-species soil (Table S4, Fig. S1). In other words, individual plants produced the lowest biomass in the homogeneous treatments with conspecific soil. However, in the heterogeneous-conspecific treatment, alien species achieved a higher biomass and a higher biomass proportion than when grown in the homogeneous-alien-species treatment (Table S3a and S4, Fig. 3a and S1). As a consequence, the biomass inequality between competing alien and native plants was larger on heterogeneous conspecific soil than on homogeneous-alien-species soil (i.e. homogeneous conspecific soil of alien species; Table S3b, Fig. 3b). Moreover, the proportional biomass of alien species was the highest when they were growing on homogeneous-native-species soil, and it was intermediate both on unconditioned soil and on heterogeneous soils (Fig. 3a).

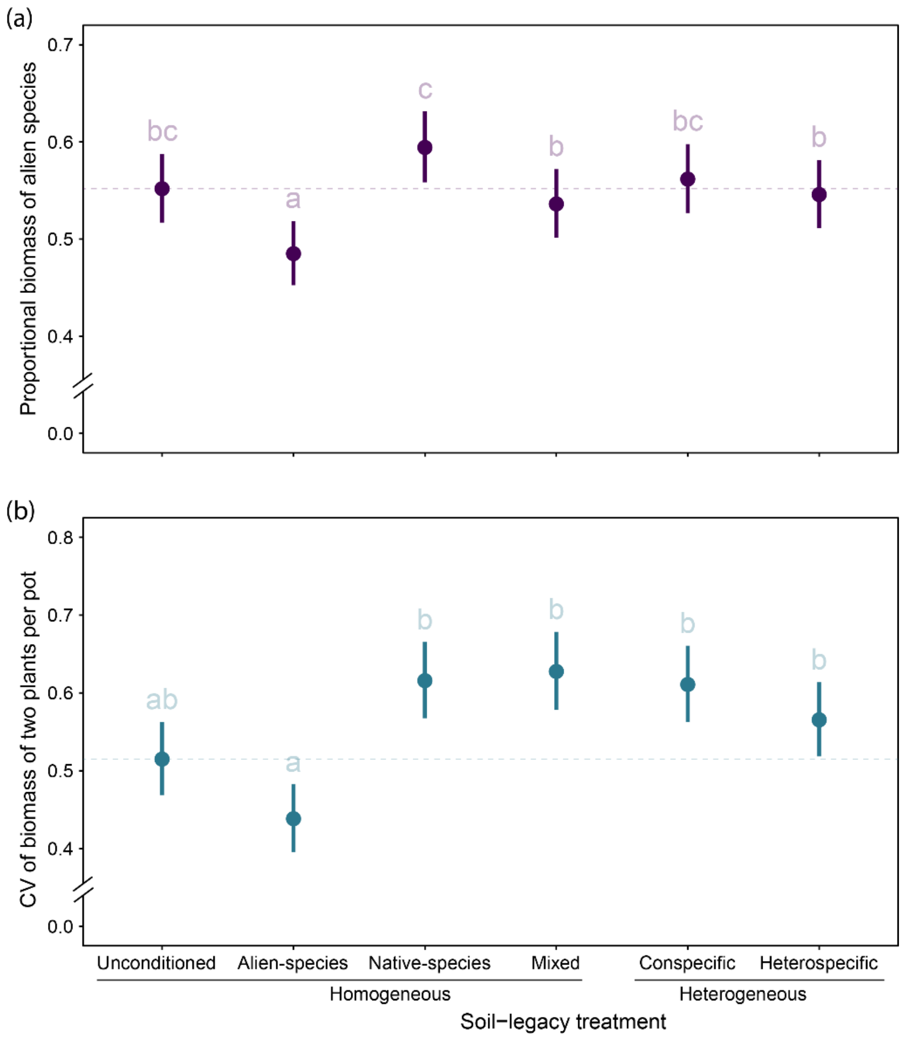

Figure 3 Effects of soil-legacy treatment on (**a**) proportional biomass of the alien plant in the presence of a native competitor and (**b**) the coefficient of variation (CV) of biomass of the two plants per pot for alien-native species combinations. Shown are modelled values (±SEs) after back-transformation. Horizontal dashed line indicates mean values of the control. The letters indicate the significant difference ($P < 0.05$) of multiple comparisons among soil-legacy treatments.

Discussion

Our study tested how four homogeneous and two heterogeneous soil legacies, and species origins affect biomass and size inequality of competing plants. We found that all conditioned soils, relative to the unconditioned soil, negatively affected biomass production of the competing plants, irrespective of their origins. There was little variation in biomass production per pot among the five soil-legacy treatments with conditioned soils. However, the biomass was not equally partitioned between the two plants per pot. When the two competing plants grew on soil that had only been conditioned by the species that was larger in the unconditioned soil-legacy treatment, the inequality in biomass was the smallest. This indicates that the conspecific soil legacy of the larger-sized plant reduced its growth advantage relative to the smaller competitor, which should contribute to coexistence of the two species. Alien plants, just like native plants, suffered significantly from conspecific soil legacies. Consequently, alien-native species combinations had the smallest biomass inequality and the lowest proportional alien biomass when grown on homogeneous-alien-species soil. However, biomass and biomass proportion of the alien species were higher on heterogeneous-conspecific soil than on homogeneous-alien-species soil (i.e. homogeneous-conspecific soil for alien species). This suggests that soil-legacy heterogeneity can mitigate the negative effects of conspecific soil legacies on alien plants.

Consistent with numerous experimental plant-soil-feedback studies (e.g. Bonanomi *et al.* 2005; Mangan *et al.* 2010; Bennett *et al.* 2017; Aldorfová *et al.* 2020) and meta-analyses (e.g. Kulmatiski *et al.* 2008; Crawford *et al.* 2019), we found that conditioned soils overall negatively affected plant growth (Fig. 2a). This likely reflects that the pathogens and allelopathic secondary metabolites accumulated by the conditioning plants, negatively affected the plants in the test phase (Packer & Clay 2000; Bever *et al.* 2015). It could also reflect that the conditioning plants had depleted the soil of nutrients. However, as we fertilized the pots during both the conditioning and test phases, nutrient depletion is likely to have played only a minor role.

For the subset of alien-native species combinations, we found that relative to unconditioned control soil, the biomass reductions of the alien species were not lower than those of the native species on their conspecific soils (-31.8% vs -32.5%) and on all the conditioned soils (-17.6% vs -10.6%; Fig. S1). This suggests that alien plant individuals did not experience weaker soil-legacy effects than native ones. This finding is surprising as it is frequently assumed that alien species have been released from many of their species-specific natural enemies (Keane & Crawley 2002; Mitchell & Power 2003), and therefore should experience less negative plant-soil feedbacks (Klironomos 2002; MacDougall *et al.* 2011). Possibly, the soil inoculum that we had collected in the field contained mainly generalist pathogens, which affected both the native and alien plant species negatively. Furthermore, it could be that because the naturalized alien species used in our study have been in Europe for a relatively long time (Table S1; Capinha *et al.* 2023) and have closely related native species, they may by now have accumulated specialist natural enemies to similar degrees as the native species.

In line with our expectation, we found that competing plants had the smallest size inequality (CV) on soils conditioned by the relatively larger species (Fig. 2b). In other words, when a plant of the species that has a larger size under unconditioned control conditions, experienced a conspecific soil legacy, its biomass was strongly reduced, making it more similar to the biomass of the competitor (Fig. 2a). When the soil had been conditioned by the smaller of the two species, the size inequality was larger than when the soil had been conditioned by the larger one. However, with the exception of the alien-native species pairs, size inequality was on average not increased relative to the unconditioned control treatment. This could reflect that slow-growing species are frequently better defended than fast-growing, acquisitive species, and that they therefore accumulate fewer soil pathogens, and experience less negative conspecific plant-soil feedback (Lemmermeyer *et al.* 2015). Furthermore, based on the expectation that native species should suffer more from soil-legacy effects than alien species, we had expected that the effects of soil-legacy treatments on size inequalities should be highest when the two plants in a pot included a native species. However, as alien plants did not suffer less than natives from soil-legacy effects, this was not the case. On the contrary, the soil-legacy effects on size inequality were the most pronounced when both plants were alien species. Possibly, this reflects that many of the naturalized alien species are fast-growing species that are less well defended and might therefore suffer more from soil-legacy effects mediated by generalistic soil pathogens.

For the subset of alien-native species pairs, we found that size inequality was smallest when the alien and native plants grew on homogeneous soil conditioned by the alien species only (Fig. 3b). Specifically, homogeneous-alien-species soil reduced the growth of the alien species considerably, whereas growth of the native species was not affected (Fig. S1). As the alien species tended to be larger overall than the native species, the reduced growth of the alien species resulted in a reduced size inequality. However, in the soil-legacy treatments that included soil conditioned by the native species, the size advantage of the alien species over the native species remained. The performance of the native plants did not differ between the homogeneous-native-species treatment, where the natives grew on conspecific soil, and the heterogeneous-conspecific treatment. This suggests that a heterogeneous soil legacy may reduce the alien species' negative conspecific plant-soil feedback. Indeed, studies have shown that soil-legacy heterogeneity can create refuges for plants where they experience less negative plant-soil feedback effects (Hendriks *et al.* 2015; Burns *et al.* 2017). Possibly, alien plants, due to their frequently superior plasticity relative to natives (Richards *et al.* 2006; Davidson *et al.* 2011; Keser *et al.* 2014; Chen *et al.* 2019), were likely to take more advantage of this when competing with native plants. As a consequence, in our study, alien plants achieved a higher biomass proportion on heterogeneous-conspecific soil (Fig. 3a), where native plants correspondingly had a reduced biomass.

Total biomass per pot, individual size inequality and also proportional biomass of alien plants in the heterogeneous soil-legacy treatments were similar to those in the homogeneous-mixed treatment. Possibly, this indicates that the soil-microbial communities in the two halves of the heterogeneous treatments rapidly mixed, so that the resulting microbial communities in the two heterogeneous soil-legacy treatments became similar to the one in the homogeneous-mixed treatment. For proportional alien biomass, the values in the homogeneous-mixed and heterogeneous treatments were intermediate to the ones in the homogeneous-alien-species and homogeneous-native-species treatments. This is not surprising because the soil legacy of the individual species was diluted, and consequently the abundance of species-specific pathogens was also reduced correspondingly. Therefore, the competition outcomes between alien and native species may not change much when they experience soil legacies of both species.

In conclusion, our study showed that soil legacies negatively affected plant growth, irrespective of their origins. The growth inequalities between competing alien plants were smaller when the soil they grew on had been conditioned by the larger-sized one. However, when alien species experienced conspecific soil legacies in the presence of native competitors, they performed better when there were also soil legacies of the native competitor. Our findings show that the performance of competing plants may depend on their origins as well as soil legacies. This may ultimately affect the competition and coexistence of alien and native plants in natural environments where soil legacies are likely to be heterogeneous.

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Author contributions

DC conceived the idea. MvK and DC designed the experiment. DC conducted the experiment. DC analyzed the data and wrote the first draft of the manuscript with further inputs from MvK.

Supporting information

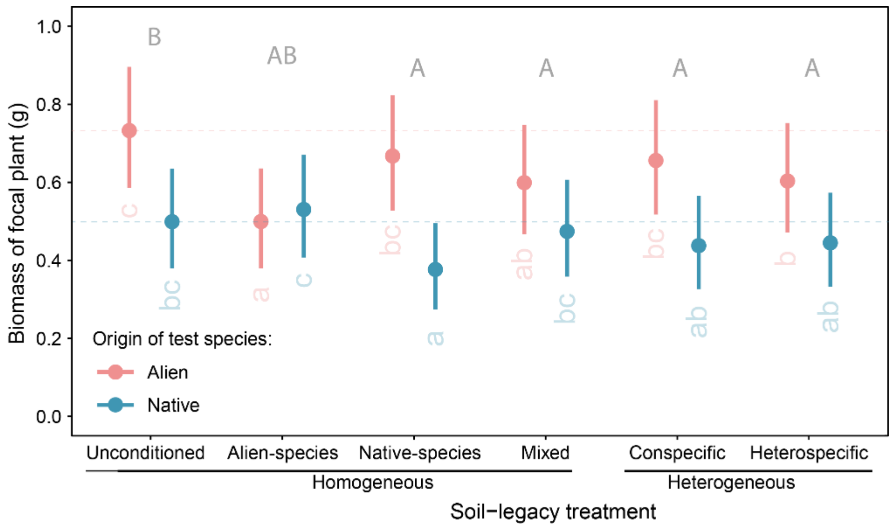

Figure S1 Effects of soil-legacy treatments on biomass of alien and native focal plants in the subset of pots with alien-native species combinations. Shown are modelled values (±SEs) after back-transformation. Horizontal dashed lines indicate mean values of the control. The capital letters indicate the significance ($P < 0.05$) of pairwise multiple comparisons among the soil-legacy treatments (irrespective of the origin combination), and the lowercase letters indicate significances ($P < 0.05$) of pairwise multiple comparisons among the soil-legacy treatments for each of the three origin combinations separately.

Table S1 Plant species used in the experiment.

Species	Family	Origin	First record year in Europe †	Native range	Sowing date		Seed source
					Soil-conditioning phase	Test phase	
Bidens frondosa L.	Asteraceae	Alien	1814	North America	21.04.2021	12.07.2021	University of Konstanz, Germany
Lepidium virginicum L.	Brassicaceae	Alien	1848	North America	21.04.2021	12.07.2021	University of Konstanz, Germany
Lolium multiflorum Lam.*	Poaceae	Alien	1814	Europe	21.04.2021	12.07.2021	Rieger-Hofmann GmbH, Germany
Onobrychis viciifolia Scop.*	Fabaceae	Alien	1791	Europe	21.04.2021	12.07.2021	Rieger-Hofmann GmbH, Germany
Solidago canadensis L.	Asteraceae	Alien	1648	North America	12.04.2021	05.07.2021	University of Konstanz, Germany
Bromus sterilis L.	Poaceae	Native	-	-	21.04.2021	12.07.2021	University of Konstanz, Germany
Centaurea jacea L.	Asteraceae	Native	-	-	12.04.2021	12.07.2021	Rieger-Hofmann GmbH, Germany
Daucus carota L.	Apiaceae	Native	-	-	19.04.2021	12.07.2021	Rieger-Hofmann GmbH, Germany
Plantago lanceolata L.	Plantaginaceae	Native	-	-	19.04.2021	12.07.2021	University of Konstanz, Germany
Trifolium pretense L.	Fabaceae	Native	-	-	19.04.2021	12.07.2021	Rieger-Hofmann GmbH, Germany

* The species is native in part of Europe but alien in Germany

† Capinha, C., Essl, F., Porto, M. & Seebens, H. (2023) The worldwide networks of spread of recorded alien species. *Proceedings of the National Academy of Sciences*, **120**, e2201911120.

Table S2 Effects of soil-legacy treatment (homogeneous-unconditioned, homogeneous-larger-species, homogeneous-smaller-species, homogeneous-mixed, heterogeneous-conspecific or heterogeneous-heterospecific), origin-combination treatment (alien-native, alien-alien or native-native) and their interaction on (a) combined aboveground biomass of the two plants per pot and (b) the coefficient of variation (CV) of the biomass of the two plants per pot. Values are in bold when P < 0.05 and in italic when 0.05 ≤ P < 0.1.

	(a) Combined biomass per pot			**(b)** CV of individual biomass per pot		
	df	χ^2	*P*	*df*	χ^2	*P*
Fixed effects						
Combined initial leaf area per pot	1	43.477	**<0.001**	1	0.945	0.331
Soil treatment	5	44.015	**<0.001**	5	76.088	**<0.001**
Origin combination	2	0.702	0.704	2	1.927	0.382
Soil × Origin	10	16.152	*0.095*	10	55.187	**<0.001**
Random effects		SD			SD	
Species-pair identity		0.178			0.128	
Identity of the conditioning-pot combination		0.042			0.050	
Residual		0.123			0.202	

Table S3 Effects of soil-legacy treatment (homogeneous unconditioned, homogeneous-alien-species, homogeneous-native-species, homogeneous-mixed, heterogeneous-conspecific or heterogeneous-heterospecific) on (a) proportional biomass of alien species and (b) the coefficient of variation (CV) in biomass of the two plants per pot in the subset of pots with alien-native species combinations. Values are in bold when *P* < 0.05.

	(**a**) Proportional biomass of alien species			(**b**) CV of biomass of alien and native plants per pot		
	df	χ^2	*P*	*df*	χ^2	*P*
Fixed effects						
Proportional initial leaf area of alien species	1	68.612	**<0.001**	-	-	-
Combined initial leaf area per pot	-	-	-	1	3.875	**0.049**
Soil treatment	5	52.017	**<0.001**	5	28.792	**<0.001**
Random effects		SD			SD	
Species-pair identity		0.110			0.146	
Identity of the conditioning-pot combination		0.026			0.053	
Residual		0.050			0.206	

Table S4 Effects of the origin of the focal plant (alien or native), soil-legacy treatment (homogeneous unconditioned, homogeneous-alien-species, homogeneous-native-species, homogeneous-mixed, heterogeneous-conspecific or heterogeneous-heterospecific) and their interaction on biomass of alien and native plants in the subset of pots with alien-native species combinations. Values are in bold when $P < 0.05$.

	df	χ^2	*P*
Fixed effects			
Initial leaf area of focal plant	1	78.135	**<0.001**
Origin	1	0.753	0.385
Soil treatment	5	17.790	**0.003**
Origin × Soil treatment	5	55.289	**<0.001**
Random effects		SD	
Focal plant species identity		0.180	
Competitor species identity		0.083	
Identity of the conditioning pot		<0.001	
Pot identity		<0.001	
Residual		0.154	

General discussion

As the number of alien species continues to increase, it is crucial to comprehend the factors that affect their performance and how they are linked. My thesis revealed the joint effects of plant-plant and plant-soil interactions on initial as well as subsequent invasions through a series of ecological experiments. In Chapter 1, I found that alien invaders were more competitive than native invaders at later stages of growth, but that they also suffered stronger negative effects from native communities over time, especially in multispecies communities. Expanding on these findings, in Chapter 2, I further showed that the success of subsequent invaders was related to previous invaders and the diversity of native communities. Although subsequent alien plants benefited from previously occurring alien plants through soil-legacy effects, more diverse communities limited and reduced such positive interactions indirectly. This suggests that the diversity of native plants plays a role in the invasion success. In Chapter 3, by adding additional competitors, I found that the negative effects of the conspecific soil legacy on alien plants can also be mitigated by other co-occurring alien plants, although it was not completely eliminated. This suggests that positive interactions can be found not only between previous and subsequent alien plants, but also between contemporaneous alien plant species. In Chapter 4, I found that soil spatial heterogeneity increased growth inequalities between alien and native plants in the presence of negative soil-legacy effects, which give the alien species an advantage in the competition with native plant species.

Resident community limits invasion success

Ecosystem stability is closely related to the species composition of the community (Ernest & Brown 2001; Donohue *et al.* 2013). The classic diversity-invasibility hypothesis proposed a pattern of negative correlations between invaders and resident community diversity (Elton 1958). When the species richness in the community is higher, its internal structure is more stable, so that it will be more difficult for alien species to establish. In Chapter 1, I found that multispecies communities more negatively affected invaders at the late growth stage, but their own performance was limited by invaders correspondingly. Although small differences were found between the two-species and four-species communities, their development trends over time already suggest that diversity plays a role in deterring invasions, and that this negative effect on invaders may be pronounced over time. Subsequently, in Chapter 2, I demonstrated that when a secondary invasion occurred, the secondary invaders were indeed influenced by the previous native community, and the diversity advantages were manifested through their soil legacies. This suggests that negative effects of the resident community on invaders is long-term, and may even span several generations of invaders. The findings of the first two chapters provide evidence for long-term interaction patterns between resident community and multigenerational invaders and also provide new insight into the mechanisms of the relationship between community diversity and invasibility through plant-soil interactions.

Subsequent invasion benefits from positive interactions between alien species

Continued increases in the number of alien species in the future will result in more frequent subsequent invasions in the invaded ecosystems (Kuebbing & Nuñez 2016; Banks *et al.* 2018; Seebens *et al*. 2021). Although the performance of invaders is constrained by the diversity of residents in invaded ranges, in Chapter 2, I clearly demonstrated that there was also a positive interaction between previous and subsequent alien invaders, and this facilitation can be achieved indirectly through soil legacies. In Chapter 3, I further found that positive interactions can occur between co-growing alien species, and thereby the focal alien plant suffered less negative feedback effects. In other words, positive interactions between aliens can take place between different generations of aliens or between contemporaneous co-occurring individuals. These finding support the invasional meltdown hypothesis (Simberloff & Von Holle 1999). While some studies have shown that the invasional meltdown can happen between plants (e.g. Tecco et al. 2006; Thouvenot et al. 2013), between plants and herbivores (e.g. Bourgeois *et al.* 2005; Relva *et al.* 2010; Green *et al.* 2011) or between primary and secondary consumers (e.g. Adams *et al.* 2003; Jackson 2015), my findings expand our understanding by highlighting that this facilitation can occur between co-growing or not co-growing alien plants through soil legacies.

Soil conditions affects coexistence of alien and native plants

Soil organisms develop dynamically over time and are closely related to plant activity (Diez *et al.* 2010; Thakur *et al.* 2021). Across the last three chapters, the negative effects of soil legacy on plant growth were found. When this comes to alien species, their performance, due to being released from negative inter-trophic relationships of their native ranges (Keane & Crawley 2002; Mitchell & Power 2003), is often expected to be better in naturalized ranges. Alien plants are therefore considered to be subject to less soil-legacy effects than native plants. In my thesis, however, this dominance of alien plants may depend on both the soils they grew on and the plants they grow with. Chapters 2 and 3 demonstrate that the presence of other alien species (either previous or co-competing) can largely mitigate the negative soil-legacy effects on the focal alien species. Chapter 4 demonstrates that the spatial heterogeneity of soil legacies increases growth asymmetry between alien and native species, and gives alien species a competitive advantage. Although the emphases are different, Chapters 3 and 4 both show that the superiority of alien species in competition with native species comes from less growth loss under negative soil-legacy effects. Taken together, the performance of alien species is largely related to the specific soil conditions and co-occurring competitors, which may provide future research some new insights into the competition and coexistence of alien and native plants.

Concluding remarks

Since the publication of the groundbreaking book by Charles Elton (1958), the research on invasion ecology has become increasingly multidimensional and systematic. With the gradual exploration of the interaction between plants and soil (Bever 2003), research on the interactions between alien species and soils has further built a bridge between invasion ecology and soil ecology. In the four studies of my thesis, I showed that the outcome of invasion can benefit from facilitations between alien species, can be constrained by the properties of resident communities, and also depends on the interactions with soils. These three work together to pin down the performance of alien species across temporal and spatial scales. Consequently, combining plant-plant and plant-soil interactions into future research will further improve the understanding of the coexistence of alien and native species.

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