

Invasional meltdown mediated by plant–soil feedbacks may depend on community diversity

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Summary

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- 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 to date.
- 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 the legacy effects of previous native invaders. However, this effect decreased when the soil had been co-conditioned by a multispecies community.
- Our findings suggest that plant–soil feedback-mediated invasional meltdown may depend on community diversity and therefore provide some evidence that diverse communities could increase resistance against subsequent alien invasions.

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.*, 2000; 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 has shown 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 to date.

Charles Elton proposed that species-rich communities should be more resistant to invaders than species-poor communities (Elton, 1958). Some studies, particularly those that have focused 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, have focused on the relationship between community diversity and co-competing current invaders, and consequently still little information 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 the 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 noninvaded 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?

Materials and Methods

Study species

For the soil-conditioning phase of the experiment, we used mesocosms with invaded and noninvaded plant communities. As invaders, we selected five alien species from the Asteraceae, Brassicaceae, Fabaceae and Poaceae (i.e. alien invaders; Supporting Information 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 10 invader species as in the conditioning phase. However, as two of the 10 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 naturalised 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 (Tables S1, S2).

Experimental set up

The experiment was conducted in the glasshouse 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., Sinntal-Altengronau, Germany). According to prior knowledge about the time required for germination of each species, the species were sown on different dates (Tables S1, S2), so that all seedlings would be in similar growth stages at transplanting. The trays were placed in a glasshouse 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 (3 l, 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 (seven monocultures), two species (seven combinations) and four species (seven 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 10 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 centre in square formation. For the pots with invaders, we planted one invader seedling in the centre 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 glasshouse compartments (24°C : 18°C, day : night temperature;

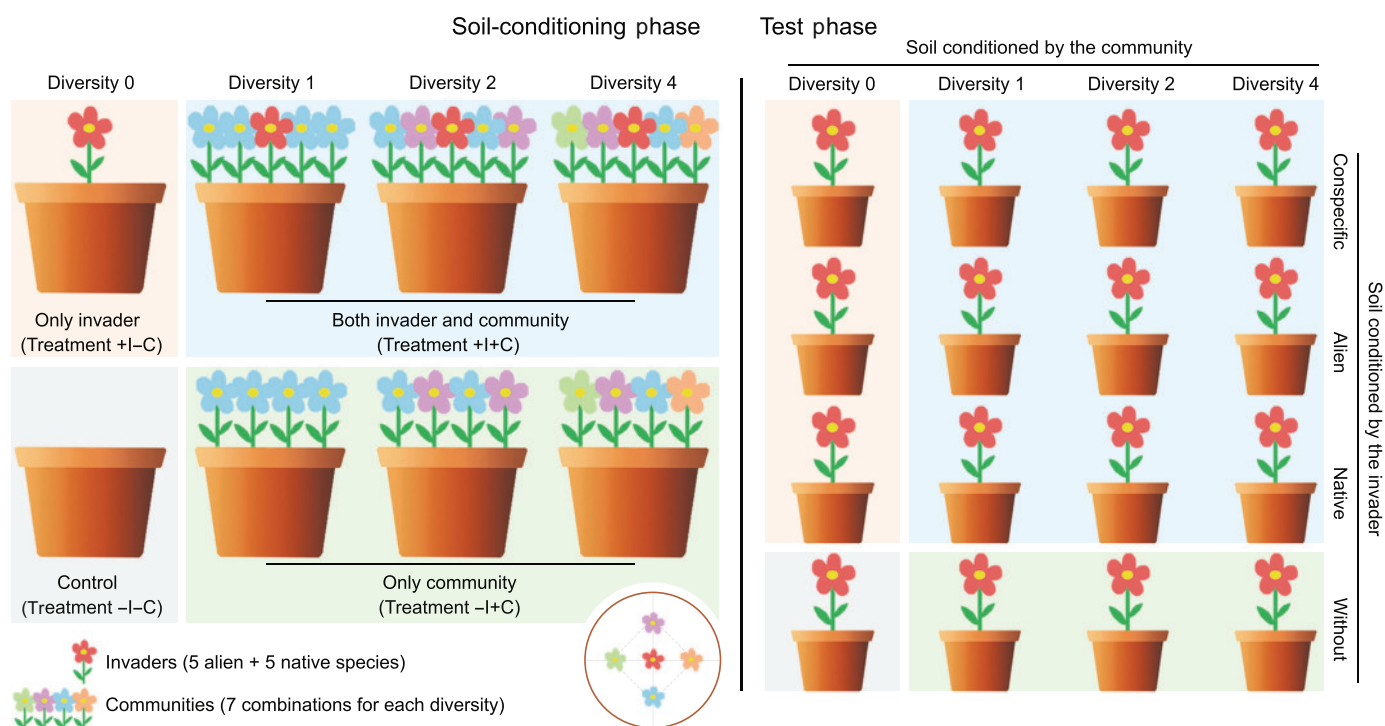


Fig. 1 Overview of the experimental design. In the soil-conditioning phase, four soil treatments were created by the presence of an invader (I) and/or a community (C). To test the effects of community diversity on the test species, four diversity levels were created (0 (no community), 1, 2 and 4 species).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

16 h : 8 h, day : night light). Seedlings that died within 2 wk after transplantation were replaced. We watered all pots, including the pots without plants, every 1–2 d, and fertilised the pots four times with a water-soluble fertiliser (1‰ m/v, Universol Blue). Positions of the pots were re-randomised 6 wk after start of the soil-conditioning phase. From 13 to 29 May 2020, 10 wk 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 sterilised 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 a 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 (please refer to the section ‘Study species’ above). As we did not have enough soil from the conditioning phase to fill all 2100 pots (0.5 l; 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. In 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 10 pots of the test phase. In other words, we grew each of the 10 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 the situation in which 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 10 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): (1) soil conditioned by a conspecific plant, (2) soil conditioned by a nonconspecific alien invader, and (3) soil conditioned by a nonconspecific 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 10 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 (totalling 1260 pots). The corresponding soils conditioned by

Table 1 Overview of the combinations of the test species in the test phase and the previous invaders used to condition the soils.

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						
		<i>Lep.vir</i>	<i>Set.fab</i>	<i>Lup.pol</i>	<i>Bro.car</i>	<i>Sol.gig</i>	<i>Lep.cam</i>	<i>Set.vir</i>	<i>Tri.pra</i>	<i>Bro.ste</i>	<i>Sen.jac</i>		
Alien	<i>Amb.art</i>	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
	<i>Set.fab</i>	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
	<i>Lup.pol</i>		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
	<i>Bro.car</i>	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
	<i>Sol.gig</i>		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	<i>Lep.cam</i>	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
	<i>Set.vir</i>	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
	<i>Tri.pra</i>		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
	<i>Bro.ste</i>	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
	<i>Hyp.per</i>		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

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 10 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 10 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.

invaders only (i.e. treatment +I–C), the 21 soils conditioned by native communities only (i.e. treatment –I+C) and the nonconditioned soil (i.e. treatment –I–C) were replicated three times (totalling 630 (treatment +I–C) + 180 (treatment –I+C) + 30 (treatment –I–C) = 840 pots). Ideally, we would therefore 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 ($\varnothing = 15$ cm) and randomly allocated to positions in two glasshouse compartments (24°C : 18°C, day : night temperature; 16 h : 8 h, day : night light). All pots were watered every 1–2 d, and re-randomised once (13 July 2020, i.e. 5 wk after start of the soil-conditioning phase). To reduce the potential effects of nutrient depletion that might have happened during the conditioning phase, we fertilised all pots in the test phase two times with 80 ml of a water-soluble fertiliser (1‰ m/v, Universol Blue). From 10 to 14 August 2020, 9 wk 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 a 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 in 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}, that is 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 nonindependence of the test species, and nonindependence of plants belonging to the same family, we included species identity and family of the test species as random effects. To account for nonindependence of plants growing on soils conditioned by the same invaders or communities, we also included the identity of invader species and communities in the conditioning phase as random effects. Furthermore, to account for nonindependence 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: (1) without an invader, (2) with an invader belonging to the same species as the test species (i.e. conspecific), (3) with an invader of another species (i.e. allospecific) that is alien or (4) with an invader of another species that is native. Then we generated three orthogonal contrasts for the invader treatment: without vs with invader ($\text{Invader}_{\text{Without/With}}$), conspecific 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 (conspecific, 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 with 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 $0.05 \leq P < 0.1$.

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

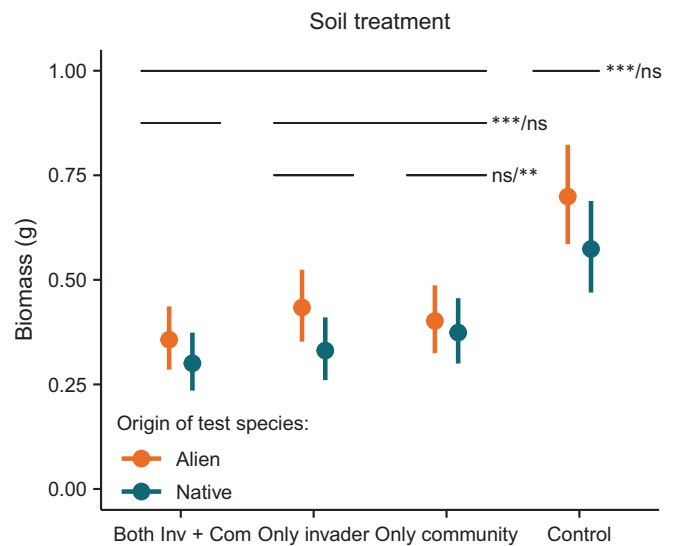


Fig. 2 Total biomass of alien and native test species in the four main soil-conditioning treatments. Shown are modelled means (\pm SEs) after back-transformation ($n = 1852$). The hierarchical contrasts between treatments are indicated by the horizontal lines, and the significance of the main effect 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; Inv, invader; Com, community.

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).

Fixed effects	df	χ^2	<i>P</i>
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		Standard deviation	
Test species family		0.001	
Test species species ^a		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 $0.05 \leq P < 0.1$.

^aStandard deviations for all the test species are shown in Supporting Information Table S7.

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 $\text{Origin} \times \text{Soil}_{\text{Invader/Community}}$ 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 $\text{Origin} \times \text{Invader}_{\text{Alien/Native}}$ in Table 2; Fig. 3a).

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, therefore including diversities 0 (i.e. invader

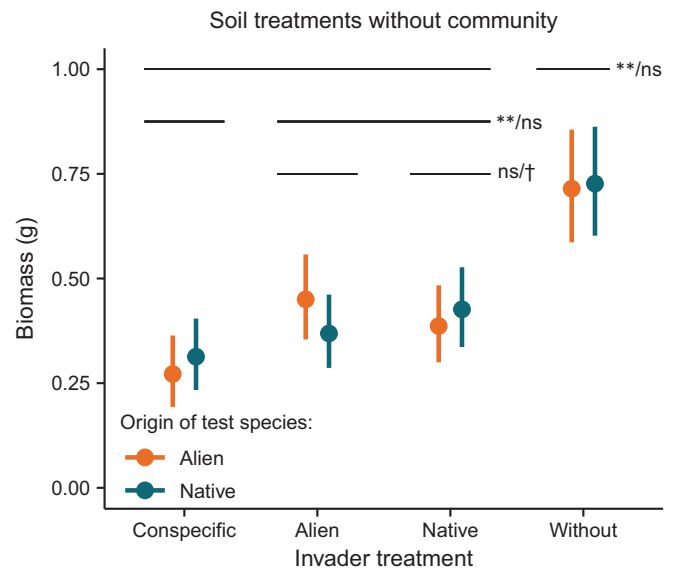


Fig. 3 Total biomass of alien and native test species in the four soil-conditioning invader (I)-type treatments (without, conspecific, allospecific alien, allospecific native) of the pots that were not conditioned by native communities (C) (i.e. treatments +I–C and –I–C; $n = 162$). Shown are modelled means (\pm 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.

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 with the soil treatment without community (i.e. diversity 0), this conspecific vs allospecific conditioning effect was smaller in the presence of a community (–16.5% vs –6.7%; significant $\text{Invader}_{\text{Con/Allo}} \times \text{Community}_{\text{Without/With}}$ 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 multispecies communities than on soil conditioned by allospecific native invaders and single-species communities (–5.6%), whereas this was not the case on soils conditioned by allospecific native invaders (marginally significant $\text{Invader}_{\text{Alien/Native}} \times \text{Community}_{\text{Mono/Multi}}$ 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 $\text{Origin} \times \text{Invader}_{\text{Alien/Native}}$ 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 multispecies communities than on soil conditioned by single-species communities (–5.5%), whereas this was not the case for native test species (significant $\text{Origin} \times \text{Community}_{\text{Mono/Multi}}$ in Table 3; Fig. 4b).

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).

Fixed effects	df	χ^2	<i>P</i>
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} × CD _{Without/With}	1	0.281	0.596
I _{Alien/Native} × CD _{Mono/Multi}	1	3.785	<i>0.052</i>
I _{Alien/Native} × CD _{Div2/Div4}	1	0.722	0.396
O × I × CD	6	1.453	0.963
O × I _{Con/Allo} × CD _{Without/With}	1	1.051	0.305
O × I _{Con/Allo} × CD _{Mono/Multi}	1	0.100	0.752
O × I _{Con/Allo} × CD _{Div2/Div4}	1	0.011	0.916
O × I _{Alien/Native} × CD _{Without/With}	1	0.170	0.680
O × I _{Alien/Native} × CD _{Mono/Multi}	1	0.002	0.969
O × I _{Alien/Native} × CD _{Div2/Div4}	1	0.119	0.730
Random effects		Standard deviation	
Test species family		<0.001	
Test species species ^a		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 $0.05 \leq P < 0.1$.

^aStandard deviations for all the test species are shown in Supporting Information Table S7.

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).

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 a 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 multispecies 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 fertilised 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

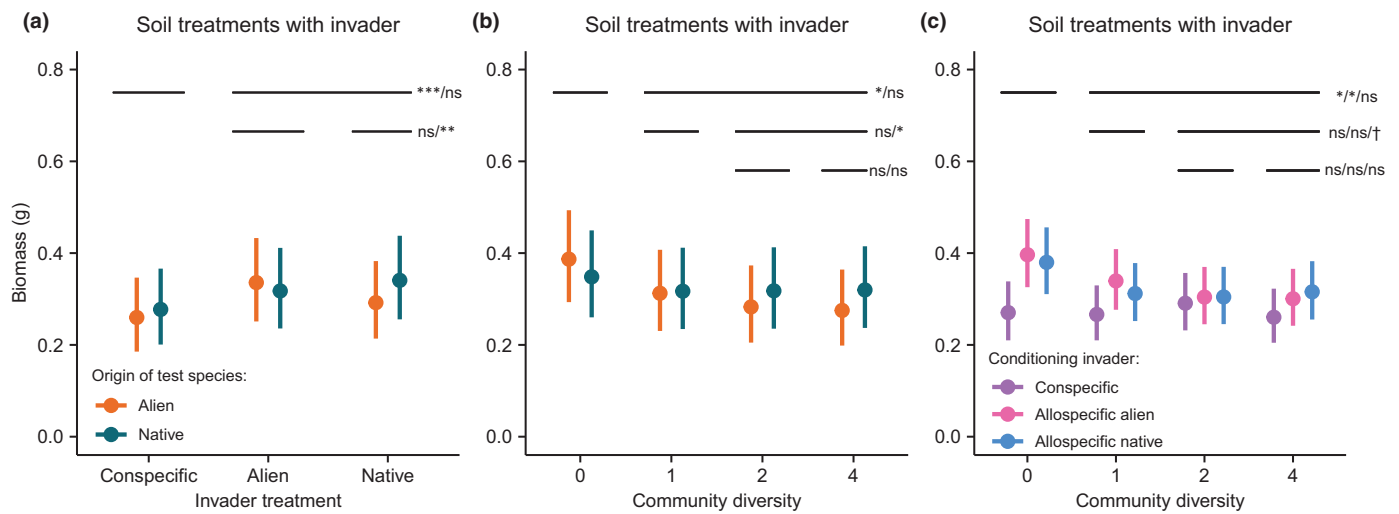


Fig. 4 Effect of soil treatments with conditioning invaders (I) on the test species. (a) Total biomass of alien and native test species in the three soil-conditioning invader-type treatments (conspicuous, allospecific alien, allospecific native) of the pots conditioned by both invaders and communities (C) and conditioned by invaders only (i.e. treatments ++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 and conditioned by invaders only (i.e. treatments ++C and +I–C; $n = 980$). (c) Total biomass of the test species in the three soil-conditioning invader treatments (conspicuous, allospecific alien, allospecific native) with native community of different diversities (0, 1, 2, 4 species; treatments ++C and +I–C; $n = 980$). Shown are modelled means (\pm 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.

effect of alien invaders on other alien test species (Fig. 3). In other words, compared with native plants, alien plants tended to have less negative soil-legacy effects on subsequent alien test species. This indicates that alien species, in contrast with 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 posits 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 (Adams *et al.*, 2003; O’Dowd *et al.*, 2003; Bourgeois *et al.*, 2005; Green *et al.*, 2011; Hohenadler *et al.*, 2018) have shown that alien invaders might facilitate the 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 with 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 the 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-microbe-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, multispecies 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 analysed the data. DC wrote the manuscript with input from MvK.

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Fig. S1 Total biomass of alien and native test species in the four soil-conditioning community-diversity treatments of the pots that were not conditioned by invaders.

Fig. S2 Effect of aboveground biomass produced by plants in the soil-conditioning phase on the biomass of the test plants.

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

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.

Table S3 Effects of the origin of the test species, soil treatment of the soil-conditioning phase and their interactions on total biomass of the test species.

Table S4 Effects of the origin of the test species, soil treatment of the soil-conditioning phase and their interactions on total biomass of the test species (including square-root-transformed total aboveground biomass of conditioning plants as a covariate).

Table S5 Effects of the origin of the test species, invader treatment of the soil-conditioning phase, the diversity of native community and their interactions on total biomass of the test species in the subset of pots that experienced conditioning with an invader (including square-root-transformed total aboveground biomass of conditioning plants as a covariate).

Table S6 Effects of the origin of the test species, the diversity of the native community and their interactions on total biomass of the test species in the subset of pots that did not experience conditioning with the invaders.

Table S7 The standard deviations for the 10 test species in the test phase from the models shown in Tables 2, 3, S3–S6.