Plant-soil feedback as a pathway of invasional meltdown

Zhijie Zhang[1], Yanjie Liu[2*], Caroline Brunel[3,4], Mark van Kleunen[1,3]

[1]Ecology, Department of Biology, University of Konstanz, 78464 Konstanz, Germany

[2]Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy Sciences, Changchun 130102, China

[3]Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, China

[4]IRD, IPME; 911 avenue Agropolis, BP 64501, 34394, Montpellier, France

*Email of corresponding author: liuyanjie@iga.ac.cn +86 431 85542266

Abstract

Invasive alien species are frequently assumed to be more competitive than natives. Hundreds of experiments have investigated this using pairs of species. However, species compete in highly diverse communities, and it remains unknown whether invasive aliens are more competitive than natives in multispecies communities. We tested whether and how a third plant species affects the competition between alien and native plants through plant-soil feedback. We first conditioned soil with one of ten species (six natives and four aliens) or without plants. Then, we grew on these 11 soils, five aliens and five natives without competition, and with intra- or interspecific competition (all pairwise alien-native combinations). Overall, aliens were not more competitive than natives when grown on soil conditioned by other natives or on non-conditioned soil. However, aliens were more competitive than natives on soil conditioned by other aliens. Soil conditioned by aliens changed the competitive outcomes by affecting intrinsic growth rates (i.e. growth rates without competition) of aliens less negatively than that of natives, rather than by affecting the strength of competition. Rhizosphere-microbiome analysis indicated that the less negative effect of aliens on other aliens is mediated by fungal endophytes. The dissimilarity of fungal endophyte communities, which positively correlated with strength of plant-soil feedback, was higher between two aliens than between aliens and natives. Our study suggests that coexistence between alien and native plants might be less likely with more aliens. Such invasional meltdown through plant-soil feedback is most likely mediated by spill-over of fungal endophytes.

Keywords: enemy release, invasion, invasional meltdown, microbes, multispecies, novelty, plant-soil feedback

Introduction

What determines invasion success of alien species is a central and urgent question in ecology[1]. Charles Elton, in his famous book, posited superior competitive ability as one of the mechanisms[2]. Since then, hundreds of experiments have studied competition between native and alien species. Now we know that many successful alien species are indeed more competitive than natives [3-5]. Most studies, however, focused on pairwise interactions[6] (i.e. interaction between two species; but see refs[7,8] for two exceptions), although in nature most species interact with multiple species. With regard to invasive alien organisms, only few hypotheses guide us in testing interactions among more than two species. One of the few that do is the invasional meltdown hypothesis, which posits that alien species facilitate other aliens over natives[9]. Nevertheless, this hypothesis has so far mainly been tested for pairs of alien species without involving native species[9,10]. Therefore, it remains unknown whether a third plant species could affect the outcome of pairwise competition between alien and native plants.

A major question in community ecology is whether we can predict competitive outcomes in multispecies communities (i.e. which species will dominate) based on pairwise experiments. Many studies assume that interactions remain pairwise in all systems[11-13], which allows to scale up from two-species systems to more diverse ones. For a hypothetical example, consider adding a third species into a two-species community (Fig. 1a). If we know from a previous pairwise experiments that the third species strongly suppresses one of the two species, we would predict that it will indirectly release the other species from competition. Although this ‘bottom-up’ approach is supported by several experiments on microbes[14,15], competition *per se* (i.e. the strength of

competition, or how individuals tolerate and suppress others) may be modified by a third species[16,17] (Fig. 1b&c). For example, it was shown that *Skeletonema costatum*, a cosmopolitan diatom, does not directly affect the growth of *Karenia brevis*, a dominant dinoflagellate in the Gulf of Mexico, but undermines the allelopathic effects of *K. brevis*[18]. This would also lessen the effect of *K. brevis* on other phytoplankton species, and interactions might consequently not always remain pairwise. Therefore, we need to study explicitly how a third species can affect the competition between alien and native species.

Plant competition occurs through different processes, which makes it more challenging to study. The most widely studied process is resource competition[19], partly because competition for space, food and other resources is the most intuitive. Nevertheless, growing evidence shows that resource use alone cannot always explain success of alien species [20-22]. Competition can also act through other trophic levels, such as soil microbes. We already know that plants species modify soil microbial communities with consequences for their own development. This process of plant-soil feedback (PSF) affects performance of plants that grow subsequently on the soil[23,24], the strength of competition[25] and species coexistence [26].

In particular, PSF studies have opened up new avenues to test mechanisms of plant invasion[27], such as enemy release[28-30] and novelty of aliens[31,32]. Based on these mechanisms, we would expect that the origin (alien or native) of the third species matters in how they affect competitive outcomes between alien and native species. First, enemy release posits that alien plants are released from their enemies[33], and therefore soils conditioned by alien plants should accumulate few soil pathogens. Consequently, aliens

would free natives that grow later on that soil from pathogens. However, if aliens instead of natives grow later on the soil, they might not be affected. This is because they are already released from pathogens. Following this logic, soil conditioned by aliens would subsequently favor natives over aliens. Second, aliens are assumed to be novel to natives[32], and therefore natives should accumulate soil pathogens that are more likely shared with other natives than with aliens. Following this logic, soil conditioned by natives would subsequently favor aliens over natives. Whether these two expectations hold remains unknown.

To test how a third alien or native plant species affects competition between alien and native species, we conducted a large multi-species PSF experiment. We first conditioned soil with one of ten species (six natives and four aliens) or, as a control, without plants. Then, on the conditioned soil, five alien and five native test species were grown without competition, and with intraspecific or interspecific competition, using all pairwise alien-native combinations. We also analyzed differences in the microbial communities of the conditioned soils. We addressed the following questions: (1) Does a third species (i.e. a soil-conditioning species) affect the competitive outcome between alien and native test species through plant-soil feedback, and does the origin (native or alien) of the third species matter? (2) If so, does the third species affect competitive outcomes through direct effects on growth of the test species (Fig. 1a), or through effects on the strength of competition (Fig. 1b&c)? (3) Do differences in the composition of soil microbial communities among the conditioned soils explain the effects of plant-soil feedback on competition between alien and native species?

Materials and Methods

Study species

We conducted a two-phase plant-soil feedback experiment in which we conditioned soils with one of four alien and six native plant species (soil-conditioning species), and tested the feedback on five alien and five native species (test species; Table S1). Multiple species were used to increase our ability to generalize the results[34]. The classification of the species as native or alien in Germany was based on the FloraWeb database[35]. All species are widespread in Germany and can be locally abundant. So, the alien species can be classified as naturalized (and probably invasive) in Germany *sensu* Richardson, et al. [36]. All species mainly occur in grasslands and overlap in their distributions according to FloraWeb, and thus are very likely to co-occur in nature. Seeds of the native species and one of the alien species (*Onobrychis viciifolia*) were purchased from Rieger-Hofmann GmbH (Blaufelden-Raboldshausen, Germany), and seeds of the other species were from the seed collection of the Botanical Garden of the University of Konstanz. We initially planned to use the same species in the soil-conditioning and test phases. However, in the soil-conditioning phase, seeds of one of the six native species (*Cynosurus cristatus*) were contaminated with other species, and germination success of two of the four aliens (*Solidago gigantea* and *Salvia verticillata*) was low. Therefore, we replaced these three species in the test phase with three alien species (*S. canadensis*, *Senecio inaequidens* and *Epilobium ciliatum*), to have five native and five alien test species.

Experimental setup

Soil-conditioning phase

In the period from 18 June to 2 July 2018 (Table S1), we sowed the four alien and six native soil-conditioning species separately into trays (10cm × 10cm × 5cm) filled with

potting soil (Topferde[@], Einheitserde Co., Sinntal-Altengronau, Germany). Because we wanted that the different species would be in similar developmental stages at the beginning of the experiment, we sowed the species at different times (Table S1), according to their germination speed known from previous experiments. We placed the trays in a greenhouse under natural light conditions, with a temperature between 18 and 25°C.

For each species, we transplanted 135 seedlings individually into 1.5-L pots. This was done for eight out of ten species, and done from 9 to 11 July 2018. For the other two species, *S. verticillata* and *S. gigantean*, we transplanted 61 and 115 seedlings respectively, from 25 July to 12 August (Table S1). This is because these two species germinated more slowly and irregularly than expected. We also added 330 pots that did not contain plants as a control treatment. In a complete design, we would have had 1680 pots. However, because we had fewer pots in *C. cristatus* (due to seed contamination), *S. gigantea* and *S. verticillata*, we had 1521 pots in the end. The substrate that we used was a mixture of 37.5% (v/v) sand, 37.5% vermiculate and 25% field soil. The field soil served as inoculate to provide a live soil microbial community, and had been collected from a grassland site in the Botanical Garden of the University of Konstanz (47.69^{o}N, 9.18^{o}E) on 12 June 2018. Immediately after removing plant material and large stones by sieving the field soil through a metal grid with a 1cm mesh width, we stored it at 4°C until the transplanting.

After the transplanting of the seedlings, we randomly assigned the pots to positions in four greenhouse compartments (23°C/18°C day/night temperature, no additional light). Each pot sat on its own plastic dish to preserve water and to avoid cross-contamination

through soil solutions leaking from the bottoms of the pots. Seedlings that died within two weeks after transplanting were replaced by new ones. All pots, including both the ones with and without plants, were watered as needed, randomized twice, and fertilized seven times during the experiment with an NPK water-soluble fertilizer (Universol Blue®, Everris, Nordhorn, Germany) at a concentration of 1‰ m/v. From 22 to 26 Oct 2018, 15 weeks after the start of soil-conditioning phase, we harvested the soil by first cutting off the shoot and then removing the roots from the soil by sieving it through a 5-mm mesh. The mesh was sterilized in between using 70% ethanol. For the pots in the control treatment, soils were also sieved through the 5-mm mesh. Then, we put the sieved soil into 1-L pots, which were used in the test phase.

Test phase

From 9 to 18 Oct 2018, we sowed the five alien and five native test species (Table S1) separately into trays filled with the same type of potting soil as used for sowing in the soil-conditioning phase. On 29 and 30 Oct, we transplanted the seedlings into the 1-L pots filled with soil from the soil-conditioning phase. Three competition treatments were imposed (Fig. 2): 1) no competition, in which individuals were grown alone; 2) intraspecific competition, in which two individuals from the same species were grown together; 3) interspecific competition, in which two individuals, one from an alien and one from a native species, were grown together. We grew all ten species without competition and in intraspecific competition, and in all 25 possible native-alien pairwise combinations of interspecific competition. For the plants that were grown in non-conditioned soil, we replicated each species without competition 12 times and with intraspecific competition six times, and each interspecific native-alien combination also

six times. For the plants that were grown on conditioned soil, we had three replicates for each combination of a competition treatment (10 without competition, 10 with intraspecific competition, 25 with interspecific competition) and soil-conditioning species (six native and four alien). Because we had fewer replicates for soil conditioned with *C. cristatus*, *S. gigantea* and *S. verticillata*, the final design had 1521 pots (and 2639 individuals) in the test phase.

After the transplanting, we randomly assigned the pots to positions in three greenhouse compartments. Each pot sat on its own plastic dish. We replaced seedlings that died within two weeks after transplanting by new ones. All plants were watered as needed, and fertilized four times during the experiment with an NPK water soluble fertilizer (Universol Blue®, Everris, Nordhorn, Germany) at a concentration of 1‰ m/v. The pots were re-randomized once on 10 Dec 2018. On 8 and 9 Jan 2019, ten weeks after the start of the test phase, we harvested all aboveground biomass, and washed belowground biomass of plants that were grown without competition free from soil. The roots of plants that were grown in competition were so tangled that we could not separate them. The biomass was dried at 70°C to constant weight, and weighed with an accuracy of one milligram.

Soil sampling, DNA extraction, amplicon sequencing and bioinformatics

From 22 to 26 October 2018, when we collected soils from the soil-conditioning phase, we randomly selected six pots for each of the ten soil-conditioning species. For each of these pots, we collected 10-20ml rhizosphere soil. We additionally collected soil from six of the pots without plants. The 66 samples were collected in sterile 50-ml cylindrical tube, and immediately stored at -80□ until DNA extraction.

We extracted DNA from 0.25g of each soil sample using the DNeasy® PowerSoil® Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. PCR amplifications and amplicon sequencing were then performed by Novogene (Beijing China). The V3-V4 region of bacterial 16S rDNA gene was amplified in triplicate with the universal primers 341F/806R (forward primer: 5'-CCTAYGGGRBGCASCAG-3'; reverse primer: 5'- GGACTACNNGGGTATCTAAT-3', [37]. The ITS2 region of the fungal rDNA gene was amplified in triplicate with the primers specific to this locus (forward primer: 5'-GCATCGATGAAGAACGCAGC-3'; reverse primer: 5'-TCCTCCGCTTATTGATATGC-3' [38].

We processed the raw sequences with the *DADA2* pipeline designed to resolve exact biological sequences (Amplicon Sequence Variants). After demultiplexing, we removed the primers and adapter with the *cutadapt* package [39]. We trimmed 16S sequences to uniform lengths. Sequences were then dereplicated, and the unique sequence pairs were denoised (using the *dada* function from the *dada2* package, [40]. We then merged paired-end sequences, and removed chimeras. We assigned the sequences to taxonomic groups using the UNITE[41] reference databases for fungi. We rarefied bacteria and fungi to 30,000 and 9,500 reads, respectively, to account for differences in sequencing depth. Three samples with lower reads for bacteria or fungi, and two samples with low amplicon concentration for fungi were excluded from analyses. In addition, we identified putative fungal functional groups that could affect plant fitness using the FUNGuild database[42]. Sequence variants assigned to arbuscular mycorrhiza fungi, plant pathogens and endophytes, respectively, represented < 0.1%, 11.4% and 15.7% of the total read abundance. Because relative abundance of assigned arbuscular mycorrhizal fungi was

extremely low and they were not detected in 37 out of 62 soil samples, we did not analyze the data of arbuscular mycorrhizal fungi.

Statistical analyses

Analyses of plant performance

To test whether soil-conditioning plants affected the strength of competition (Fig. 1b&c) and competitive outcomes between alien and native species in the test phase, we used a linear mixed-effect model, as implemented in the *nlme*[43] package with the R 3.6.1[44]. The model included aboveground biomass of test plants as the response variable, and soil-conditioning treatment (none, native plant, alien plant), competition treatment (no, intra- and interspecific competition), origin of test species (native, alien) and their interactions as the fixed effects. To account for non-independence of species belonging to the same family and of plants belonging to the same species, family and species identity of the test (focal and competitor plant) and soil-conditioning plants as random effects. A significant interaction between competition and soil-conditioning treatments would indicate that the presence of a soil-conditioning plant or its origin affects the strength of competition. A significant interaction between soil-conditioning treatment and origin of the test species would indicate that the presence of a soil-conditioning plant or its origin affects biomass production of alien and native test speciess differently, averaged across competition treatments. In other words, it would indicate that the soil-conditioning treatment affects the competitive outcome between aliens and natives. Competitive outcome here refers to which species will exclude or dominate over other species at the end point for the community[45]. Most studies infer it by only growing the species in mixture[46]. However, we inferred it from the average of three types of cultures, that is, plants without

competition, in monocultures and in mixtures. This method has two advantages. First, it better mimics the dynamics of species populations across space and time[5,47]. Second, it should increase the precision of estimating competitive outcomes[45].

To test whether soil-conditioning plants directly affected growth of alien and native species (Fig. 1a), we used a linear mixed-effect model to analyze the test plants that were grown without competition. The model included aboveground, belowground or total biomass of test plants as the response variable, and soil-conditioning treatment, origin of the test species and their interaction as fixed effects. Family and species identity of the test and soil-conditioning plants were included as random effects.

In all models, to account for the fact that soil-conditioning plants were transplanted at different times, we included transplanting date as a covariate. To improve homoscedasticity of residuals, we allowed soil-conditioning species, test species and soil-conditioning treatments to vary their residuals by using the *varComb* and *varIdent* function[48]. To improve normality of the residuals, we square-root transformed biomass of the test plants. To assure model convergence, we increase the maximum number of iterations and evaluations when necessary. The significance of fixed effects in the mixed models was assessed with likelihood-ratio tests when comparing models with and without the effect of interest.

The soil-conditioning treatment had four levels: 1) the soil was not conditioned by plants (non-conditioned soil), 2) the soil was conditioned by the same species as the focal test plant (home soil), and if the soil was conditioned by another species, this was either 3) an alien species (alien soil) or 4) a native species (native soil). We created three

dummy variables [49] to split up these four soil-conditioning treatments into three orthogonal contrasts to address the questions: 1) Does it matter whether the soil was conditioned by plants or not ($Soil_{Conditioned/Non\text{-}conditioned}$)? 2) When the soil was conditioned by plants, does it matter whether the soil was conditioned by the same species as the focal test plant or by a different species ($Soil_{Home/Away}$)? 3) When the soil was conditioned by a species different from the focal test plant, does it matter whether the soil was conditioned by an alien or a native species ($Soil_{Alien/Native}$)?

Likewise, for the first model, which used data from all competition treatments, we created two dummy variables to split up the three competition treatments – no, intra- and interspecific competition – into two contrasts to address the questions: 1) Does it matter whether the test plant was grown with competition ($Comp_{Yes/No}$)? 2) When the test plant was grown with competition, does it matter whether the competitor belonged to the same species or not ($Comp_{Intra/Inter}$)? In a few cases of the interspecific competition treatment (103 out of 1573 pots), competitor species were the same as the soil-conditioning species. Therefore, these pots are testing a two-species rather than a three-species interaction. However, removing these data points does not affect the results, indicating that our results are robust (Table S2).

Analyses of the soil microbial community

To test the effect of soil-conditioning species on soil bacterial and fungal communities, and their subsequent effects on the test plants, we used three methods. First, we tested whether the presence of a soil-conditioning plant affected the composition of soil microbial communities, and whether this effect depended on the origin of the soil-conditioning species. To do so, we used permutational analysis of variance

(PERMANOVA), as implemented in the *adonis* function of the *vegan* package[50]. The model included Bray-Curtis dissimilarities of bacteria and fungi, which were calculated using reads relative abundances, as the response variable, and soil-conditioning treatment as explanatory variable. Transplanting date, family and species identity of the soil-conditioning plants were also included as explanatory variables to control for non-independence. We split up the three soil-conditioning treatments into two contrasts to address the questions: 1) Does it matter whether the soil was conditioned by plants or not? 2) When the soil was conditioned by plants, does it matter whether the species is alien or native? We used nonmetric multidimensional scaling (NMDS) to visualize differences in the soil microbial communities of the plant species.

Second, we tested whether alien and native species accumulated fungal pathogens to different degrees. To do so, we used linear mixed models that included the richness, Shannon diversity or relative abundance of fungal pathogens as the response variable, and soil-conditioning treatments, which were again split up into two contrasts, as the fixed effect. Transplanting date was included as a covariate, and family and species identity of soil-conditioning plants as random effects. We allowed the different soil-conditioning species to have different variances in the models testing relative abundance to improve homoscedasticity, and we logit-transformed relative abundance to improve normality of the residuals.

To link soil enemies and responses of test plants, and because the enemy release hypothesis predicts that alien species respond less negatively to enemies than native species [28,51], we further tested whether alien and native test species responded differently to diversity and abundance of fungal pathogens. To do so, we used linear mixed models

and included the strength of plant-soil feedback ($\ln PSF_{ij}$) as the response variable, and diversities (or logit-transformed relative abundance), origin of test species and their interaction as fixed effects. Family and species identity of soil-conditioning and test species were included as random effects. The strength of plant-soil feedback, $\ln PSF_{ij}$, was calculated as

$$\ln PSF_{ij} = \ln biomass_{ij} - \ln biomass_{i0}.$$

Here, $\ln biomass_{ij}$ and $\ln biomass_{i0}$ are mean aboveground biomass of test species i when grown without competition on soil conditioned by species j and on soil not conditioned by plants, respectively. Positive values indicate that soil-conditioning species j improved growth of test species i. We excluded conspecific plant-soil feedback (i.e. $\ln PSF_{ii}$), as its evolutionary history may differ from that of heterospecific plant-soil feedback. We allowed different soil-conditioning and test species to have different variances to improve homoscedasticity.

Third, we analyzed how conditioned soil communities differed 1) between plants from the same alien plant species, 2) between plants from the same native species, 3) between different alien species, 4) between different native species, and 5) between alien and native species. To do so, we used linear mixed models and included averaged Bray-Curtis dissimilarities as the response variable, and the five above-mentioned categories of plant combinations as the fixed effect. Family and species identity of soil-conditioning species were included as random effects. The Bray-Curtis dissimilarities of bacteria, fungi, fungal pathogens or fungal endophytes were first calculated between all pairwise samples, and then averaged across replicates to get average values for each within-

species pair or between-species pair. We allowed the soil-conditioning species to have different variances to improve homoscedasticity, and we logit-transformed the Bray-Curtis dissimilarities to improve normality of the residuals. We split up the five categories of plant combinations into four contrasts to address the questions: 1) Are soil communities more similar (or different) when conditioned by the same plant species than by another species? 2) When conditioned by the same species, are soil communities more similar for alien species than for native species? When conditioned by different species, 3) are soil communities more similar between two alien species than between an alien and a native species, and 4) are soil communities more similar for the latter than between two native species? We used heatmaps to visualize the community dissimilarities, whose values were mean-centered and then bounded to range from -1 to 1. This was done with the *corrplot* package[52].

To link microbial community dissimilarity to responses of test plants, we further tested whether the biomass response of test species was related to their microbial community dissimilarity with the soil-conditioning species. To do so, we used linear mixed models and included strength of plant-soil feedback ($\ln PSF_{ij}$) as the response variable, and included logit-transformed Bray-Curtis dissimilarities between soil-conditioning and test species as the fixed effect. Family and species identity of soil-conditioning and test species were included as random effects. Again, we excluded conspecific plant-soil feedback. We allowed different soil-conditioning and test species to vary their residuals to improve homoscedasticity. This analysis was restricted to the seven species that were used in both the soil-conditioning and test phase, because it required data from both soil microbial communities and strength of plant-soil feedback.

Results

Do soil-conditioning species affect competitive outcomes (differences in biomass production)?

Averaged across the three competition treatments (no, intra- and inter-specific competition), plants produced less aboveground biomass (-67.2%; χ^2 = 10.31, *P* = 0.001) on conditioned soil than on non-conditioned soil, and on home soil (i.e. soil conditioned by the same plant species) than on away soil (-22.7%; χ^2 = 4.54, *P* = 0.033; Fig. 3a; Table 1). Averaged across soil-conditioning treatments, biomass of alien and native plants did not significantly differ (χ^2 = 0.083, *P* = 0.774; Fig. 3a; Table 1). However, when grown on alien soil (i.e. soil conditioned by an alien plant), alien plants produced significantly more aboveground biomass (+18.2%) than native plants, whereas on native soil, this difference was smaller (+9.9%; Origin × $\text{Soil}_{\text{Alien/Native}}$ interaction: χ^2 = 4.74, *P* = 0.029; Fig 3a; Table 1). This indicates that soil conditioning with an alien plant pushes the competitive outcome more strongly towards subsequent alien plants than soil conditioning with a native plant does.

Do soil-conditioning species affect growth or the strength of competition?

For the subset of plants grown in the absence of competition, aboveground biomass (-59.8%; χ^2 = 13.38, *P* < 0.001) was lower on conditioned soil than on non-conditioned soil (Fig. 3b; Table S3). The competition-free plants also tended to produce less biomass on home soil than on away soil (Fig. 3b). This effect was not significant for aboveground biomass, but it was marginally significant for belowground biomass (-15.0%; χ^2 = 2.93, *P* = 0.087; Fig. 3b & S1; Table S3). Averaged across all soil-conditioning treatments, alien and native competition-free plants did not differ in biomass production (χ^2 = 0.025, *P* = 0.875). However, aliens achieved more aboveground biomass (+17.3%) than natives on

alien soil, whereas on native soil, this difference was smaller (+8.5%; Fig. 3b; Table S3). Although this finding was only marginally significant for aboveground biomass (χ^2 = 2.90, P = 0.088) and belowground biomass (χ^2 = 3.23, P = 0.072), it was significant for total biomass (χ^2 = 4.56, P = 0.033; Table S3; Fig. S1). This result indicates that soil conditioning with an alien plant advances the growth of subsequent alien plants more strongly than that of subsequent native plants.

Competition reduced aboveground biomass (-35.1%; χ^2 = 3.74, P = 0.053; Fig. 3c; Table 1), and was more intense when the test plants were grown on alien soil than on native soil (-39.3% *vs.* -33.0%; χ^2 = 4.85, P = 0.028; Fig 3c; Table 1). However, the strength of competition was not affected by the other soil-conditioning treatments. Alien and native test species did not differ in their biomass responses to competition (χ^2 = 0.25, P = 0.618), and this finding holds for every soil-conditioning treatment. We also found that intra- and inter-specific competition did not differ in strength (χ^2 = 0.80, P = 0.373), and that this finding holds for alien and native test species, and for each soil-conditioning treatment (Fig 3c; Table 1).

How do soil-conditioning species affect soil microbial communities?

Overall, the presence of plants significantly modified the composition of soil bacterial and fungal communities (Supplement S3.1). Moreover, alien and native plant species modified the composition of bacterial and fungal communities differently (Supplement S3.1). However, neither the presence of plants nor the origin of plants significantly affected diversities and relative abundance of fungal pathogens (Supplement S3.2). Further analyses showed that, diversities and relative abundance of fungal pathogens did

not affect the strength of plant-soil feedback, and that this holds for both native and alien test species (Supplement S4.1).

Dissimilarity of rhizosphere bacterial communities was higher (i.e. more blue colors in Fig. 4) between plants of different species than between plants of the same species (χ^2 = 4.31, P = 0.038; Fig. 4a & e; Table S7). Although this does not hold for rhizosphere fungal communities, their dissimilarity depended on the origins of the species in the between-species combination (Fig. 4b-d & f-h; Table S7). Specifically, dissimilarities of fungal communities as a whole and of the subset of fungal endophytes were generally higher between two alien plant species than between an alien and a native species (Fungi: χ^2 = 4.00, P = 0.045; Fungal endophyte: χ^2 = 12.11, P = 0.001). Furthermore, the dissimilarity of fungal endophyte communities was higher between an alien and a native species than between two natives (χ^2 = 10.53, P = 0.001; Fig. 4d & h; Table S7). Analyses for the subset of species used in both the conditioning and test phase showed that dissimilarity of fungal endophyte communities correlated positively with the magnitude of the negative plant-soil feedback (χ^2 = 6.10, P = 0.014; Fig. 5d; Table S10). In other words, when two plant species built up very different communities of fungal endophytes, they affected each other less negatively through plant-soil feedback. For the other groups of microbiota, i.e. bacteria, fungi overall and fungal pathogens, dissimilarity of soil communities did not significantly affect plant-soil feedback (Fig. 5a-c; Table S10).

Discussion

We found that when grown on soil that had not been conditioned by plants, aliens and natives produced, across competition treatments, similar biomasses. The same was true or on soil that had been conditioned by native plants. This indicates that aliens were not more competitive than natives on those soils. However, on soil that had been conditioned by aliens, aliens produced more biomass than natives and thus were more competitive. Analysis of biomass of plants grown without competition, indicated that conditioning by aliens changed the competitive outcomes by decreasing intrinsic growth rates (i.e. growth rates without competition) of aliens less than that of natives. The strength of competition, however, was not affected by the soil-conditioning treatment. Analysis of soil microbiomes, revealed that dissimilarity of fungal endophyte communities between plant species was positively correlated with the strength of plant-soil feedback, and that dissimilarities of fungal endophyte communities were higher between two aliens than between an alien and a native species. This suggests that the less negative effect of conditioning by aliens on aliens is at least partly driven by a lower chance of spill-over of shared fungal endophytes between aliens.

Invasional meltdown in a multi-species context

The similar aboveground biomass of aliens and natives on soil that had not been conditioned or had been conditioned by native plants, indicates that on those soils aliens are not more competitive than natives. This result is in line with the recent finding that alien and native species do not differ in their competitive abilities if both of them are widespread and abundant species[5]. However, on soil conditioned by aliens, aliens were more competitive than natives. This finding supports the predictions of the invasional

meltdown hypothesis[3,9,53]. So far, over 13,000 plant species have become naturalized outside their natural ranges[54,55], and in some regions more than half of the flora consists of naturalized alien species[56]. These numbers are still increasing[57], which means that interactions between alien species are likely to become more and more frequent. Our findings indicate that the facilitation between aliens, mediated by plant-soil feedback, may accelerate the naturalization of aliens and their impacts on natives.

A major question in ecology is whether it is possible to predict competitive outcomes in multispecies communities based on pairwise interactions. The results of our experiment suggest that this is possible. For example, from the data of plants that were grown without competition, which tested pairwise interactions between soil-conditioning and test species, we showed that alien test species produced more biomass than natives on soil that had been conditioned by aliens. This finding still hold when we also included the data of plant that were grown with competition to assess competitive outcomes in multispecies communities. Moreover, the soil-conditioning species rarely changed the strength of competition. When they did, they affected the strength of competition equally for alien and native species, and thus did not affect competitive outcomes. This finding echoes those of other previous empirical studies. For example, a phytoplankton experiment by Prince, et al. [18] found that the strength of competition was modified only in two out of the ten species in their study. Friedman, et al. [15] found that competitive outcomes in three-species bacterial communities were predicted by pairwise outcomes with an accuracy of 90%. Therefore, we might in most cases be able to scale up from pairwise interactions to three-species interactions.

Although we might be able to scale up to three-species systems, we might not yet be able to scale up to more diverse systems. Friedman, et al. [15] found that pairwise outcomes alone poorly predict outcomes of seven- or eight-species bacterial communities. This could indicate that with increasing diversity it the likelihood increases that the strength of pairwise competition is modified by at least one of the many other species in the community. Future experiments that test competition between alien and native organisms in more diverse communities could shed light on this. However, because naturalized alien plant species often occur in species-poor communities[58], we believe that our experiment and results are representative for plant invasions in the real world.

Potential mechanisms underlying invasional meltdown

We did not find evidence for an enemy release effect in plant-soil feedback. At the end of the soil-conditioning phase, alien and native plant species did not differ in the diversity and relative abundance of fungal pathogens. In addition, the performance of alien and native species in the test phase did not change with diversity and relative abundance of fungal pathogens in the soil. The lack of evidence for enemy release contrasts with the findings of meta-analyses that found that enemy release contributed to plant invasion[59,60]. This discrepancy could be explained by the fact that the studies included in the meta-analyses mainly focused on aboveground enemies and on herbivores. Belowground microbial enemies are more diverse, and many of them might be rare or less harmful. Therefore, diversity and relative abundance of soil pathogens may be less likely to capture the mechanism underlying plant-soil feedback process than the actual identities of the pathogens. Indeed, we found that alien and native plants modified the composition of soil microbial communities (including the composition of fungal pathogens;

Supplement S3.2) in different ways. This suggests that it plant-soil feedback is mainly mediated by the composition of the soil microbial communities than the diversity and abundance.

Interestingly, we found that the dissimilarity of fungal endophyte communities was higher between alien plant species than between aliens and natives, and higher than between natives. We found, however, a similar pattern when the field-soil inoculate used in the soil-conditioning phase had been sterilized (Fig. S6; Table S8). This suggests that the high dissimilarity of fungal endophyte communities between aliens is likely driven by endophytes that were already present in the plants before transplanting (e.g. as seed microbiota) rather than by those that were in the field-soil inoculum. There are three potential reasons why dissimilarity of fungal endophyte communities was on average higher between two aliens than between an aliens and a native, and higher than between two natives. First, given that phylogenetic distance correlated positively with dissimilarity of soil fungal endophyte communities (Supplement S5), it could be that the phylogenetic distance between aliens was higher than between aliens and natives, and higher than between natives. However, as this was not the case (Supplement S5), this explanation can be discarded. A second potential reason is that natives have co-occurred with each other for a longer time, and thus share more similar endophytes[61]. A third potential reason could be that if the alien species brought endophytes with them from their native ranges, these endophytes jumped over to native hosts. Such host shifts of endophytes are more likely to involve native plants than other alien plants, as alien-native interactions are still more common than alien-alien interactions. Regardless of the exact

reason, the observed dissimilarities in fungal endophyte communities suggest that they might play a role in plant-soil feedback differences.

In line with this idea, dissimilarity of fungal endophyte communities correlated positively with the strength of plant-soil feedback. In other words, when two plant species built up very different communities of fungal endophytes, they affected each other less negatively through plant-soil feedback. Plant species that build up very different endophyte communities might have less spill-over of endophytes, and consequently the conditioning species affects the test species less negatively. The association between the strength of plant-soil feedback and dissimilarity of fungal endophyte communities, together with the higher dissimilarity of soil communities between alien plant species than between alien and native plants species, provides a mechanistic explanation for invasional meltdown. Still, plant-endophyte interactions are not well understood, and can range from mutualistic to pathogenic[62]. More empirical evidence for their role in plant-soil feedback and invasion is still required. Manipulative studies based on synthetic microbial communities[63], might shed light on the roles of endophytes in plant competition and invasion success.

Conclusions

Our results indicate that the accumulation of alien species may be accelerated in the future, because aliens could favor other aliens over natives through plant-soil feedback. Since Charles Darwin[32], novelty has been posited as an important mechanism of invasion, as it allows aliens to occupy niches that are not used by natives[64-66]. Here, we unveiled another role of novelty. Due to novelty, there might be little spill-over of endophytes, many of which might be pathogenic, between alien plant species. Consequently, alien

species in our study suppressed each other less than they suppressed natives. This could lead to invasional meltdown.

Acknowledgements

We thank L. Arnold, S. Berg, O. Ficht, M. Fuchs, S. Gommel, E. Mamonova, V. Pasqualetto, C. Rabung, B. Rüter, B. Speißer, H. Vahlenkamp and E. Werner for practical assistance. ZZ was funded by the China Scholarship Council (201606100049) and supported by the International Max Planck Research School for Organismal Biology. YL was funded by the Chinese Academy of Sciences (Y9H1011001, Y9B7041001).

Author contributions

ZZ conceived the idea. ZZ, YL and MvK designed the experiment. ZZ, YL and CB performed the experiment. ZZ analyzed the data and wrote the manuscript with input from all others.

References

1 van Kleunen, M., Bossdorf, O. & Dawson, W. The Ecology and Evolution of Alien Plants. *Annual Review of Ecology, Evolution, and Systematics* **49**, 25-47, doi:10.1146/annurev-ecolsys-110617-062654 (2018).

2 Elton, C. S. *The ecology of invasion by animals and plants*. (Methuen, 1958).

3 Kuebbing, S. E. & Nunez, M. A. Invasive non-native plants have a greater effect on neighbouring natives than other non-natives. *Nature plants* **2**, 16134, doi:10.1038/nplants.2016.134 (2016).

4 Golivets, M. & Wallin, K. F. Neighbour tolerance, not suppression, provides competitive advantage to non-native plants. *Ecology Letters* **21**, 745-759, doi:10.1111/ele.12934 (2018).

5 Zhang, Z. & van Kleunen, M. Common alien plants are more competitive than rare natives but not than common natives. *Ecology Letters* **22**, 1378-1386, doi:10.1111/ele.13320 (2019).

6 White, E. M., Wilson, J. C. & Clarke, A. R. Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions* **12**, 443-455, doi:10.1111/j.1366-9516.2006.00265.x (2006).

7 Feng, Y. & van Kleunen, M. Phylogenetic and functional mechanisms of direct and indirect interactions among alien and native plants. *Journal of Ecology* **104**, 1136-1148, doi:10.1111/1365-2745.12577 (2016).

8 Metlen, K. L., Aschehoug, E. T. & Callaway, R. M. Competitive outcomes between two exotic invaders are modified by direct and indirect effects of a native conifer. **122**, 632-640, doi:10.1111/j.1600-0706.2012.20792.x (2013).

9 Simberloff, D. Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters* **9**, 912-919, doi:10.1111/j.1461-0248.2006.00939.x (2006).

10 Braga, R. R., Gómez-Aparicio, L., Heger, T., Vitule, J. R. S. & Jeschke, J. M. Structuring evidence for invasional meltdown: broad support but with biases and gaps. *Biological Invasions* **20**, 923-936, doi:10.1007/s10530-017-1582-2 (2018).

11 Maynard, D. S., Miller, Z. R. & Allesina, S. Predicting coexistence in experimental ecological communities. *Nature Ecology & Evolution* **4**, 91-100, doi:10.1038/s41559-019-1059-z (2020).

12 May, R. M. Will a Large Complex System be Stable? *Nature* **238**, 413-414 (1972).

13 Godoy, O., Stouffer, D. B., Kraft, N. J. B. & Levine, J. M. Intransitivity is infrequent and fails to promote annual plant coexistence without pairwise niche differences. *Ecology* **98**, 1193-1200, doi:10.1002/ecy.1782 (2017).

14 Vandermeer, J. H. The Competitive Structure of Communities: An Experimental Approach with Protozoa. *Ecology* **50**, 362-371, doi:10.2307/1933884 (1969).

15 Friedman, J., Higgins, L. M. & Gore, J. Community structure follows simple assembly rules in microbial microcosms. *Nature Ecology & Evolution* **1**, 0109, doi:10.1038/s41559-017-0109 (2017).

16 Case, T. J. & Bender, E. A. Testing for Higher Order Interactions. *The American Naturalist* **118**, 920-929 (1981).

17 Levine, J. M., Bascompte, J., Adler, P. B. & Allesina, S. Beyond pairwise mechanisms of species coexistence in complex communities. *Nature* **546**, 56-64, doi:10.1038/nature22898 (2017).

18 Prince, E. K., Myers, T. L., Naar, J. & Kubanek, J. Competing phytoplankton undermines allelopathy of a bloom-forming dinoflagellate. *Proceedings. Biological sciences / The Royal Society* **275**, 2733-2741, doi:10.1098/rspb.2008.0760 (2008).

19 Tilman, D. *Resource competition and community structure*. (Princeton university press, 1982).

20 Dawson, W., Fischer, M. & van Kleunen, M. Common and rare plant species respond differently to fertilisation and competition, whether they are alien or native. *Ecology Letters* **15**, 873-880, doi:10.1111/j.1461-0248.2012.01811.x (2012).

21 Godoy, O., Valladares, F. & Castro-Díez, P. Multispecies comparison reveals that invasive and native plants differ in their traits but not in their plasticity. *Functional Ecology* **25**, 1248-1259, doi:10.1111/j.1365-2435.2011.01886.x (2011).

22 Liu, Y. & van Kleunen, M. Nitrogen acquisition of Central European herbaceous plants that differ in their global naturalization success. **33**, 566-575, doi:10.1111/1365-2435.13288 (2019).

23 Bever, J. D., Westover, K. M. & Antonovics, J. Incorporating the soil community into plant population dynamics: The utility of the feedback approach. *Journal of Ecology* **85**, 561-573, doi:10.2307/2960528 (1997).

24 Kulmatiski, A., Beard, K. H., Stevens, J. R. & Cobbold, S. M. Plant-soil feedbacks: a meta-analytical review. *Ecology Letters* **11**, 980-992, doi:10.1111/j.1461-0248.2008.01209.x (2008).

25 Lekberg, Y. *et al.* Relative importance of competition and plant-soil feedback, their synergy, context dependency and implications for coexistence. *Ecol Lett* **21**, 1268-1281, doi:10.1111/ele.13093 (2018).
26 Crawford, K. M. *et al.* When and where plant-soil feedback may promote plant coexistence: a meta-analysis. *Ecol Lett* **22**, 1274-1284, doi:10.1111/ele.13278 (2019).
27 Dawson, W., Schrama, M. & Austin, A. Identifying the role of soil microbes in plant invasions. *Journal of Ecology* **104**, 1211-1218, doi:10.1111/1365-2745.12619 (2016).
28 Callaway, R. M., Thelen, G. C., Rodriguez, A. & Holben, W. E. Soil biota and exotic plant invasion. *Nature* **427**, 731, doi:10.1038/nature02322

https://www.nature.com/articles/nature02322#supplementary-information (2004).
29 Ke, P. J. & Wan, J. Effects of soil microbes on plant competition: a perspective from modern coexistence theory. *Ecological Monographs* **0**, e01391, doi:10.1002/ecm.1391 (2019).
30 Kuebbing, S. E., Classen, A. T., Call, J. J., Henning, J. A. & Simberloff, D. Plant–soil interactions promote co-occurrence of three nonnative woody shrubs. *Ecology* **96**, 2289-2299, doi:10.1890/14-2006.1 (2015).
31 Callaway, R. M. *et al.* Novel weapons: Invasive plant suppresses fungal mutualists in america but not in its native europe. *Ecology* **89**, 1043-1055, doi:10.1890/07-0370.1 (2008).
32 Darwin, C. *On the origin of species*. (J. Murrary, 1859).
33 Keane, R. M. & Crawley, M. J. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* **17**, 164-170, doi:10.1016/s0169-5347(02)02499-0 (2002).
34 van Kleunen, M., Dawson, W., Bossdorf, O. & Fischer, M. The more the merrier: Multi-species experiments in ecology. *Basic and Applied Ecology* **15**, 1-9, doi:10.1016/j.baae.2013.10.006 (2014).
35 Bundesamt für Naturschutz. (http://www.floraweb.de/, 2003).
36 Richardson, D. M. *et al.* Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* **6**, 93-107, doi:10.1046/j.1472-4642.2000.00083.x (2000).
37 Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research* **41**, e1-e1, doi:10.1093/nar/gks808 %J Nucleic Acids Research (2012).

38 Orgiazzi, A. *et al.* Unravelling Soil Fungal Communities from Different Mediterranean Land-Use Backgrounds. *PLOS ONE* **7**, e34847, doi:10.1371/journal.pone.0034847 (2012).

39 Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *2011* **17**, 3 %J EMBnet.journal, doi:10.14806/ej.17.1.200 (2011).

40 Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**, 581-583, doi:10.1038/nmeth.3869 (2016).

41 Nilsson, R. H. *et al.* The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* **47**, D259-D264, doi:10.1093/nar/gky1022 %J Nucleic Acids Research (2018).

42 Nguyen, N. H. *et al.* FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**, 241-248, doi:https://doi.org/10.1016/j.funeco.2015.06.006 (2016).

43 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. Linear and nonlinear mixed effects models. *R package version* **3**, 57 (2018).

44 *R: A language and environment for statistical computing* (R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/. 2019).

45 Gibson, D., Connolly, J., Hartnett, D. & Weidenhamer, J. Designs for greenhouse studies of interactions between plants. *Journal of Ecology* **87**, 1-16 (1999).

46 Aschehoug, E. T., Brooker, R., Atwater, D. Z., Maron, J. L. & Callaway, R. M. The Mechanisms and Consequences of Interspecific Competition Among Plants. *Annual Review of Ecology and Systematics* **47**, 263-281, doi:doi:10.1146/annurev-ecolsys-121415-032123 (2016).

47 Hart, S. P., Burgin, J. R. & Marshall, D. J. Revisiting competition in a classic model system using formal links between theory and data. *Ecology* **93**, 2015-2022, doi:10.1890/11-2248.1 (2012).

48 Zuur, A., Ieno, E., Walker, N., Saveliev, A. & Smith, G. Mixed effects models and extensions in ecology with R. Gail M, Krickeberg K, Samet JM, Tsiatis A, Wong W, editors. *New York, NY: Spring Science and Business Media* (2009).

49 Schielzeth, H. Simple means to improve the interpretability of regression coefficients. *Methods Ecol. Evol.* **1**, 103-113, doi:10.1111/j.2041-210X.2010.00012.x (2010).

50 Oksanen, J. *et al.* The vegan package. (2019).

51 Chun, Y. J., van Kleunen, M. & Dawson, W. The role of enemy release, tolerance and resistance in plant invasions: linking damage to performance. *Ecology Letters* **13**, 937-946, doi:10.1111/j.1461-0248.2010.01498.x (2010).

52 Wei, T. & Simko, V. corrplot: Visualization of a correlation matrix (Version 0.84). (2017).

53 Simberloff, D. & Von Holle, B. Positive Interactions of Nonindigenous Species: Invasional Meltdown? *Biological Invasions* **1**, 21-32, doi:10.1023/A:1010086329619 (1999).

54 van Kleunen, M. *et al.* Global exchange and accumulation of non-native plants. *Nature* **525**, 100-103, doi:10.1038/nature14910 (2015).

55 Pyšek, P. *et al.* Naturalized alien flora of the world. *Preslia* **89**, 203-274 (2017).

56 Essl, F. *et al.* Drivers of the relative richness of naturalized and invasive plant species on Earth. *AoB PLANTS* **11**, doi:10.1093/aobpla/plz051 (2019).

57 Seebens, H. *et al.* Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences* **115**, E2264, doi:10.1073/pnas.1719429115 (2018).

58 Stotz, G. C. *et al.* Not a melting pot: Plant species aggregate in their non-native range. *Global Ecology and Biogeography* **n/a**, doi:10.1111/geb.13046 (2019).

59 Liu, H. & Stiling, P. Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions* **8**, 1535-1545, doi:10.1007/s10530-005-5845-y (2006).

60 Zhang, Z. *et al.* Contrasting effects of specialist and generalist herbivores on resistance evolution in invasive plants. *Ecology* **99**, 866-875, doi:10.1002/ecy.2155 (2018).

61 Dickie, I. A. *et al.* The emerging science of linked plant–fungal invasions. *New Phytologist* **215**, 1314-1332, doi:10.1111/nph.14657 (2017).

62 Hardoim, P. R. *et al.* The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiol Mol Biol Rev* **79**, 293-320, doi:10.1128/MMBR.00050-14 (2015).

63 Großkopf, T. & Soyer, O. S. Synthetic microbial communities. *Current Opinion in Microbiology* **18**, 72-77, doi:https://doi.org/10.1016/j.mib.2014.02.002 (2014).

64 Divíšek, J. *et al.* Similarity of introduced plant species to native ones facilitates naturalization, but differences enhance invasion success. *Nature communications* **9**, 4631, doi:10.1038/s41467-018-06995-4 (2018).

65 Feng, Y., Fouqueray, T. D., van Kleunen, M. & Cornelissen, H. Linking Darwin's naturalisation hypothesis and Elton's diversity-invasibility hypothesis in experimental grassland communities. *Journal of Ecology* **107**, 794-805, doi:10.1111/1365-2745.13061 (2019).

66 Li, S. P. *et al.* The effects of phylogenetic relatedness on invasion success and impact: deconstructing Darwin's naturalisation conundrum. *Ecol Lett* **18**, 1285-1292, doi:10.1111/ele.12522 (2015).

Tables and figures

Table 1 Effects of soil treatments, competition treatments, origin of test species and their interactions on aboveground biomass of plants.

Significant effects ($P < 0.05$) are in bold and marked with asterisks, and marginally significant effects ($0.05 < P < 0.1$) are in italics.

	χ^2	**P**
Transplanting date	12.815	**<0.001***
$Soil_{Conditioned/Non\text{-}conditioned}$	10.306	**0.001***
$Soil_{Home/Away}$	4.535	**0.033***
$Soil_{Alien/Native}$	0.107	0.744
Origin (O)	0.083	0.774
$Comp_{Yes/NO}$	3.738	*0.053*
$Comp_{Intra/Inter}$	0.795	0.373
O : $Soil_{Conditioned/Non\text{-}conditioned}$	1.395	0.238
O : $Soil_{Home/Away}$	1.669	0.196
O : $Soil_{Alien/Native}$	4.741	**0.029***
$Soil_{Conditioned/Non\text{-}conditioned}$: $Comp_{Yes/NO}$	0.956	0.328
$Soil_{Conditioned/Non\text{-}conditioned}$: $Comp_{Intra/Inter}$	0.176	0.675
$Soil_{Home/Away}$: $Comp_{Yes/NO}$	0.121	0.728
$Soil_{Home/Away}$: $Comp_{Intra/Inter}$	2.273	0.132
$Soil_{Alien/Native}$: $Comp_{Yes/NO}$	4.846	**0.028***
$Soil_{Alien/Native}$: $Comp_{Intra/Inter}$	0.321	0.571
O:$Comp_{Yes/NO}$	0.249	0.618
O:$Comp_{Intra/Inter}$	0.371	0.542
O : $Soil_{Conditioned/Non\text{-}conditioned}$: $Comp_{Yes/NO}$	0.511	0.475
O : $Soil_{Conditioned/Non\text{-}conditioned}$: $Comp_{Intra/Inter}$	0.001	0.972
O : $Soil_{Home/Away}$: $Comp_{Yes/NO}$	1.725	0.189
O : $Soil_{Home/Away}$: $Comp_{Intra/Inter}$	0.156	0.693
O : $Soil_{Alien/Native}$: $Comp_{Yes/NO}$	0.197	0.657
O : $Soil_{Alien/Native}$: $Comp_{Intra/Inter}$	0.000	0.990
Random effects	SD	
Family (focal test)	0.165	
Species (focal test)	0.199	
Family (competitor test)	0.065	
Species (competitor test)	0.076	
Family (soil)	0.038	
Species (soil)	0.031	
Residual	0.187	

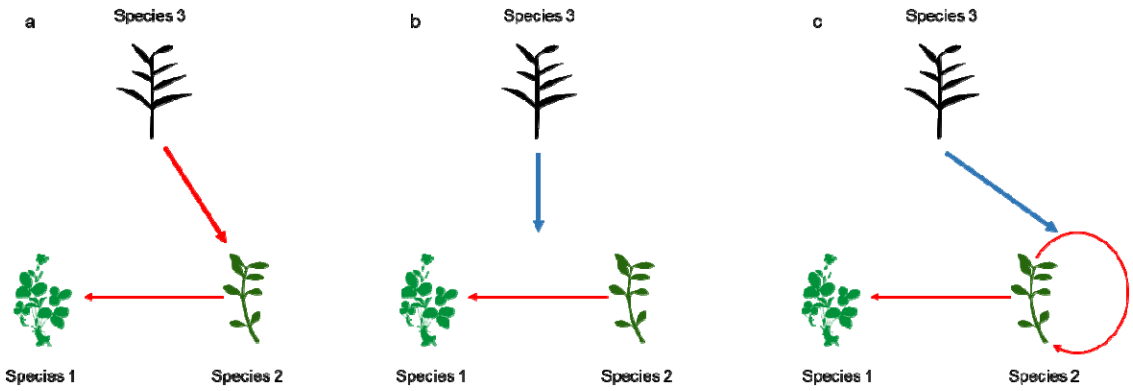

Figure 1 Graphical illustration of how competitive outcomes between two species can be affected by a third species. a, Species 3 (the third species) can release species 1 from competition by the strongly suppressing species 2. **b** & **c,** Species 3 can affect species 1 by modifying **b** the competitive effect of species 2 on species 1 (i.e. interspecific competition) and **c** the competitive effect of species 2 on itself (i.e. intraspecific competition). Red arrows indicate pairwise interspecific or intraspecific interactions. Blue arrows indicate the effect of species 3 on the strength of competition.

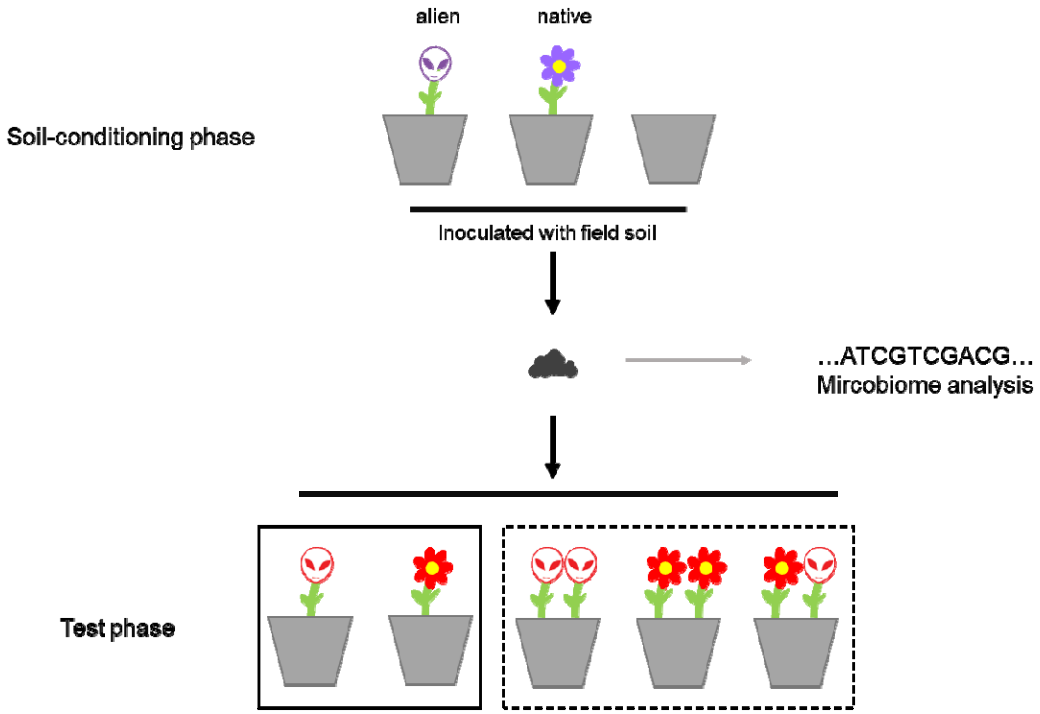

Figure 2 Graphical illustration of the experimental design. In the soil-conditioning phase, soil was conditioned by alien or native species, or not conditioned. Then, test species were grown on the soil without competition or with intra- or interspecific competition. Soil DNA samples were collected after soil conditioning, and sequenced for analysis of soil microbial communities. Competitive outcomes were calculated as differences between alien and native species in the test phase across competition treatments. Plants grown without competition (solid-line box) were used to test how soil-conditioning species directly affect the growth of test species (Fig. 1a). The differences between plants grown in competition (dashed-line box) and the ones grown without competition were used to test how soil-conditioning species affect the strengths of intra- and interspecific competition (Fig. 1b&c).

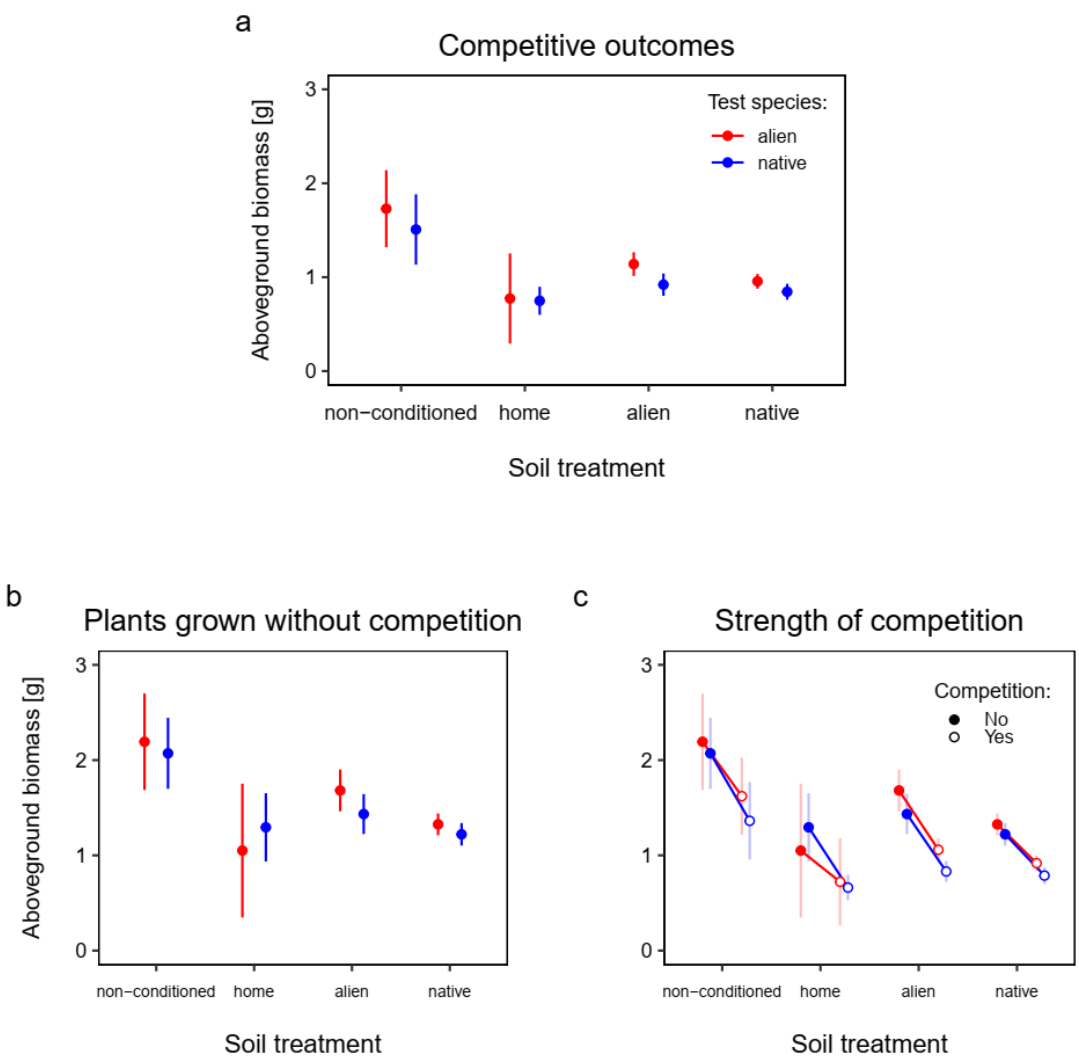

Figure 3 Effect of soil-conditioning treatments on aboveground biomass of alien (red) and native (blue) test species. a, mean values (± SEs) were calculated across competition treatments, and alien test species are considered more competitive than natives when they had a higher aboveground biomass. **b**, mean values were calculated based on plants grown without competition. **c**, Slopes indicate the strength of competition, that is, the difference between plants grown without competition (solid dots, the same values as in b) and in competition (open dots). For the soil-conditioning treatments, 'non-conditioned' refers to soil that was not conditioned by plants, 'home' to soil conditioned by the same species as the test species, and 'alien' and 'native' to soils conditioned by other species than the test species, and which were alien or native, respectively.

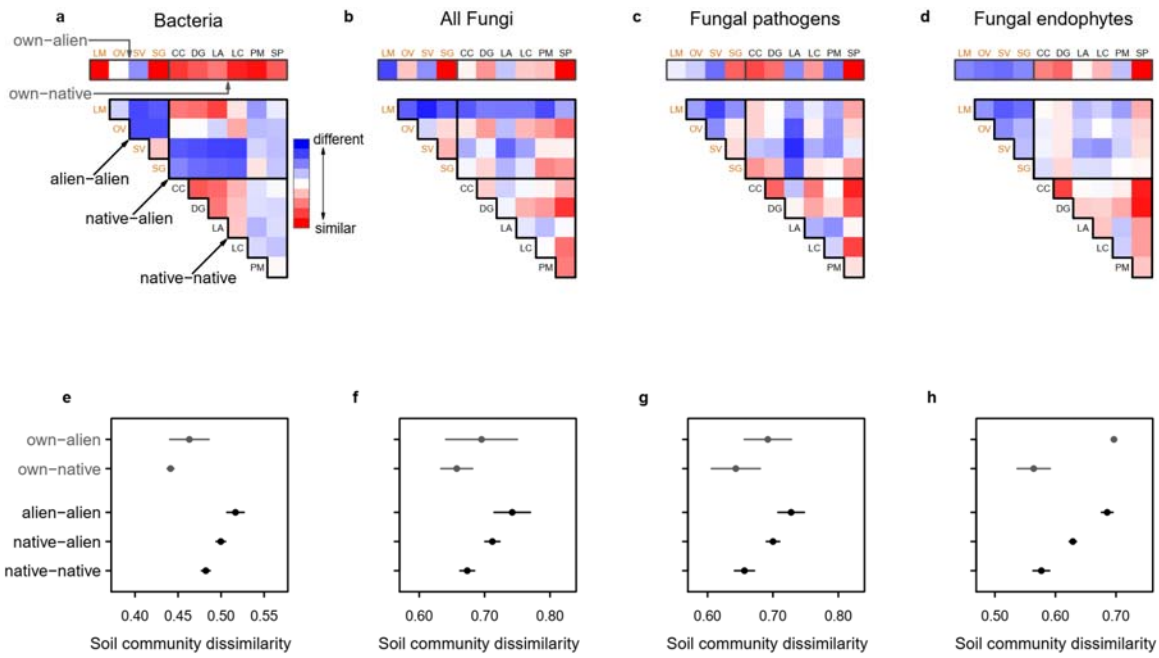

Figure 4 Dissimilarities of soil microbial communities within and between plant species. a & **e**, bacterial communities; **b** & **f**, fungal communities, **c** & **g**, fungal pathogen communities; **d** & **h**, fungal endophyte communities. The upper panels show the heatmaps of community dissimilarities of all within-species (top horizontal bars) and between-species combinations (triangular matrices), which are divided into five categories (own-alien, own-native, alien-alien, native-alien, native-native) with black borders. Labels at the top and along the diagonal provide abbreviations of species names (full names in Table S1) of aliens (orange) and natives (black). The lower panel shows the mean values (±SEs) of each of the five categories. Own-alien: between plants of the same alien plant species; own-native: between plants of the same native species; alien-alien: between plants of different alien species; alien-native: between plants of alien and native species; native-native: between plants of different native species.

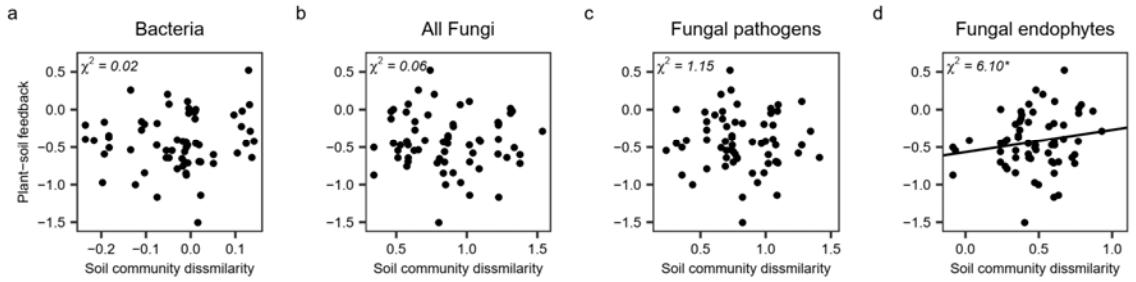

Figure 5 Relationship between soil-community dissimilarity and strength of plant-soil feedback. a, bacterial communities; **b**, fungal communities; **c**, fungal pathogen communities; **d**, fungal endophyte communities. Negative values of the strength of plant-soil feedback indicate that plants grow worse on conditioned soil than on non-conditioned soil. Soil-community dissimilarity was logit-transformed. A significant effect of community dissimilarity was found for fungal endophytes, as indicated by an asterisk.