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In their native range, invasive plants are held in check by negative soil-feedbacks

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Abstract. The ability of some plant species to dominate communities in new biogeographical ranges has been attributed to an innate higher competitive ability and release from co-evolved specialist enemies. Specifically, invasive success in the new range might be explained by release from biotic negative soil-feedbacks, which control potentially dominant species in their native range. To test this hypothesis, we grew individuals from sixteen phylogenetically paired European grassland species that became either invasive or naturalized in new ranges, in either sterilized soil or in sterilized soil with unsterilized soil inoculum from their native home range. We found that although the native members of invasive species generally performed better than those of naturalized species, these native members of invasive species also responded more negatively to native soil inoculum than did the native members of naturalized species. This supports our hypothesis that potentially invasive species in their native range are held in check by negative soil-feedbacks. However, contrary to expectation, negative soil-feedbacks in potentially invasive species were not much increased by interspecific competition. There was no significant variation among families between invasive and naturalized species regarding their feedback response (negative vs. neutral). Therefore, we conclude that the observed negative soil feedbacks in potentially invasive species may be quite widespread in European families of typical grassland species.

Key words: biotic interactions; enemy release; invasive species; native range; naturalized species; plant invasions; plant–soil feedbacks; soil inoculation; soil sterilization.

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INTRODUCTION

Ecosystems throughout the world are increasingly being threatened by human-mediated deliberate or accidental introduction of non-indigenous species (Sax et al. 2007). In many areas, non-native species have become invasive threatening native biodiversity and leading to economic loss (Mooney and Cleland 2001, Liao et al. 2007, Lin et al. 2007). Increased global trade and traffic, as well as species shifts as a result of global warming are major drivers of the introduction process. Results from recent studies suggest that plants that shift ranges towards higher latitudes and altitudes also include potential invaders (Engelkes et al. 2008, Beaumont et al. 2009, van Grunsven et al. 2010).

The potential of native range-expanders and non-native invasive species to become a dominant component of newly invaded ecosystems

might depend on biotic interactions (Joshi and Vrieling 2005, Suttle et al. 2007, Engelkes et al. 2008). Here, we define invasive species as species that have spread beyond their point of introduction in new ecosystems to become dominant (Valéry et al. 2008). In contrast, naturalized species as defined here have been introduced into new biogeographical ranges, where they are self perpetuating, but do not become dominant (Richardson et al. 2000). These two contrasting types may be viewed as extremes along a continuum from highly invasive species to species that are only naturalized.

Several hypotheses have been proposed and various studies conducted in an attempt to find traits that allow a species to become invasive rather than only naturalized. Among these, the enemy release hypothesis proposes that the success of invasive species can be explained by their release from natural enemies found in their native range (Keane and Crawley 2002). Recent studies that investigated interactions between plants and soil organisms observed that negative feedbacks play a significant role in plant performance in mixed grassland communities (Bever 1994, Bever et al. 1997, Klironomos 2002, Kardol et al. 2007, van der Putten et al. 2007, Petermann et al. 2008, Harrison and Bardgett 2010). Negative soil feedbacks can enhance species coexistence due to a form of a Janzen-Conell effect (Bever et al. 1997, Packer and Clay 2000, Petermann et al. 2008, McCarthy-Neumann and Kobe 2010). Kulmatiski et al. (2008) and Callaway et al. (2011) suggested that missing plant–soil feedbacks may explain why some non-native plants become invasive and dominant in a new biogeographical range whereas others do not. The advantage of invasive species over native species in new ranges may, in addition, be due to negative soil-feedbacks affecting the native, but not the invasive plants (Klironomos 2002, van Grunsven et al. 2007), although this effect may weaken over time in the new ranges as new enemies adjust to invasive species (Diez et al. 2010).

In the present study, we tested if soil-feedbacks within six plant families in the native range were more negative for species that have become invasive than for species that have become naturalized in new ranges. This would indicate that potentially invasive species are prevented from becoming dominant in their native range by negative soil-feedbacks. Once released from these feedbacks, these species have the potential to become dominant and invasive in new ranges. As the degree of negative feedback experienced in native soil may differ between early- and mid-successional plant species (Kardol et al. 2006), we classified our species into these two categories. Furthermore, we expected that negative soil-feedbacks might be more strongly expressed in interspecific competition (Petermann et al. 2008). We therefore grew the plants in two diversity treatments, one maximizing intra- and the other interspecific interactions. To test for negative soil-feedbacks, we grew plants either in sterilized soil to remove soil feedbacks and compared this with a control of sterilized soil re-inoculated with unsterilized native soil inoculum. As we only used populations from the native European range in our study, we only could detect invasion strategies that pre-adapt species to become dominant in new ranges.

Material and Methods

Species pool and seed origin

We searched within the central European grassland flora for families with species that are known to have become invasive or naturalized on other continents (Uva et al. 1997, Mack and Erneberg 2002, Mack 2003*a*, Cappuccino and Arnason 2006, USDA 2008, WEEDS 2008, ISSG 2009). We found six families with a total of 16 mid- or early-successional species that fulfilled the specification that they contained both potentially invasive and potentially naturalized species (Table 1). The species were classified as early or mid-successional according to Lauber and Wagner (1996). Seeds from the native range were obtained from the St. Gallen Botanical Garden (Switzerland) and from commercial providers of local genotypes (UFA, Winterthur, Switzerland; Rieger-Hoffman, Blaufelden-Raboldshausen, Germany; Appels-Wilde, Darmstadt, Germany; Secret seeds, Devon, UK).

Soil treatments

We mixed 50% sterilized sand with 50% sterilized germination soil (Ricoter; Aarberg, Switzerland) for seed germination and germinated plants. The plant growth medium in the pots was a mix of 50% sterilized sand with 50%

Table 1. Species list of grassland species native to Europe used in this study. Nomenclature follows Lauber and Wagner (1996) and APG III (2009).

Family	Naturalized mid-successional	Invasive early-successional	Invasive mid-successional
Asteraceae	*Bellis perennis*	*Hypochoeris radicata*	*Hieracium aurantiacum*
Caryophyllaceae	*Silene vulgaris*	*Stellaria media*	*Silene pratensis*
Lamiaceae	*Prunella vulgaris*	*Lamium amplexicaule*	*Glechoma hederacea*
Plantaginaceae	*Veronica officinalis*	*Veronica arvensis*	
Poaceae	*Anthoxanthum odoratum*	*Poa annua*	*Poa trivialis*
Ranunculaceae	*Ranunculus acris*		*Ranunculus bulbosus*

sterilized potting soil (BF 4, De Baat; Holland). The soil was sterilized by gamma radiation (max. 50 kGray, min. 29 kGray). These sterile mixes were used as a treatment to remove potential negative soil feedbacks originating from soil biota (Petermann et al. 2008). Before mixing, the soil and sand were left under sterile conditions to stabilize for four weeks after the gamma-radiation treatment. We inoculated half of the prepared sterile soil mix with a non-sterilized soil-inoculum at 4% of the total mass of the mix to represent the native grassland soil control. Bulked soil samples (0–5 cm depth) were taken from five ruderal sites, five grassland sites and two agricultural fields in north-eastern Switzerland for the soil inoculum. These soil samples were mixed, earthworms and roots were removed and the soil was sieved with a 2-mm sieve. In the subsequent text we refer to the two soil types as sterile and inoculated.

Germination conditions

Plants were grown in germination trays and then transplanted into pots (see below). Germination trays, pots and saucers were washed and sterilized with 70% ethanol. Seeds were surface-sterilized by soaking them in a 7% sodium hypochlorite solution for 3 min and then rinsing for 2 min with autoclaved water (Bartelt-Ryser et al. 2005). After a 7-day cold-stratification period at 4°C, seeds were placed on germination trays in the glasshouse on 8 February 2009. Each germination tray contained 150 cell plugs of 15 ml volume. These were filled with the two soil treatments, sterile and inoculated as described in the previous section. No nutrients were added. Seeds and seedlings were watered twice a day with autoclaved water. Seeds were germinated in a 10.5-h day regime with 14–19°C day- and 10–16°C night temperature.

Experimental setup

Four weeks after germination, on 7–11 March 2009, the sterile and inoculated soil plugs with the germinated seedlings were transferred from the germination trays into 28.5 cm diameter, 10 L pots containing the soil mix described above. If a plug contained more than one seedling, the additional seedlings were randomly removed. The experimental communities included monocultures and two different mixtures from the species pool. Monocultures consisted of 20 individuals of a single species together with 16 edge and one central individual of three grass species. These individuals were used as buffer plants to standardize edge effects. The 20 test individuals were planted at regular intervals within the pot (Fig. 1). The 16 buffer plants and the central plant were randomly chosen from the three grass species *Anthoxanthum odoratum*, *Poa annua* and *Poa trivialis*. We used mixes of the three grass species in all pots because in nature they commonly occur in the 'matrix' vegetation around the test species. Each monoculture species × soil treatment combination was replicated three times resulting in a total of 72 pots (12 species × 2 soil treatments × 3 replicates). For the families Ranunculaceae and Plantaginaceae germination was too low to yield enough seedlings for monocultures.

Mixed communities also consisted of 20 individuals equally distributed among five naturalized and five invasive species with 17 buffer plants. For a comparison between invasive plants of different successional status, two different mixtures were assembled: one containing two individuals each of all naturalized and early-successional invasive species and one containing two individuals each of all naturalized and mid-successional invasive species. Each mixture was replicated five times for each soil treatment resulting in a total of 20 pots (2 mixes × 2 soil treatments × 5 replicates). The individual species in the mixtures were randomly

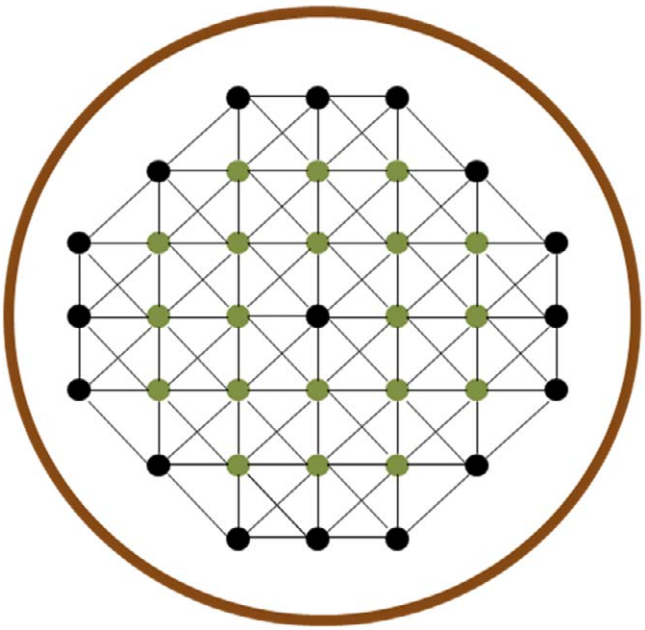

Fig. 1. Spatial arrangement of 20 experimental (green) and 16 buffer and central individuals (black) in large pots.

assigned to the 20 planting positions.

There were 92 pots and 1840 experimental individuals in total. Seven to eight pots were randomly placed on 12 trolley tables. The two different soil treatments were placed on separate tables to avoid cross-contamination, however, mixture and monoculture pots were mixed on single tables. Plants were manually watered three times a week with tap water to keep soil moisture constant. Each pot was placed on its own saucer to avoid contact with water from another mixture or monoculture. The plants were watered in the saucers to make sure that each pot was given an equal amount of water. Separate nozzles were used for each soil treatment to avoid contamination between treatments. Tables and pots within tables were randomized every two weeks. After transplanting, plants experienced natural day length and day temperatures of 14–19°C and night-temperatures of 12–17°C.

Measurements and harvest

Five weeks after germination (one week after transplanting the seedlings into the large pots) the number of leaves, excluding the cotyledons, was counted on all 1840 test individuals. Eight weeks after germination, the height of all 270 plant populations was measured. Separate measuring instruments and surgical gloves were used for each treatment group. All equipment was sterilized before and after use and stored in sealed plastic bags. Bacterial analysis of the soil was conducted during the experiment as a control to test if our experimental conditions were effective (Appendix: Table A1). Fourteen weeks after germination, when plants started to set seeds, all individuals were harvested at ground level and individually bagged. The aboveground biomass of all 270 populations was determined after drying at 70°C for 24 h. Roots were harvested, washed and stored with a drop of sterilized water in plastic bags at 4°C before staining with black ink and vinegar (see Appendix: Table A2) to test for the presence of arbuscular mycorrhizal fungi (Vierheilig et al. 1998) as a further control that our experimental conditions were effective.

Statistical analysis

Statistical analyses were conducted using the software products R, version 2.7.2 (R Development Core Team 2008), and GenStat, version 12 (VSN International 2010). General mixed models using residual maximum likelihood (REML) were fitted and results assembled in analyses of variance (ANOVA) tables (Payne et al. 2010). Significance tests were based on approximate *F*-

tests using appropriate error terms and denominator degrees of freedom, which can be fractional in REML analysis. The fixed terms in the models were soil treatment (sterile vs. inoculated), diversity (monoculture vs. mixture), invasiveness (invasive vs. naturalized species), and interactions among these. Table, pot within table, family, species within family and the interactions species within family × diversity and species within family × diversity × soil were used as random terms. To test for significant family × invasiveness × soil treatment interactions, family was treated as fixed effect in alternative models. However, because this three-way interaction was never significant the corresponding model results are not presented in tables. Similarly, the term successional status (early- vs. mid-successional species) was also tested in alternative models but omitted in the final analyses (except for the analysis of deviance for plant survival) because it had little explanatory power.

The effects of negative feedbacks were additionally tested as in Petermann et al. (2008): the log-ratio of mean number of leaves / biomass of each species in communities grown on inoculated soil was divided by the log-ratio of mean number of leaves / biomass of each species in communities grown on sterile soil to get a proportional measure of feedback. The log-transformation returns zero when there is no difference between inoculated and sterile soils, and negative values for negative feedbacks. The log-ratio was then used as the response variable in an ANOVA. The analysis of log-ratios uses less information because it is calculated from means and therefore the results from the analysis of the original data are more precise. The use of log-ratios however provides an easier illustration of the negative soil feedbacks because it reduces the complexity of the statistical analysis by one dimension, i.e., the soil treatment. We fitted general mixed models using residual maximum likelihood (REML). The fixed terms were diversity (monoculture vs. mixture), invasiveness (invasive vs. naturalized species), and the interaction among these. Family and species within family were used as random terms. To test for significant family × invasiveness interactions, family was treated as fixed effect in alternative models. However, as this two-way interaction was never significant, the corresponding model results are not presented in tables.

Results

Differences between invasive vs. naturalized species in sterile vs. inoculated soil

One week after transplantation into pots, invasive species had 30% more leaves in sterile than in inoculated soil whereas naturalized species had a similar number of leaves in the two soil types ($P = 0.034$ for interaction soil treatment × invasiveness; Table 2). The soil treatment × invasiveness interaction was also significant for plant height after 4 weeks and aboveground biomass after 10 weeks (Table 3). Invasive plants produced 31% more aboveground biomass in sterile than in inoculated soil whereas no such difference was found in naturalized species (Fig. 2).

There were no significant three-way interactions family × invasiveness × soil treatment in statistical models with family as fixed effect. This suggests that differences between invasive and naturalized species in their responses to soil treatments were similar for the six plant families.

Plant height was on average 71% greater in mixtures than in monocultures ($P = 0.003$ for diversity main effect in Table 3, Fig. 2). The effects of diversity (monocultures vs. mixtures) were not observed in the number of leaves or in biomass (Table 3). Invasive plants tended to have a higher mortality than naturalized plants in mixtures growing on inoculated soil, whereas no such difference was observed in monocultures or on sterile soil ($P = 0.052$ for three-way interaction in Table 4).

Analysis of negative soil feedbacks

Log-ratios of leaf number and of aboveground biomass between inoculated and sterile soil were significantly more negative for invasive than for naturalized species ($P = 0.007$ and $P = 0.032$ for main effect of invasiveness in Table 5; Fig. 3) indicating stronger negative soil feedbacks on invasive species. This difference was enhanced in mixtures, where invasive species had even more negative log-ratios for leaf number than in monocultures ($P = 0.018$ for two-way interaction in Table 5). Although there appeared to be some variation in the difference between invasive and naturalized species among the six families in

Table 2. Results of mixed-effects ANOVA for the number of plant leaves one week after transplanting plants into pots (numDf: degree of freedom of term, denDf: degree of freedom of error term [which can be fractional in REML analysis], *F*: deviance ratio, *P*: error probability with (*) indicating marginal significance and * indicating significance); $n = 1840$.

Variables	numDf	denDf	*F*	*P*
Diversity (D)	1	40.7	1.99	0.166
Soil treatment (T)	1	37.2	1.53	0.224
Invasiveness (I)	1	8.9	4.85	0.055(*)
D × T	1	39.3	0.23	0.632
D × I	1	32.2	1.67	0.206
T × I	1	31.1	4.91	0.034*
D × T × I	1	30.7	1.27	0.269

their log-response ratios (Fig. 3), the corresponding interaction family × invasiveness in statistical models with family as fixed effect was never significant.

Discussion

Invasive success in the new range might be explained by release from biotic negative soil-feedbacks, which control potentially dominant species in their native range. Hence, plant species that have become invasive might be held in check by negative soil interactions in their home range. To test the hypothesis whether invasive plants are indeed controlled by negative soil-feedbacks in their native range and may therefore potentially be released in new biogeographical ranges, we compared the performance of invasive with that of only naturalized species within six plant families in sterile soils representing the release from negative feedbacks and in non-sterilized, inoculated soils of the native range. Even though naturalized species have not been used so far in this context, we believe that they are a necessary control group to test the hypothesis (Cappuccino and Arnason 2006, van Kleunen et al. 2010).

We indeed found that European species that have become invasive in other parts of the world would have the potential to reach a higher abundance within ecosystems in the native range if their performance were not reduced by negative soil-feedbacks. This suggests that highly competitive plant species may coexist with less competitive plant species as a result of negative soil-feedbacks. The negative soil-feedback effect on plant growth was observed if sterilized soil was inoculated with unsterilized soil inoculum from the native range. This is consistent with the findings of Bever (2003), suggesting that highly competitive plant species can coexist as a result of negative soil-feedbacks. Whereas the invasive species in our experiment performed better in sterile compared with inoculated soil, the naturalized species did not. This suggests that naturalized species lack the potential to reach a higher abundance and become invasive if released from negative soil-feedbacks.

Differences in performance between invasive and naturalized species were already visible during early plant growth for leaf number and plant height as well as for biomass at the stage when most species were producing seeds and therefore the plants had to be harvested. On average, the advantage of invasive over naturalized species was three times larger in sterile than in inoculated soil. This indicates that the invasive species in our study had the potential to become dominant in new ranges where soils do not contain the specialized plant enemies controlling

Table 3. Results of mixed-effects ANOVA for plant height 4 weeks and for aboveground biomass 10 weeks after transplanting plants into pots (numDf: degree of freedom of term, denDf: degree of freedom of error term [can be fractional in REML analysis], *F*: deviance ratio, *P*: error probability with (*) indicating marginal significance and */** indicating significance); $n = 270$.

	Height				Biomass			
Variables	numDf	denDf	*F*	*P*	numDf	denDf	*F*	*P*
Diversity (D)	1	10.5	14.81	0.003**	1	10.4	3.29	0.099
Soil treatment (T)	1	24.7	0.2	0.657	1	23	7.97	0.01*
Invasiveness (I)	1	9	0.4	0.545	1	8.8	4.69	0.059(*)
D × T	1	54.7	0	0.997	1	30.7	0.34	0.567
D × I	1	9.8	0.62	0.451	1	11.2	2.4	0.149
T × I	1	23.9	5.08	0.034*	1	21.5	5.61	0.027*
D × T × I	1	51.6	1.45	0.234	1	29.6	0.42	0.521

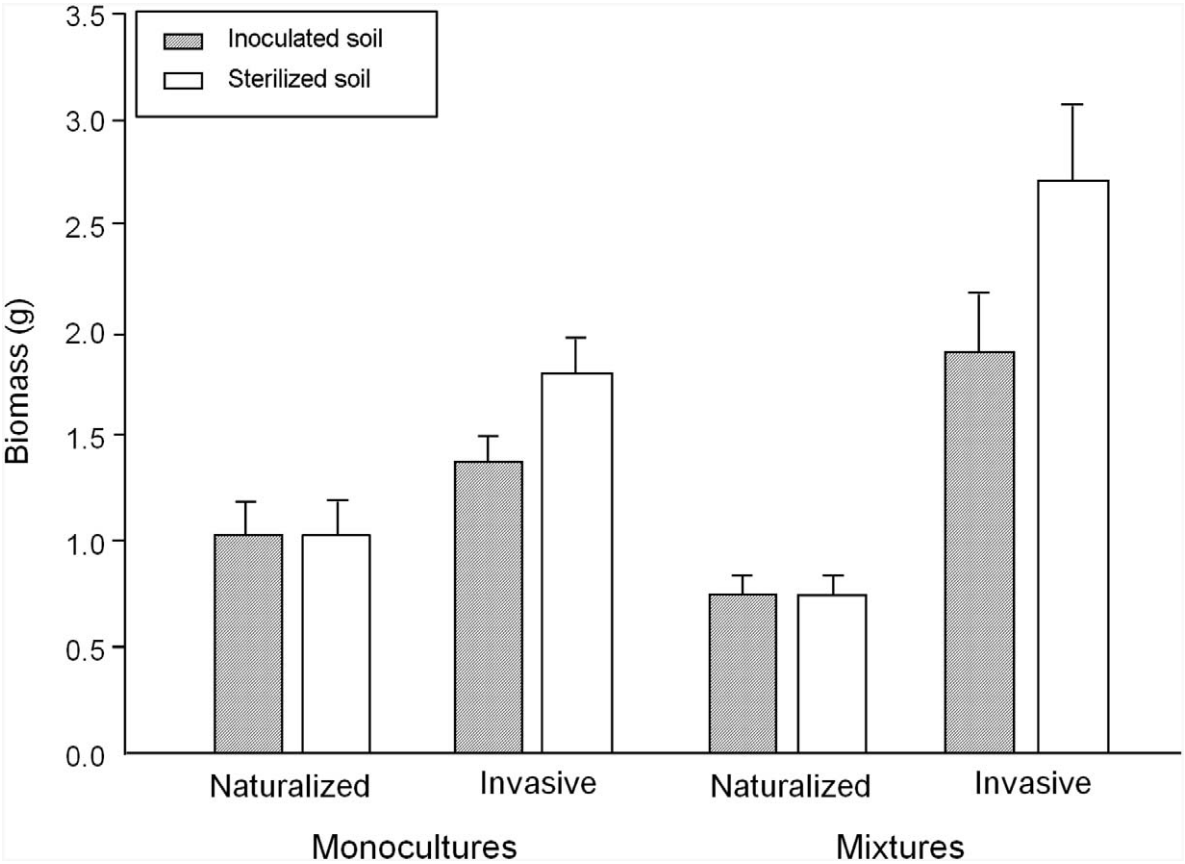

Fig. 2. Aboveground biomass (+ 1 SEM) 10 weeks after transplanting into pots. Treatments: monocultures vs. mixtures × naturalized vs. invasive species × inoculated vs. sterile soil.

these species in the native range. It suggests that these species are not dominant in their native range partially because they are held in check by negative soil-feedbacks. Bever (2002) and Callaway et al. (2011) observed that negative feedbacks could override beneficial effects of native-range arbuscular mycorrhizal fungi. This may be why the invasive species in our study did not show any benefit from soil mutualists in native range soil inocula. Moreover, the beneficial effects of many soil mutualists (e.g., mycorrhizal fungi and nitrogen fixing bacteria) decrease with increasing soil fertility and many soil mutualists decline in abundance in fertile soils (Kiers and van der Heijden 2006). Hence, in fertile soils, negative plant-soil feedbacks are more likely to occur. The use of a relatively fertile soil mixes in this experiment might have reduced the importance of such soil mutualists, thus enhancing the probability for negative plant–soil feedbacks. The low root-colonization levels of arbuscular mycorrhizal fungi found in this experiment (12%) did indeed indicate that the effects of these mutualists were low.

Table 4. Results of generalized mixed-effects ANOVA for number of plants surviving 10 weeks after transplanting plants into pots (numDf: degree of freedom of term, denDf: degree of freedom of error term [can be fractional in REML analysis], *F*: deviance ratio, *P*: error probability with (*) indicating marginal significance); $n = 270$.

Variables	numDf	denDf	*F*	*P*
Diversity(D)	1	27.9	0.16	0.695
Soil treatment (T)	1	24	0.12	0.729
Invasiveness (I)	1	8.4	0.26	0.624
D × T	1	29.8	0.91	0.348
D × I	1	21.6	0.77	0.391
T × I	1	19.3	0.51	0.483
D × T × I	1	20.1	4.28	0.052(*)

Table 5. Results of mixed-effects ANOVA for soil feedback (log-ratio of values in inoculated vs. sterile soil) calculated from mean log-transformed numbers of plant leaves one week and from mean log-transformed aboveground biomasses 10 weeks after transplanting plants into pots (numDf: degree of freedom of term, denDf: degree of freedom of error term [can be fractional in REML analysis], *F*: deviance ratio, *P*: error probability with (*) indicating marginal significance and */** indicating significance); $n = 32$.

	Log-ratio leaves				Log-ratio biomass			
Variables	numDf	denDf	*F*	*P*	numDf	denDf	*F*	*P*
Invasiveness (I)	1	14.2	10.15	0.007**	1	11	6.01	0.032*
Diversity (D)	1	12.1	1.19	0.296	1	16.2	0.08	0.782
I × D	1	12.3	7.48	0.018*	1	16.2	0.55	0.468

Our results not only support the empirically derived hypothesis; they are also in agreement with theoretical models. These predict that invaders, once released from negative soil feedbacks, may achieve higher population densities (Levine et al. 2006, Eppstein and Molofsky 2007) and may also profit from pathogen-control of potential competitors in the new range, enabling a large fitness differential compared with resident competitors (Turnbull et al. 2010).

No significant variation among plant families

It has often been pointed out that it is unlikely that there is a single strategy that can make plants globally invasive (Callaway and Maron 2006, van Kleunen et al. 2010). As phylogeny can influence the potential strategies species could evolve (Mack 2003*b*, Brandt et al. 2009, Cadotte et al. 2009), it is important to test general hypotheses across a number of species from different taxonomic groups. We replicated the comparison between invasive and naturalized species across six plant families. We did not, however, find any significant variation in the response of invasive species to the release from negative soil feedbacks compared to naturalized species among these families. It is conceivable that such variation was not detected because of low statistical power at the level of testing the corresponding three- or two-way interactions in the analysis of the original data or the log-response ratios, respectively (see Materials and methods). From the results as presented in Fig. 3 it appears that stronger support for our hypothesis came from the families Asteraceae, Lamiaceae and Caryophyllaceae. These are characterized by rapid growth if not controlled by enemies. Among them, Asteraceae contribute almost 40% of the world's weeds (Radosevich et al., 1997).

The three families which according to Fig. 3 apparently did not support our hypothesis as strongly (Plantaginaceae, Poaceae, Ranunculaceae) might depend more on soil mutualists or they might intrinsically invest more in defense and thus have a lower potential to invest in growth in the absence of enemies (Koricheva 2002, Joshi and Vrieling 2005). During the invasion process, they might have to evolve reduced defense and increased growth, and therefore have competitive potential (Blossey and Nötzold, 1995, Joshi and Vrieling 2005). As we deliberately analyzed populations from the native range in our study, we could only detect strategies that pre-adapt species to become dominant in new ranges. In the family Poaceae both naturalized and invasive species did not grow as well in sterile compared to in inoculated soil suggesting that they may have suffered from a loss of beneficial soil organisms, i.e., mutualists. Thus, in this case the invasive species may be associated with new mutualists in the new range. Again, this possibility was deliberately excluded in our study by using sterile soil rather than soil with a non-native inoculum.

Conclusion

"Since so many of the World's major invaders come from Europe" (Pyšek et al., 2004), the focus of our study on native European species is relevant to today's biodiversity crisis. The results of our study provide new evidence that some species, which are not particularly dominant in vegetation of native ranges, may be "pre-adapted" to become dominant and thus invasive in new ranges. These species are apparently held in check by negative soil feedbacks in the native range. Once they are no longer controlled, as in sterile soil or in new soils

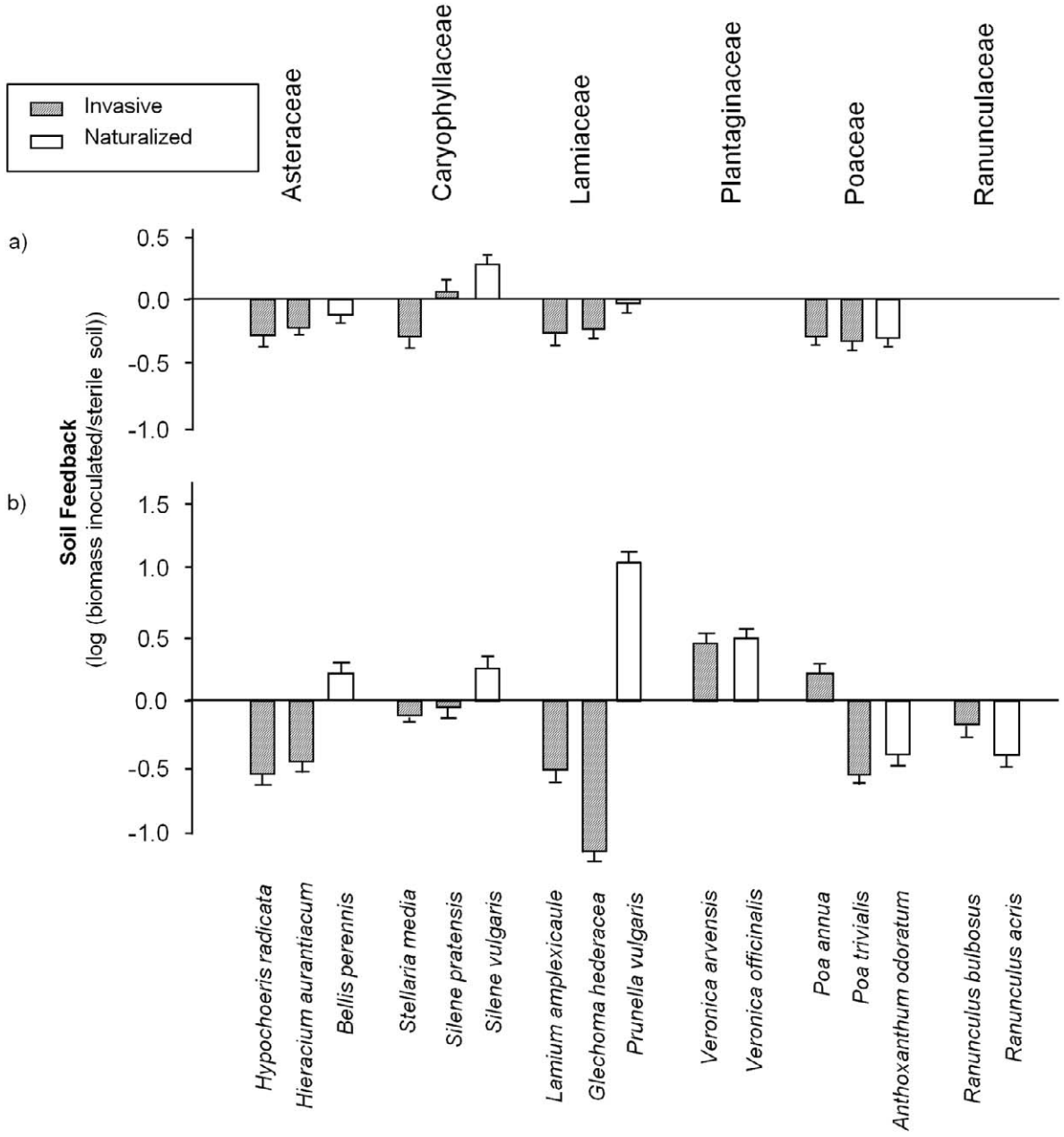

Fig. 3. Values for soil feedbacks (log-ratio of values in inoculated vs. sterile soil) calculated from mean log-transformed aboveground biomasses 10 weeks after transplanting plants into pots for invasive vs. naturalized species (hatched vs. open bars) + 1 SEM. Plants were grown in monocultures (a) or mixtures (b). The species are ordered according to family.

without specific enemies, they can thrive due to their intrinsically high growth capacity.

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Appendix

Supplementary methods

Inoculum controls.—The following soil samples were collected to control for bacterial growth in the different soil treatments: (1) sterile germination soil (treatment "sterile", 3 samples), (2) sterile germination soil with soil inoculum

(treatment "inoculated", 3 samples), (3) sterile and inoculated soil of large pots at harvest (15 samples per treatment "sterile" and "inoculated").

One-half gram of soil was added to 10 ml 0.9% NaCl solution. Attached bacteria were suspended in the solution by first mixing the solution using Vortex Genie Model K550.GE (Scientific Industries; New York, USA) followed with an ultrasonic bath (Transsonic 460/H, Elma; Singen, Germany). This solution was diluted 1×10^3 and 1×10^6, respectively, at the start of the experiment, and at harvest. An amount of 50 µl of each sample was spread on LB Agar (Atlas, 2005). The petri dishes were incubated at 30°C for 24 h after which the number of colony forming units (CFUs) was counted using a Scienceware (New Jersey, USA) colony counter to determine bacterial densities. After 12 further hours of incubation the number of CFUs was recounted.

The number of CFUs in inoculated germination soil contained significantly more CFUs than sterile germination soil ($F_{1,8} = 378$, $P < 0.001$; Table A1). CFUs grew in only two of the nine samples of the sterilized soil dilution. The fraction of CFUs in sterilized soil compared to inoculated soil indicated that the soil inoculum was successful in introducing soil bacteria into the treatment.

Further bacterial soil culture controls revealed that colonization increased in the sterile soil treatment during the course of the experiment, but always was lower than in the inoculated soil treatment (Table A1). At harvest, inoculated soil still had 28% more CFUs than sterile soil.

Roots were harvested, washed and stored with a drop of sterilized water in plastic bags at 4°C before staining with black ink and vinegar to test for the presence of arbuscular mycorrhizal fungi (Vierheilig et al. 1998). A thumb-top size sample of root was placed in a 15-ml centrifuge tube with 10 ml 10% KOH (Erne chemicals; Dällikon, Switzerland) to fully cover the roots. The roots were cleared in a water bath at 85°C for 30–35 min. Roots were rinsed several times with tap water. We added 10 ml ink solution (5% ink (Parker) and 95% white table vinegar (Migros Budget)) to the roots. The cleared roots in the ink solution were heated for 10–15 min in a water bath at 85°C. Roots were again rinsed in tap water. Arbuscular mycorrhizal fungal colonization was recorded when hyphae, vesicules or arbuscules were visible (Vierheilig et al. 1998). Arbuscular mycorrhizal fungi were found only in the roots of plants grown in sterile soil with an inoculum of native-range soils (Table A2).

Table A1. The number of Colony Forming Units (CFUs) assessed for a 0.5-g soil sample of (1) sterile germination soil, (2) sterile germination soil with inoculum, (3) sterile and inoculated soil of large pots at harvest.

Bacteria	Sterile soil mean	Inoculated soil mean	*P*
CFUs/g: 1, 2	3×10^5	6×10^7	<0.001
CFUs/g: 3	8×10^{10}	1×10^{11}	<0.001

Table A2. Mycorrhizal colonization for all plant species grown in monocultures to test for mycorrhizal infection.

AMF	Sterile soil	Inoculated soil	*P*
Not colonized	10	4	<0.001
Colonized	0	6	<0.001