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Invasive plants escape from suppressive soil biota at regional scales

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Summary

1. A prominent hypothesis for plant invasions is escape from the inhibitory effects of soil biota. Although the strength of these inhibitory effects, measured as soil feedbacks, has been assessed between natives and exotics in non-native ranges, few studies have compared the strength of plant–soil feedbacks for exotic species in soils from non-native versus native ranges.

2. We examined whether 6 perennial European forb species that are widespread invaders in North American grasslands (*Centaurea stoebe*, *Euphorbia esula*, *Hypericum perforatum*, *Linaria vulgaris*, *Potentilla recta* and *Leucanthemum vulgare*) experienced different suppressive effects of soil biota collected from 21 sites across both ranges.

3. Four of the six species tested exhibited substantially reduced shoot biomass in 'live' versus sterile soil from Europe. In contrast, North American soils produced no significant feedbacks on any of the invasive species tested indicating a broad scale escape from the inhibitory effects of soil biota.

4. Negative feedbacks generated by European soil varied idiosyncratically among sites and species. Since this variation did not correspond with the presence of the target species at field sites, it suggests that negative feedbacks can be generated from soil biota that are widely distributed in native ranges in the absence of density-dependent effects.

5. *Synthesis*. Our results show that for some invasives, native soils have strong suppressive potential, whereas this is not the case in soils from across the introduced range. Differences in regional-scale evolutionary history among plants and soil biota could ultimately help explain why some exotics are able to occur at higher abundance in the introduced versus native range.

Key-words: exotic species, grasslands, invasion, natural enemies hypothesis, negative soil feedback, plant–soil (below-ground) interactions, soil biota, soil pathogens

Introduction

Soil biota has important effects on the distribution and abundance of plant species and the structure and functioning of plant communities (Ehrenfeld, Ravit & Elgersma 2005; Callaway & Rout 2011; Mordecai 2011). Soil pathogens are a major component of these impacts because pathogens often suppress plant recruitment, growth and survival (Bell, Freckleton & Lewis 2006; Mangan *et al.* 2010), reduce the relative abundance of species in communities (Bever 1994; Klironomos 2002), mediate competitive interactions between species (Van der Putten & Peters 1997; Petermann *et al.* 2008), alter succession (Van der Putten, Van Dijk & Peters 1993; Kardol, Bezemer & Van der Putten 2006; Kardol *et al.* 2007) and decrease community productivity (Van der Heijden, Bardgett & Van Straalen 2008; Maron *et al.* 2011; Schnitzer *et al.* 2011). The suppressive effects of soil biota can amplify over time because plants often alter the composition and structure of soil microbial communities in ways that intensify the net negative impacts of all interactions between soil biota and a particular plant species (i.e. plant–soil feedbacks; Bever, Westover, & Antonovics 1997). Importantly, if these species-specific inhibitory effects of soil biota on native plants are commonly relaxed for exotic plant species, this mechanism might enable some exotic plants to become invasive (Agrawal *et al.* 2005; Kulmatiski *et al.* 2008).

One approach to examining the role of soil biota in exotic plant invasions has been to contrast the strength of plant–soil feedbacks among co-occurring native and exotic species (Van der Putten *et al.* 2013). These studies have repeatedly found that natives suffer from greater negative effects of soil biota than co-occurring exotics do (Klironomos 2002; Agrawal *et al.* 2005; Kardol *et al.* 2007; Kulmatiski *et al.* 2008; MacDougall, Rillig & Klironomos 2011) and that these negative feedbacks can affect competitive interactions among

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natives (Petermann *et al.* 2008; Pendergast, Burke & Carson 2013). Simulation models also suggest that differences in the strength of negative soil feedbacks experienced by resident natives and an invading exotic can lead to high equilibrium abundance of the invader (Turnbullo *et al.* 2010). Although some abundant exotic species can accumulate soil pathogens through time (Eppinga *et al.* 2006; Nijjer, Rogers & Siemann 2007; Diez *et al.* 2010), there appears to be a general inequality in the strength of plant–soil feedbacks between natives and exotics (Kulmatiski *et al.* 2008). Moreover, it is also the case that some invasive plants suffer from strong negative feedbacks where they are native (Zuppinger-Dingle *et al.* 2011; Yang *et al.* 2013).

While these studies have added much to our understanding of how soil biota might contribute to invasion, another approach is to explicitly compare the relative effects of biotic interactions or other processes on plants in their introduced *and* native ranges (Hierro, Maron & Callaway 2005). Of particular interest is whether plants are more self-limited in their native versus exotic ranges. In other words, do interactions with soil biota produce negative feedbacks on species in their native range, yet produce neutral or even positive feedbacks where these species are introduced? Although answering this question could shed light on whether altered biotic interactions are responsible for the spectacularly greater abundance of some invasives in invaded versus native communities, such biogeographical comparisons in the strength of plant–soil feedbacks remain rare.

Perhaps, the best documented case of strong biogeographical differences in the way soil biota has affected invasion success is that of *Prunus serotina*. In the native range of North America, *P. serotina* is strongly suppressed by pathogenic soil biota (Packer & Clay 2000, 2002; Reinhart *et al.* 2003). In the non-native range of Europe, where *P. serotina* forms stands that are roughly 10 times more dense than in North America, soil biota produce *positive* feedbacks that strongly contrast with negative soil-feedbacks in the native range (Reinhart *et al.* 2003, 2005). Reinhart *et al.* (2010) found that *Pythium* pathogens from the native range caused far more root rot and mortality of seedlings from both North American and European populations than *Pythium* from the non-native range. In studies with soil samples from just a few sites in both ranges, stronger negative effects of soil biota from the native ranges of exotic invasive species have been shown for *Centaurea stoebe* (Callaway *et al.* 2004a), *Acer platanoides* and *A. negundo* (Reinhart & Callaway 2004), *Robinia pseudoacacia* (Callaway *et al.* 2011a) and *Triadica sebifera* (Yang *et al.* 2013) but not for other species (Beckstead & Parker 2003; Andonian *et al.* 2011).

One impediment to studying the general importance of enemy escape for plant invasion in a biogeographical context is that it is logistically difficult to compare the effects of soil pathogens or herbivores on multiple plant species at many sites across both native and introduced ranges. Such widespread sampling is necessary, however, to obtain a representative picture of how generally suppressive enemies might be across the native range, and whether escape from these enemies is consistently advantageous across the introduced range. The few exemplary studies that have compared insect or pathogen impacts on the demography or performance of single plant species have examined these effects in just a handful of populations from the native and introduced range (Callaway *et al.* 2004b, 2011a; DeWalt, Denslow & Ickes 2004; Hierro, Maron & Callaway 2005; Williams, Auge & Maron 2010).

We explored biogeographical differences in the effects of whole soil biota on the performance of six perennial forb species that are widespread invaders in North America with strong local impacts. These six species occupy mesic grassland habitats in their native range in Europe and also invade semi-arid grasslands in North America. We sampled soil from 21 geographically dispersed sites across both the native and introduced range of these species (Table 1) and quantified the strength of plant–soil feedbacks. Our goal was to determine whether soil biota is generally suppressive in the native range and to contrast this with what occurs at uninvaded sites across the non-native range. Towards this end, we examined the following: (i) whether biogeographical patterns in the impacts of soil biota were consistent across multiple invasive species and (ii) the degree of variability in the strength of these effects across a substantial number of native and introduced sites.

Materials and methods

FOCAL SPECIES

We performed plant–soil feedback experiments using the following six plant species, all of which are widespread exotic invaders in grasslands in North America: *Centaurea stoebe* (Gugler) Hayek (Asteraceae), *Euphorbia esula* L. (Euphorbiaceae), *Hypericum perforatum* L. (Clusiaceae), *Linaria vulgaris* Mill. (Scrophulariaceae), *Potentilla recta* L. (Rosaceae) and *Leucanthemum vulgare* Lam. (Asteraceae). These species are all native to Europe and co-occur in similar grassland habitat in both ranges.

SOIL COLLECTION

In April to May 2008, we collected soil from 11 unmown grassland sites across Europe (native range) and 10 undisturbed grasslands across North America (non-native range; Table 1). All sites were native-dominated and were selected because (i) they represented suitable habitat for all of the focal species, and (ii) the focal species either did not currently occur at these sites, or if they did occur at a site only one of the six species was present and was uncommon. Our goal was to determine the potential for our focal species to promote feedbacks with soil collected from suitable habitat from each continent, but not from habitat in which soils had been exposed to target species and density-dependent accumulation of pathogens.

At each site, we used a bulb planter (6 cm diameter) to collect soil 'plugs' from the top 10 cm of soil. Soil samples were stratified across a large area at each site (approximately 5000 m^2) with an aggregate of 8 L of soil collected per site (which represented approximately 30–40 soil 'plugs'). After sampling a site, we rinsed the bulb planter with alcohol so that pathogens would not be transferred among samples taken at different sites. We subsampled half (4 L) of the bulk soil sampled from each site, placing this mixed pooled subsample in

Table 1. Location of soil collection sites in Europe and North America

Location	Country	State/Province	Range	Elevation	Latitude	Longitude
David's Valley	Romania		N	110	47.18	27.93
Perieni	Romania		N	164	46.26	26.63
Campalung Moldovanesc	Romania		N	626	47.52	25.55
Maramures	Romania		N	535	47.67	20.43
Hortobagy	Hungary		N	88	47.56	21.13
Brenner	Austria		N	1345	47.04	11.50
Partenkirchen	Germany		N	710	47.50	11.08
Puy de Valey	France		N	778	45.05	3.88
Moulins	France		N	211	46.52	3.33
Leoting	France		N	460	45.36	3.13
Clermont-Ferrand	France		N	422	45.46	3.08
The Dalles	USA	Oregon	I	100	45.63	−121.21
Lind	USA	Washington	I	417	46.97	−118.56
Rose Lake	USA	Idaho	I	661	47.52	−116.51
Southern Bitterroot	USA	Montana	I	1184	46.13	−114.04
Butte	USA	Montana	I	1688	46.01	−112.53
Clearwater	USA	Montana	I	1165	47.02	−113.36
Miles City	USA	Montana	I	722	46.41	−105.84
Devil's Lake	USA	North Dakota	I	443	48.11	−98.86
George Reserve	USA	Michigan	I	290	42.47	−84
Ontario-Guelph LTMRS	Canada	Guelph	I	334	43.54	−80.25

N: Native range of focal plant species; I: introduced range of focal species. Elevation in metres.

a Ziploc bag. Soil was kept cold and subsequently shipped on ice to the University of Guelph where feedback experiments were conducted.

FEEDBACK EXPERIMENTS, FIRST ROUND

For the first round of soil conditioning, for each focal plant species we inoculated 21 groups of 16 Deepot cells (Stuewe & Sons, Inc., Targent, OR, USA; 5 cm by 25 cm in size, 410 mL volume), with each group of 16 Deepots receiving inocula from one of the 21 soil collection sites (16 pots × 21 soil collection sites × 6 species = 2016 Deepot cells total). Since individual soil samples collected at each site were pooled, replicate containers containing soil from a given site were not 'true replicates'. Rather, these pots contained similar soil biota that was then subjected to replicate 'training' through time. Each cell was filled with a 300 mL homogenized mix of 20% sterile sand, 60% Turface (composed of pure calcinated clay, Profile Products, Buffalo Grove, IL, USA), and 20% sterilized soil (autoclaved soil collected from an old field near Guelph and used to attain appropriate soil texture and as a source of nutrients for the mix) with 10–20 mL of soil inoculum added to the top of this planting medium. We autoclaved field soil rather than using gamma irradiation (which we used to kill biota in soil previously trained prior to the second round of the experiment; see below) because this was logistically more convenient (and did not require the expense that gamma irradiation did). We then sprinkled a very thin layer of sunshine mix (a standard potting soil mix) on top of the soil inoculum to help keep the soil/inoculum mixture in each cell moist.

Seeds of each species were surface sterilized with dilute sodium hypochlorite solution, rinsed with water and plated onto 1.5% water agar petri plates. Seeds were then cold stratified at 4 °C for 3 weeks and germinated under artificial light and ambient temperature. After germination, three seedlings of the same species were transplanted to each replicate cell and subsequently weeded down to one seedling per pot.

Pots were randomized by treatment within the greenhouse (and re-randomized at monthly intervals), and plants were grown for 12 weeks under a light:dark regime of 14:10 h. The temperature in the greenhouse ranged between 16 and 18 °C/23–26 °C (night/day). We ceased watering approximately 2 weeks prior to harvest to dry soils so that plants could be more easily extracted from soil. Shoots were clipped, and replicates from each unique plant species/soil treatment combination were aggregated, homogenized (large root material removed, small fine roots chopped up but left in soil substrate), and divided into two portions. One portion remained non-sterile, and the other portion was gamma irradiated to 32 kGy. Soils were aggregated prior to irradiation because it was not feasible to irradiate many separate samples. Soils were then stored at 4 °C for approximately 4 weeks until the second round of soil training began. Gamma irradiation has advantages as a method of killing soil biota compared to autoclaving soil because, unlike autoclaving, it does not result in a large release of nitrogen (J. Klironomos, unpubl. data).

FEEDBACK EXPERIMENTS, SECOND ROUND

For the second round of soil training, we established 8 replicate Deepots for each soil location × sterilization x plant species combination. We filled each Deepot with soil substrate consisting of 8% sterile soil (collected from the same old field near Guelph as in the first round), 72% Turface and 20% sterile silica sand. To the top of this mixture, we added 70 mL of soil inoculum (either sterilized or unsterilized), and a layer of sterile sunshine mix (approximately 50 mL) was added to the top of the pot. We then placed surface sterilized and cold stratified seeds (sterilized and cold stratified as described above) of one of the respective focal species on the soil surface. After germination, we weeded these down to one individual per pot. Plants were well-watered and a low concentration of 20-20-20 fertilizer was added every 2 weeks.

Plants were grown for different periods of time to accommodate each species' unique growth rate (*Linaria* 78–85 days, *Euphorbia*

117–126 days, *Hypericum* 135–142 days, *Centaurea* 150–157 days, *Potentilla* 162–169 days and *Leucanthemum* 175–182 days) and then harvested. We obtained shoot biomass of each species and for *E. esula*, *L. vulgaris* and *H. perforatum* we also determined root biomass. For the remaining three species, it was not possible to accurately estimate root biomass because roots were so entangled with fine soil particles.

For each species except *C stoebe* and *L. vulgare*, we sampled a subset of the fine roots from each pot. These were washed free of soil, cut into 5 cm fragments and fixed in 60% ethanol. The root fragments were cleared (10 min in boiling 10% KOH) and then mounted on microscope slides using glycerol. Roots were assessed at 150 intersections at 100× magnification for any necrotic lesions on their outer surface (McGonigle *et al.* 1990).

ANALYSES

For each species, we calculated the mean shoot and (where applicable) root biomass of all individuals grown in sterile and live conditioned soil from each soil collection location. We also calculated mean per cent of root lengths with lesions for the species for which this was assessed. To determine whether soil feedbacks had inhibitory effects on plant size (or root lesions) and to assess whether this differed between soils collected from the native versus introduced range or between species, we performed separate three-way ANOVAS (using Systat 11.0) on these means, testing for effects of species, soil sterilization, continent (Europe versus North America) and their interactions on the following: (i) shoot biomass, (ii) root biomass and iii) per cent of roots with lesions (arc-sin square root transformed). We used mean values from each site because soil samples from each site were initially pooled. To further decompose the significant species × soil sterilization x continent interactions, we performed separate two-way ANOVAS for each species, testing the effects of continent, soil sterilization and the continent x sterilization interaction on shoot or root biomass. For the species showing significant suppressive effects of live European soil biota on shoot biomass, root biomass or root lesions, we compared shoot or root biomass or root lesions of plants grown in 'live' European soil versus 'live' North American soils. To do this, we ran two-way ANOVAS with continent and species (and their interaction) as factors.

For each species and soil location, we also calculated the strength of soil feedback as (Mean $\text{biomass}_{\text{live soil}}$-Mean $\text{biomass}_{\text{sterile soil}}$)/ Mean $\text{biomass}_{\text{sterile soil}}$. We then performed two-way ANOVAS on these feedback values (for shoot and roots separately), testing for effects of species, continent and the species x continent interaction. We used *post hoc* comparisons to examine the continent effect separately by species since the two-way interaction indicated that the magnitude of feedbacks differed among species. We used one-sample t-tests to examine for each species whether the magnitude of feedbacks were significantly different from zero both when that species was grown in European soil and North American soil. To determine whether there could be differences in nutrient status of soils from Europe and North America, we used two-way ANOVA to compare shoot biomass of plants grown in sterile North American versus sterile European soil. In this test, species and continent of soil origin were the main effects. Finally, for the four species for which there were significant negative feedbacks when grown in European soil, we used their mean feedback values for each European soil location to determine whether across locations from Europe, the strength of soil feedbacks were correlated among species. If the strength of feedbacks from soil collected from particular European sites was correlated among species, this would suggest a broader and more generalist effect of the community of soil pathogens in the native range. In contrast, if the strength of feedbacks across native sites were not correlated among our focal species, it would suggest that pathogenic biota were likely species specific.

Results

Across all species and sites, plants had reduced shoot ($F_{1,226} = 34.5$, $P < 0.0001$) and root ($F_{1,114} = 22.8$, $P < 0.0001$) biomass when grown in 'live' soil inocula versus sterile soil, and in European versus North American soil (shoots, $F_{1,226} = 53.6$, $P < 0.0001$; roots, $F_{1,114} = 17.25$, $P < 0.0001$). Shoot ($F_{5,226} = 270.7$, $P < 0.0001$) and root biomass ($F_{2,114} = 514$, $P < 0.0001$) also differed among focal species. Most notably, 'live' cultured soil from Europe had strong suppressive effects on plant size, whereas this was not the case for North American soil (continent × sterilization interaction, shoot biomass, $F_{1,226}$=40.2, $P < 0.0001$; root biomass, $F_{1,116}$=12.7, $P < 0.002$). This strong biogeographical difference in the impacts of soil biota on plant biomass was not consistent across all species (significant species x continent × soil sterilization interaction, shoot biomass; $F_{5,226} = 8.74$, $P < 0.0001$; root biomass; $F_{2,114} = 6.6$, $P < 0.003$).

To further decompose the significant species x continent x soil sterilization interaction, we ran separate two-way ANOVAS for each focal species. Four of the six focal species (*Centaurea*, *Euphorbia*, *Linaria* and *Potentilla*) had substantially reduced shoot biomass when grown in 'live' versus sterile European soil (See Appendix S1 in Supporting Information for Figure showing how species differed in growth responses to sterile versus live soil), but there was no reduction in plant size between 'live' and sterile North America soil (See Appendix S2 for statistical results). For two of the three species tested for root biomass (*Euphorbia* and *Linaria*), soil from the native range was much more inhibitory than soil from the non-native range producing a significant continent by sterilization effect (See Appendix S1 for Figure and S2 for statistical results). For the four species showing significant effects of soil sterilization on shoot biomass, the average size of plants in cultured 'live' European soil was always less than the average size of plants in cultured 'live' North American soil (Appendix S1). However, there were no differences in shoot (two-way ANOVA, $F_{1,113} = 0.76$, $P = 0.38$) or root (two-way ANOVA, $F_{1,57} = 0.16$, $P = 0.69$) biomass of plants grown in sterile European versus sterile North American soil, and this was consistent across species (i.e. no significant species by continent interaction, shoots, $F_{5,113} = 1.7$, $P = 0.14$; roots, $F_{2,57} = 2.0$, $P = 0.14$).

For the four species for which shoot biomass was significantly suppressed by live European soil biota, there were significantly fewer root lesions on plants grown in sterile versus non-sterile soil ($F_{1,152} = 90.1$, $P < 0.0001$). There were also more root lesions when plants were grown in European versus North American soil ($F_{1,152} = 21.6$, $P < 0.0001$). However, these effects varied by species (sterile x species interaction; $F_{3,152} = 17.2$, $P < 0.0001$; continent × species interaction:

$F_{3,152} = 7.6$, $P < 0.0001$). In separate tests among species comparing root lesions on plants grown in 'live' European versus 'live' North American soils, only *L. vulgaris* had more lesions when grown in European versus North American soil ($F_{1,19} = 12.7$, $P < 0.003$).

The strength and direction of soil feedbacks on shoot biomass differed significantly based on continent of soil origin ($F_{1,113} = 42.3$, $P < 0.0001$) and species ($F_{5,113}$=4.87, $P < 0.0001$) with the magnitude of the continent effect differing among species (Fig. 1; continent x species interaction, $F_{5,113} = 7.5$, $P < 0.0001$). Soil feedbacks were consistently negative and significantly less than zero for four of six species that showed negative effects of growing in 'live' European soil (Fig. 1), but in North American soils, average feedbacks were not significantly different from zero for any of the species tested (Fig. 1). Similar to feedbacks on shoot biomass, plant–soil feedbacks in European soil reduced root biomass, but this was not the case in North American soils (Fig. 1; $F_{1,57} = 10.1$, $P < 0.003$). This trend was marginally significantly different among species (Fig. 1; species × continent interactions $F_{2,57} = 2.8$, $P = 0.07$).

For the four focal species that showed significant negative feedbacks when grown in their 'home' soil, there was substantial variation in the strength of these feedbacks among the European soil sampling sites (Table 2). However, there was no correlation in the strength of these feedbacks among species for soil from any given site (Table 2). That is, sites with soil that was particularly suppressive (or not) for one species were not similarly suppressive (or not) in their effects on the other three species (Table 2).

Discussion

Our experiments revealed that four of six invasive plants tested experienced strong suppressive effects from the biota in their native European soil, and yet experienced no such effects in soil from their introduced North American soil (Fig. 1). Notably, these four species were also always smaller in 'live' European than 'live' North American soil. These results are unlikely to have been caused by underlying differences in soil nutrient status between North American and European soils, since plants did not differ in size when grown in sterile European versus sterile North American soil. Furthermore, we used a very small layer of soil inoculum in each pot and thus the effects of soil from different sites on the nutrient status of plants should have been minimal.

Our results are particularly striking given that soils were collected across a large number of sites spread across a substantial geographical area in both ranges (Table 1). Although biogeographical differences in the effects of soil biota on exotic plant performance have been determined for a few individual species, based on soil from a limited number of sites (Packer & Clay 2000, 2002; Reinhart *et al.* 2003, 2010; Reinhart & Callaway 2004; Andonian *et al.* 2011; Callaway *et al.* 2011a), this is the first demonstration of such widespread effects on a suite of invasive species.

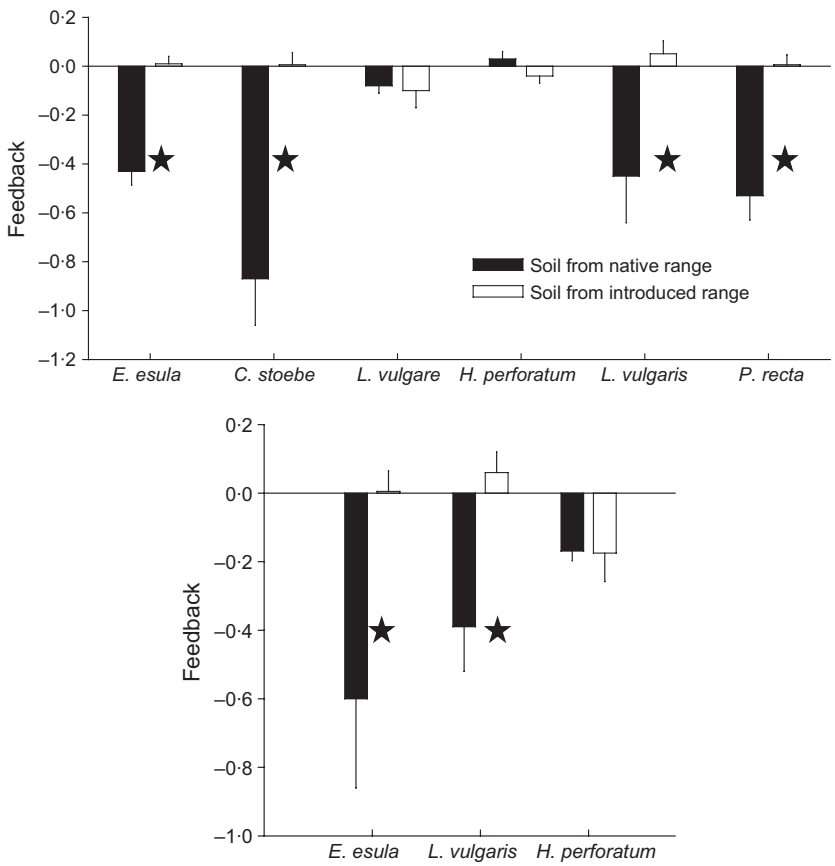

Fig. 1. Mean (±SEM) strength of soil feedbacks on top) shoot and bottom) root biomass when species were grown in soil inoculum from the native European range (black bars) and from the North American introduced range (white bars). Negative feedbacks indicate plants grew poorer in 'live' versus sterile soil biota (see Materials and methods for how feedback was calculated). Stars indicate species that show significant differences in the strength of feedbacks in European versus North American soils.

Table 2. (A) Mean strength of feedback when grown in soil from the 11 European sample sites for each of the 4 focal species that showed significant negative feedbacks in European soil. (B) Pearson correlation coefficients of the correlations among species in the mean strength of feedbacks experienced across all 11 sites

(A)

	Species			
	Euphorbia esula	*Linaria vulgaris*	*Potentilla recta*	*Centaurea stoebe*
Locations				
Campalung Moldovanesc	−0.97	−0.31	−0.75	−0.75
Hortobagy	−0.72	−2.06	−0.96	−0.64
Leoting	−0.76	−0.02	0.03	−0.61
David's Valley	−1.19	−0.88	−0.57	−0.60
Puy de Valey	−1.06	−0.098	−0.17	−0.36
Brenner	−0.40	−0.16	−0.36	−0.35
Partenkirchen	−0.10	0.24	−0.50	−0.35
Clermont-Ferrand	−0.78	−0.19	−0.34	−0.34
Perieni	−2.38	−0.74	−0.94	−0.30
Maramures	−0.07	−0.77	−0.92	−0.30
Moulins	−1.15	−0.02	−0.38	−0.13

(B)

	E. esula	*L. vulgaris*	*P. recta*	*C. stoebe*
E. esula	1.0			
L. vulgaris	0.15	1.0		
P. recta	0.17	0.70	1.0	
C. stoebe	−0.039	0.38	0.11	1.0

All values are non-significant ($P > 0.05$).

Given that we did not sample from the rhizospheres of our focal species, and indeed sampled from grasslands where our focal species were absent or very rare, it is even more striking that such large feedback effects were generated in European soil. These results suggest that inhibitory soil biota for a given species, and the evolutionary relationship between the species and soil biota, is not restricted to local communities but instead occurs over very broad regional scales. One possible explanation for this is that generalist pathogens are present in many locales, but at low abundances, and these pathogens can be enhanced through successive rounds of plant–soil feedback. For example, many fungal parasites/pathogens are also saprobes capable of living and reproducing in the soil as decomposers, but when a suitable host is present, they can also act as biotrophs and attack living plant roots. However, European sites where we found particularly large negative feedbacks on one of the four focal species (for which there were significant overall negative feedbacks of European soil on shoot biomass) were not necessarily sites where there were equally strong negative feedbacks on other focal species (Table 2). In fact, there were no significant correlations among sites in the strength of feedbacks across the four focal species. Thus, different plant species appear to culture particular agents in the soil biota that occur very broadly across regions but in a somewhat species-specific manner. For at least four of the six widespread species that we studied, it appears that negative soil feedbacks do not develop only in the context of local frequency-dependent processes.

Despite considerable variation among species in the strength of negative feedbacks when grown in native European soil, all six tested species showed consistently minimal impacts from soil feedbacks when grown in North American soils, a pattern also observed for a North American invader in Europe (Callaway *et al.* 2011a). The fact that feedbacks in North American soils were negligible is consistent with previous work showing that exotics may frequently leave pathogens behind when they are introduced to new regions (Mitchell & Power 2003 but see Parker & Gilbert 2007), and that exotic plants typically experience very minor negative soil feedbacks in their introduced range compared to natives in the introduced range (Kulmatiski *et al.* 2008). Range expanding species also appear to suffer from less negative soil feedbacks than do species that have not migrated in response to climate change (Van Grunsven *et al.* 2007; Engelkes *et al.* 2008). Together, these studies and ours suggest that evolutionary history between plants and soil biota can greatly determine the magnitude and direction of plant–soil feedbacks.

One dimension of this evolutionary relationship between plants and soil biota that might have influenced our results is the fact that we grew plants from seeds collected across the introduced range rather than from both ranges. If introduced genotypes have lost resistance to their native soil pathogens through rapid evolutionary change in the introduced range, the magnitude of negative feedback experienced by these plants grown in European soil might be greater than what would be observed had we used seeds from the native range.

To our knowledge, no study to date has tested whether there are differences in the magnitude of feedbacks experienced in native soil between native and introduced genotypes of invasive plants (but see Maron, Vilá & Arnason 2004). While there has been much work exploring whether plants introduced to new ranges have lost herbivore resistance (Bossdorf *et al.* 2005; Colautti, Maron & Barrett 2009), rapid loss of resistance to native soil pathogens remains untested.

Although our results support the 'enemy escape' hypothesis (Elton 1958), we lack several key pieces of evidence to fully test this idea. For example, we do not know the extent to which the suppressive effects of European soils we found in the greenhouse translate to meaningful reductions in key demographic parameters or plant densities in the field. Nor do we know how changes in particular demographic components that might occur due to negative interactions with European soil biota ultimately influence the abundance of our focal species in the native range. Given that the negative feedback effects of European soil biota on any given invasive species were quite variable among sites (Table 2), it would be interesting to determine the extent to which such site-specific effects might drive variation in the abundance of these plants in Europe.

If escape from suppressive soil biota at home does facilitate invasion abroad, this must be partly because co-occurring North American species are inhibited by their own native soil biota, whereas exotic invaders remain relatively unaffected (Agrawal *et al.* 2005; MacDougall, Rillig and Klironomos 2011) or even positively influenced (Klironomos 2002; Reinhart *et al.* 2003; Callaway *et al.* 2004b). Given our results, one might also assume that the effects of soil biota on native plants might be very site dependent, which might also help explain variation in invader abundance across introduced sites. Ultimately, asymmetry in the strength of interactions between soil biota and native versus invasive plants is very likely to give invaders a leg up in competition with natives, which could facilitate their competitive dominance (*sensu* Van der Putten & Peters 1997). There have been surprisingly few studies, however, comparing the strength of competition between invasives and surrounding vegetation in their home and introduced ranges. In the only field experiment of this type of which we are aware, Callaway *et al.* (2011b) showed that interspecific competition from surrounding vegetation had substantial negative impacts on *C. stoebe* biomass in its native range in Europe, but competitive effects were generally negligible in the introduced range of North American. Whether these strong biogeographical differences in the strength of competition are mediated by biogeographical differences in the negative impacts of soil biota on *C. stoebe* or interspecific competitors is not clear. Studies that examine whether competitive interactions between invaders and natives at home and abroad are potentially mediated by biogeographical differences in the strength of plant–soil feedbacks would be highly informative in this context. For native plants, recent work indicates that negative soil feedbacks can mediate competitive interactions (Reinhart & Callaway 2006; Kardol *et al.* 2007; Pendergast, Burke & Carson 2013).

We do not know the identity of the organisms in 'live' cultured European soil that caused negative impacts on plants, but other studies have implicated pathogenic soil fungi in causing negative feedback effects (Packer & Clay 2000; Klironomos 2002; Kardol *et al.* 2007). Since sterilization kills both mutualist biota (such as arbuscular mycorrhizal fungi) as well as pathogenic agents in the soil, our results are likely conservative in terms of estimating the net suppressive effects of soil biota. Furthermore, work that has attempted to tease apart specific components of the soil community have found that the effects of the community as whole are more consistent and explanatory than the component parts (Callaway *et al.* 2011a). Thus, the fact that we found positive effects on focal plants from sterilizing European soils indicates that these effects were likely driven by suppression of pathogenic biota.

Our data on root lesions only partially corresponded to patterns of plant growth in 'home' and 'away' soil. We generally found more lesions on roots when plants were grown in European versus North American soil, and sterilization reduced the percentage of assayed roots exhibiting lesions. However, only one species (*Linaria vulgaris*) had more root lesions in 'live' European versus 'live' North American soil. Although some pathogens would be expected to produce lesions on roots after attack (Johnson & Curl 1972; Bowen & Rovira 1976) many microbes that influence plant growth may not leave visible traces on plant roots. Thus, it may not be surprising that data on root lesions did not perfectly correspond with patterns of plant growth in sterile and 'live' soil.

It is becoming increasingly clear that native plants in a variety of systems suffer from strong negative soil feedbacks (Van der Putten, Van Dijk & Peters 1993; Kardol, Bezemer & Van der Putten 2006; Kardol *et al.* 2007; Kulmatiski *et al.* 2008; Mangan *et al.* 2010; Mordecai 2011; Reinhart 2012; Pendergast, Burke & Carson 2013). Our results bolster this growing literature, in that we found four of six European species are strongly suppressed when grown in European soil collected across a large geographical area. The fact that these species were not suppressed in North American soils provides relatively clear evidence that invasive plants can benefit from leaving suppressive elements of their soil biota behind when they are introduced to new regions. What is needed now are both detailed studies that identify the pathogenic agents responsible for negative feedback effects, coupled with more widespread studies where biogeographical differences in soil feedback effects are quantified, and where the greater ramifications of these differences in feedbacks on plant demography and ultimately plant abundance are determined.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Graphs showing mean shoot and root biomass of focal species grown in live cultured soil and sterile soil from Europe and North America.

Appendix S2. Two-way ANOVA table showing effect of continent of soil origin, soil sterilization and the soil origin x sterilization interaction on mean shoot and root biomass.