

Does relatedness of natives used for soil conditioning influence plant-soil feedback of exotics?

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Abstract The naturalisation hypothesis has been gaining attention recently as a possible mechanism to explain variations in invasion success. It predicts that exotic genera with native representatives should be less successful because of an overlap in resource use and of the existence of common specialised enemies. In this study, we tested whether native congenics have more negative impact on exotic species than heterogenics by increasing the effects of soil pathogens. We sampled soil in populations of three exotic species (*Epilobium ciliatum*, *Impatiens parviflora* and *Stenactis annua*) at sites with and without respective congeneric species. This soil was used as an inoculum for cultivating the first plant cohort, which included exotics, as well as native congenics and heterogenics. The conditioned soil was subsequently used for cultivating the second cohort of plants (exotics only). We found no consistent impact of relatedness of conditioning species on

exotic growth. Although soil conditioned by congeneric *E. hirsutum* had the largest reduction on the performance of *E. ciliatum*, the final biomass of *S. annua* was lowest when grown in soil conditioned by itself. There was no effect of stimulating species on the biomass of *I. parviflora*. In both experimental phases, performance of exotics was improved when cultivated with sterilised inocula, indicating the dominance of soil generalist pathogens. However, the biomass of *S. annua* was increased most by congeneric-stimulated inoculum from congeneric sites, suggesting a possible role for specialised symbionts. Our results suggest that variations in invasion success of at least some exotics may be affected by species-specific interactions mediated by the soil biota.

Keywords *Impatiens parviflora* · Naturalisation hypothesis · Phylogenetic relatedness · Plant invasions · Soil microbiota

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Introduction

At present, there is a strong interest in identifying the factors that determine which imported species are more likely to succeed and which of them will probably fail. Aside from other possible mechanisms, increasing attention is being paid to the effects of the phylogenetic relationships between exotics and colonised flora on invasion success. As previously proposed by Darwin (1859), this explanation assumes

that exotic species with native representatives should be less successful than those that do not have relatives in the native flora. The reduced success of related exotics is explained by a larger overlap in resource use among close relatives and by their common specialised enemies. Both processes may increase the biotic resistance of colonised communities against invasion (Levine et al. 2004; Strauss et al. 2006).

Darwin's naturalisation hypothesis is usually examined by linking the success of exotic species (e.g., expressed by their naturalisation probability or by the area colonised by them) to their phylogenetic similarity with the native flora. Such an approach was used by Strauss et al. (2006), who compared introduced invasive and non-invasive grasses in terms of their phylogenetic relatedness with native Californian grasses. Mack (1996), in his meta-analysis, investigated the relationship between the probability of naturalisation of introduced species and the presence of native congeners in flora found in six U.S. states. In both of these studies, the invasiveness or naturalisation probability of introduced species was associated with their lesser phylogenetic similarity with native floras. On the other hand, Duncan and Williams (2002), who analysed introduced species in New Zealand, found a higher naturalisation rate in introduced genera containing native species. Lambdon and Hulme (2006) compiled presence/absence records of congeners for all naturalised alien plant species found in islands of the Mediterranean basin and found no true influence from related natives on establishment success of alien species. Diez et al. (2008) found scale-dependent support for the naturalisation hypothesis in their study surveying data on naturalisation and abundance of exotic plants in the Auckland region: at a larger scale, the probability of naturalisation was positively correlated to the number of native species in a genus. Within habitats, however, exotic abundance was negatively related to native congeneric abundance.

Curiously, in spite of the increasing amount of research based on a correlative approach, there are very few studies that examined experimentally whether native related species reduce the performance of exotics in a new range more than non-related species (e.g., Dawson et al. 2009). This assumption was explored in studies whose main focus was not exotic species. Concerning competitive

interactions, a recent meta-analysis by Cadotte et al. (2008) indicated more intense competition between closely related species, but a study by Cahill et al. (2008) did not. With respect to specialised enemies, more closely related plant taxa are more likely to share a more similar community of specialised herbivores or pathogenic fungi (Becerra and Venable 1999; Novotný et al. 2006; Gilbert and Webb 2007). Cross-infection of closely related species by pathogens was also used to explain the differences in seedling mortality in a tropical forest (Webb et al. 2006). Webb et al. (2006) showed that phylodiversity had a positive effect on seedling survival at a 36 m² scale and a 4 m² scale, indicating that the survival rate of seedlings is enhanced if heterospecific seedlings in a neighbourhood are not closely related.

Soil microorganisms are a significant group of biota with a regulatory impact on plants. They may influence plant performance directly, but very often, their impact can be modulated by conspecific or heterospecific plants. This impact has been shown repeatedly by plant-soil feedback experiments, a technique consisting of cultivating two "plant generations." In this technique, the first plant generation is used to condition the soil, which is subsequently applied to the cultivation of the second plant generation. This technique is based on two assumptions (Kulmatiski et al. 2008); first, that plants cause species-specific changes to soil microbial communities; and second, that plants show species-specific responses (plant-soil feedback) to these changes.

It has been demonstrated that conditioning by conspecifics leads to a negative plant-soil feedback when compared to conditioning by heterospecifics (van der Putten et al. 1993; Klironomos 2002), which documents the accumulation of specialised pathogens during the first experimental phase. Negative plant-soil biota feedbacks appear to be predominant in natural systems; they serve as a frequency-dependent regulatory mechanism that helps to maintain plant species diversity according to the Janzen-Connell concept (Janzen 1970; Connell 1971; reviewed by Reinhart and Callaway 2006).

In exotic species, plant-soil feedbacks have been shown repeatedly to be less negative or even positive (Klironomos 2002; Callaway et al. 2004; van Grunsven et al. 2007). These effects can be explained by the absence of specialised soil biota with negative impacts and by stimulating generalist symbiotic

biota. What remains untested, however, is whether native species that differ in their relatedness to exotics also differ in their influence on the soil microbial community and therefore in their effects on exotic plants (but see Brandt et al. 2009). Specifically, in accordance with the naturalisation hypothesis, we assumed that more negative plant-soil feedbacks in exotics will be found if the soil is preconditioned by congenics compared to soil preconditioned by heterogenerics. To test this assumption, we carried out a plant-soil feedback experiment with three exotic species that have established themselves successfully in Central Europe, namely *Epilobium ciliatum*, *Impatiens parviflora* and *Stenactis annua*. Field surveys (Dostál, unpublished) have revealed that sites occupied by these exotics may differ in the phylogenetic structure of the plant community; specifically, exotic populations grow with or without the presence of congenics. In this paper, we reported on the results of the effect of the soil microbial community harvested at sites with and without congenics and on the relatedness of native plants used for the soil conditioning on the growth of exotics. We aimed to answer the following questions:

- 1) Does an inoculum sampled at sites with congenics have a more negative impact on exotic growth than an inoculum sampled at congeneric-free sites?
- 2) Is exotic growth reduced more in soil conditioned by congenics than by heterogeneric species? Do conditioning species and the origin of an inoculum interact to influence exotic growth?
- 3) Using soil conditioned by exotics themselves, is their performance improved in comparison to their growth in soil conditioned by native species?

Methods

Species

We studied the effect of the soil microbial community on three exotics, specifically *Epilobium ciliatum*, *Impatiens parviflora* and *Stenactis (Erigeron) annua*. *E. ciliatum* (Onagraceae) is native to North America

and Eastern Asia, and it was introduced to Central Europe in 1960 (Pyšek et al. 2002). It grows in a variety of habitats, including degraded grasslands, disturbed areas and roadsides. *I. parviflora* (Balsaminaceae) is native to Central Asia and was introduced to Central Europe in 1870 (Pyšek et al. 2002) where it is very common now (Sádlo et al. 2007). It grows mainly in the forest understory. *S. annua* (Asteraceae) is native to North America and was introduced to Central Europe in 1882 (Pyšek et al. 2002). It occupies disturbed habitats, degraded grasslands, rock outcrops and roadsides. *S. annua* is naturalised in the Czech Republic; the other two exotics are classified as invasive (Pyšek et al. 2002).

We were interested in how the effects of a soil microbial community are modulated by native species that differ in their relatedness to respective exotic species. Therefore, we used two natives, a congeneric and a heterogeneric, for each exotic species. All three species were observed to grow in an identical habitat type (Dostál, unpublished). In the case of *Epilobium ciliatum*, we used *Epilobium hirsutum* and *Achillea millefolium* (Asteraceae), for *Impatiens parviflora*, we used *Impatiens noli-tangere* and *Galeopsis speciosa* (Lamiaceae), and for *Stenactis annua*, we used *Erigeron acer* and *Geum urbanum* (Rosaceae).

Soil sampling

In May 2008, soil samples were collected at six sites for each exotic species, half of them in the presence of the exotic only and the other half in the presence of the exotic and its congeneric (see Table 1). The presence of heterogenerics were not considered in the soil sampling scheme. Only sites with exotic populations larger than 100 individuals and a minimum density of 1 plant/m² were included; the same criteria were applied for congeneric populations. It was important to ensure that habitat types did not differ consistently for an environmental variable across site types in a way that could influence the composition of the soil microbial community. To test this assumption, we compared both habitat types in terms of species composition as a proxy for site environmental characteristics. There were no significant differences in species composition between both site types, as demonstrated by a Canonical Correspondence Analysis [F -ratio = 1.12, P = 0.172; number of permutations = 499 with block-constrained permutations

Table 1 An overview of sites where the soil inoculum was sampled for the first experimental phase (soil conditioning)

Species	Site name	Co-ordinates	Habitat	Co-occurrence of congeneric
<i>Epilobium ciliatum</i>	Jablečno	N49°53', E13°46'	Wet meadow	Yes
<i>Epilobium ciliatum</i>	Pojbuky	N49°30', E14°54'	Wet meadow	Yes
<i>Epilobium ciliatum</i>	Otradovice	N49°39', E14°41'	Wet meadow	Yes
<i>Epilobium ciliatum</i>	Jankovská Lhota	N49°40', E14°43'	Wet meadow	No
<i>Epilobium ciliatum</i>	Ondřejka	N49°29', E14°56'	Wet meadow	No
<i>Epilobium ciliatum</i>	Mračl	N49°50', E14°42'	Abandoned arable field	No
<i>Impatiens parviflora</i>	Okrouhlice	N49°46', E14°44'	Mixed forest	Yes
<i>Impatiens parviflora</i>	Křešice	N49°48', E14°50'	Mixed forest	Yes
<i>Impatiens parviflora</i>	Líšno	N49°44', E14°42'	Mixed forest	Yes
<i>Impatiens parviflora</i>	Průhonice1	N49°60', E14°34'	Mixed forest	No
<i>Impatiens parviflora</i>	Struhařov	N49°46', E14°48'	Mixed forest	No
<i>Impatiens parviflora</i>	Chvojen	N49°47', E14°39'	Mixed forest	No
<i>Stenactis annua</i>	Sulice	N49°56', E14°33'	Abandoned arable field	Yes
<i>Stenactis annua</i>	Mračl	N49°50', E14°42'	Abandoned arable field	Yes
<i>Stenactis annua</i>	Mrač2	N49°50', E14°42'	Abandoned arable field	Yes
<i>Stenactis annua</i>	Mrač3	N49°50', E14°42'	Annual grassland on rock outcrop	No
<i>Stenactis annua</i>	Průhonice2	N49°60', E14°34'	Abandoned arable field	No
<i>Stenactis annua</i>	Senohraby	N49°53', E14°42'	Abandoned cherry-tree plantation	No

Co-occurrence of congeneric indicates sites where both the exotic and its related native species were present. In case of *Impatiens parviflora*, the congeneric was *Impatiens noli-tangere*, for *Epilobium ciliatum* it was *Epilobium hirsutum*, and for *Stenactis annua* it was *Erigeron acer*

(on exotic species)]. Therefore, we assumed that the habitat types did not differ consistently from each other in environmental characteristics.

At each site, we applied a systematic raster sampling to obtain soil samples. The soil was sampled at 40 locations along 5 transects, with a distance of 5 m between transects and between locations along the transects. At each location, a soil sample of 5 × 5 × 5 cm was taken, giving a total volume of approximately 5 L of soil substrate from each site. The soil was then transferred to the lab and sieved through a 5-mm mesh to remove stones and coarse roots. The soil was homogenised within the sites and used as an inoculum. Each pot (volume = 1 L) used in the first experimental phase (see below) received a mixture (in a ratio of 1:6) of soil substrate collected in the field and steam-sterilised river sand.

Soil conditioning (first experimental phase)

To analyse whether the origin of the soil inoculum (from habitats with and without a congeneric) could

influence the growth of exotic species, each exotic species was grown in 30 pots (5 pots with soil from each source site, with one plant per pot) with an unsterilised inoculum. The same number of pots was prepared with an inoculum sterilised by gamma irradiation (45 kGray) that eliminated all soil biota (McNamara et al. 2003). To avoid the confounding effect of nutrient release after the sterilisation treatment (Troelstra et al. 2001; McNamara et al. 2003; van Grunsven et al. 2007), pots with sterilised and unsterilised inoculum were fertilised weekly by a 0.25-strength Hoagland nutrient solution (Hoagland and Arnon 1950).

In addition to exotics grown with an unsterilised inoculum that were used for conspecific soil conditioning, another two groups of plants were used for soil conditioning; these were congenics and heterocongenics. These plants were processed using the same method as for the exotics. Each of the two native species associated with their respective exotic (see *Species*) was grown in 30 pots (5 pots per site of inoculum source), with one plant per pot, and an

unsterilised inoculum that was mixed with steam-sterilised river sand (in a ratio of 1:6). The pots were fertilised weekly with 0.25-strength Hoagland nutrient solution. The plants were cultivated for 8 weeks from early June to early August 2008, when they were harvested and divided into groups of above- and below-ground biomass. After drying to a constant weight (at 70°C for 48 h), the biomass was weighed. Soil from each pot was kept separately in plastic bags in the fridge for 1 week at a temperature of 5°C.

Plant-soil feedback (second experimental phase)

In this experimental stage, a new cohort of the exotic plants was grown in soil conditioned by the conspecifics, congeners and heterogenerics.

Plants of each exotic species were grown in pots with sterilised and unsterilised inocula from the first experimental phase. The inoculum was obtained by mixing the substrates from the pots such that all combinations of conditioning species and source site were produced. Part of the inoculum was then sterilised by gamma irradiation (45 kGray). Each pot contained inoculum mixed with steam-sterilised river sand (in a ratio of 1:6) and one individual of each respective exotic. There were 180 pots in total for each exotic species (2 habitat types \times 3 sites \times 3 species used for conditioning \times 2 sterilisation treatments \times 5 plant replicates).

The plants were cultivated for a period of 10 weeks from mid-August until late October 2008. Throughout the experiments, the plants were fertilised weekly with 0.25-strength Hoagland nutrient solution. At the end of October, the plants were harvested and divided into above- and below-ground biomass. After drying to a constant weight (at 70°C for 48 h), the biomass was weighed.

During both of the experimental phases (soil conditioning and feedback), the pots were kept in a greenhouse and their position was completely randomised at the beginning of the experiment. The experimental plants were grown from seeds sown directly in pots or by planting the seedlings obtained by germination on sterilised sand. Seeds used in the experiment were harvested from multiple mother plants in 2007 at soil-sampling sites and then pooled. During the second experimental phase, 11 plants of *E. ciliatum*, 8 plants of *I. parviflora* and 11 plants of *S. annua* died before harvest and were excluded from the analyses.

Statistical analyses

For both experimental phases, we analysed the total biomass of the exotics as the response variable. The output of these analyses is described in the Results section. We also performed identical analyses with shoot and root biomass, and these can be found in the Supplementary Material section.

In the first experimental phase, mixed-effects ANOVAs were performed with the fixed factors being sterilisation, soil origin (two habitat types with and without congeners) and their interaction. The sites were nested within soil origin and were entered as a random factor. Analyses were performed separately for each exotic species. The data were further merged and an analysis was done for all of the species together. In this case, the identity of the exotic species was included as an additional random factor.

In the second experimental phase, mixed-effects ANOVAs were performed with the fixed factors being sterilisation, soil origin (two habitat types with and without congeners), conditioning species and their interactions. The sites were nested within soil origin and were entered as random factor. The analyses were performed separately for each exotic species. Further analysis was done with data merged from all species. In this case, the fixed factor “conditioning category” refers to one of three species categories used (conspecifics, congeners and heterogenerics). Random factors in the overall analysis were the identity of exotic species, identity of conditioning species nested within a category and sites nested within soil origin.

Significant effects (in the case of fixed factors) were further examined by single-factor post-hoc comparisons (Student Newman-Keuls tests) to determine which specific treatment combinations were different from the others.

All analyses were done by STATISTICA 8.0, StatSoft Inc.

Results

In the first experimental phase, sterilisation of the soil inoculum significantly improved the plant growth of all three species. In *E. ciliatum*, total biomass harvested from pots with sterilised inoculum after

soil conditioning was 40% higher than biomass of plants from pots with unsterilised inoculum. In *I. parviflora* and *S. annua*, this difference was 62 and 22%, respectively (Table 2; Fig. 1). Within each species, the negative effect of the inoculum was independent of its origin; i.e., the presence of a congeneric at the site did not influence the impact of soil biota on exotic performance, nor was the interaction between soil origin and sterilisation significant. Aside from sterilisation treatment, growth also differed between individual species according to the overall test (Table 2). The pattern obtained for total biomass did not differ from the output of analyses that used root and shoot biomass as response variables. The only exception was shoot biomass of *Stenactis annua*, which was not influenced by sterilisation treatment (Supplementary Material, Table S1).

In the second experimental phase, inoculum sterilisation again improved plant performance. *E. ciliatum*, *I. parviflora* and *S. annua* plants grown in pots with sterilised inoculum were 51, 27 and 28% larger, respectively, than plants grown with unsterilised inoculum (Table 3; Fig. 2). In *E. ciliatum* and *S. annua*, total biomass depended on the conditioning species, and it interacted with sterilisation (in *E. ciliatum* this interaction was only marginally significant). In *E. ciliatum*, feedback was the most negative if the soil was conditioned by a congeneric. *S. annua* had the worst performance when it grew in soil that was conditioned by itself (Fig. 2).

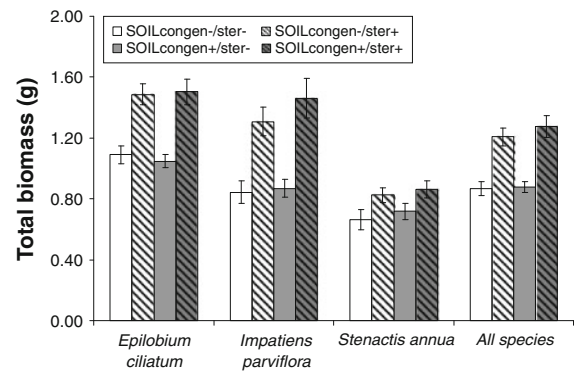


Fig. 1 Total biomass (g; mean \pm SE) of three exotics harvested after the first experimental phase. The plants were grown in pots with a soil inoculum originating from both site types, with a congeneric (SOILcongen+) and without (SOILcongen-). The inoculum was treated by sterilisation (ster+) or left untreated (ster-)

The significant effect of conditioning species was most likely due to changes in the soil microbial community and not due to allelopathy or nutritional changes. It was indicated by post-hoc comparisons showing that the identity of conditioning species induced differences in total biomass of target species, but this only occurred in plants grown with unsterilised inoculum (Fig. 2).

Further, sterilisation \times soil origin influenced the growth of *S. annua* that was most inhibited by unsterilised inoculum harvested at sites in the absence of congenics (Table 3; Fig. 3a). There

Table 2 Results of a mixed-effects ANOVA showing the effects of sterilisation, soil origin (from habitats with and without congenics) and their interaction on total biomass of exotics in the first experimental phase

Effect	<i>Epilobium ciliatum</i>			<i>Impatiens parviflora</i>			<i>Stenactis annua</i>			All species		
	df, ddf	<i>F</i>	<i>P</i>	df, ddf	<i>F</i>	<i>P</i>	df, ddf	<i>F</i>	<i>P</i>	df, ddf	<i>F</i>	<i>P</i>
Fixed terms												
Sterilisation	1, 52	38.27	<0.001	1, 52	32.28	<0.001	1, 52	9.26	0.004	1, 160	68.98	<0.001
Soil origin	1, 4	0.09	0.783	1, 4	1.21	0.334	1, 4	0.41	0.555	1, 11.35	1.08	0.321
STERIL \times ORIGIN	1, 52	0.06	0.801	1, 52	0.11	0.741	1, 52	0.25	0.618	1, 160	0.00	0.969
Random terms												
Species	–			–			–			2, 15.33	52.11	<0.001
Site (Soil origin)	4, 52	0.46	0.766	4, 52	0.66	0.625	4, 52	2.33	<i>0.068</i>	14, 160	1.08	0.379
Error	52			52			52			160		

The factor “Site” was nested in “Soil origin” in the analysis. Results are given separately for each exotic species and for all species together. In this case, the additional random term “Species” refers to the identity of exotic species. Significant effects are in bold ($P < 0.05$) and marginally significant effects are in italics ($P < 0.10$)

Table 3 Results of a mixed-effects ANOVA showing the effects of sterilisation, conditioning species, soil origin (from habitats with and without congeners) and their interactions on total biomass of exotics in the second experimental phase

Effect	<i>Epilobium ciliatum</i>			<i>Impatiens parviflora</i>			<i>Stenactis annua</i>			All species		
	df, ddf	<i>F</i>	<i>P</i>	df, ddf	<i>F</i>	<i>P</i>	df, ddf	<i>F</i>	<i>P</i>	df, ddf	<i>F</i>	<i>P</i>
Fixed terms												
Sterilisation	1, 154.77	16.21	<0.001	1, 157.42	33.41	<0.001	1, 155.42	3.50	0.063	1, 220.84	18.49	<0.001
Soil origin	1, 3.99	0.12	0.747	1, 3.85	1.67	0.269	1, 3.99	0.26	0.640	1, 13.88	0.31	0.587
Conditioning species/ category ^a	2, 154.20	3.32	0.039	2, 157.33	0.96	0.386	2, 154.41	5.85	0.004	2, 4.03	0.34	0.729
STERIL × ORIGIN	1, 154.38	0.04	0.843	1, 157.53	0.05	0.820	1, 153.84	5.13	0.025	1, 428.90	3.23	0.073
STERIL × CONDSP	2, 155.03	2.40	0.094	2, 157.33	0.63	0.536	2, 154.28	4.55	0.012	2, 124.52	1.07	0.347
CONDSP × ORIGIN	2, 154.23	0.44	0.648	2, 157.74	0.64	0.529	2, 154.53	0.93	0.398	2, 96.20	1.33	0.269
STERIL × CONDSP × ORIGIN	2, 155.16	0.35	0.704	2, 157.70	1.61	0.203	2, 154.39	4.03	0.020	2, 63.48	0.68	0.509
Random terms												
Target species	–			–			–			2, 7.46	33.04	<0.001
ID (Conditioning category)	–			–			–			4, 486.68	6.01	<0.001
Site (Soil origin)	4, 153	5.62	<0.001	4, 156	0.93	0.450	4, 153	4.27	0.003	14, 478	3.75	<0.001
Error	153			156			153			478		

The factor “Site” was nested in “Soil origin”. Results are given separately for each exotic species and for all species together. In this case, the additional random term “Target species” refers to the identity of exotic species and “ID” refers to the identity of conditioning species nested within one of three categories (conspecifics, congeners and heterogenerics). Significant effects are in bold ($P < 0.05$) and marginally significant effects are in italics ($P < 0.10$)

^a Conditioning category applicable only in analysis for all species

was a three-way interaction as the previous two factors interacted with conditioning species. If conditioned by itself and a congeneric using an inoculum from congeneric free-sites, growth was severely reduced by unsterilised inoculum. However, conditioning by a congeneric (but not by itself or a heterogeneric) and using the unsterilised inoculum from congeneric sites improved the performance of *S. annua* (Table 3; Fig. 3b).

Sterilisation treatment was the only significant fixed factor in the overall test. The effect of sterilisation × soil origin interaction was marginally significant and was influenced by the pattern found for *S. annua*. All three random factors were found to be significant (Table 3).

The pattern obtained for total biomass did not differ from the output of analyses that used root and shoot biomass as response variables. The only exception was the root biomass of *Stenactis annua* that was not influenced by the sterilisation treatment (Supplementary Material, Table S2).

Discussion

In this study, we demonstrated the negative effects of a soil microbial community on the growth of three exotic species in both experimental phases (soil conditioning and plant-soil feedback). This result indicates that soil pathogens’ effect on studied species dominated over the effect of soil microbial mutualists. Our results agree with the findings of Agrawal et al. (2005) and van Grunsven et al. (2007), whereas MacKay and Kotanen (2008) or Callaway et al. (2004) showed that symbionts were the dominant component of the soil microbiota. Unfortunately, we cannot say which species or even guilds of soil microbiota were responsible for the observed pattern. Despite improvements in molecular species identification techniques, the identities of soil microbiota that have significant impacts on plant performance in plant-soil feedback experiments remain mostly unknown (but see Packer and Clay 2000; Klironomos 2002).

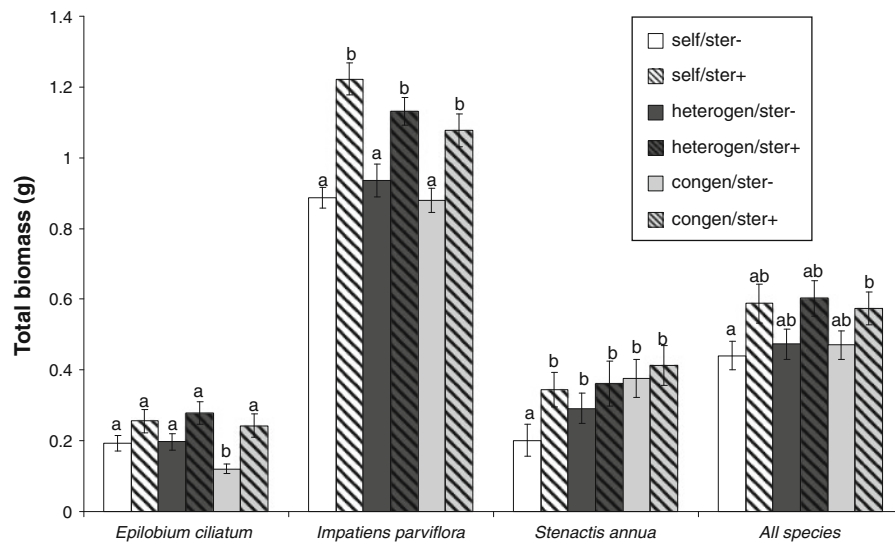


Fig. 2 Total biomass (g; mean \pm SE) of three exotics harvested after the second experimental phase. The plants were grown in pots with soil that was conditioned by conspecifics (self), heterogenics (heterogen) and congenics

(congen) with an inoculum treated by sterilisation (ster+) or left untreated (ster-). Bars sharing the same letter are not significantly different ($P > 0.05$; S-N-K posthoc test)

We also found that exotics did not show less negative feedback when grown in their own soil in comparison to soil stimulated by other species. This contrasts with the findings of Klironomos (2002) or Callaway et al. (2004), who explained the better performance of exotics when grown in their own soil by claiming that they benefited from generalist soil microbes in their new environment when no longer in the presence of specialised enemies. Our results indicate that even if exotics leave behind some pathogens, they may encounter new enemies, and those are most likely generalists.

However, the results of our study do not support the assumptions of the naturalisation hypothesis. In the first experimental phase, whether the inoculum was harvested at sites with or without congenics did not appear to affect exotic growth. In the latter experimental phase, we also did not prove the existence of a greater negative impact of soil conditioned by congenics than of soil conditioned by other two plant categories. There was also a lack of evidence showing any interaction between the category of the conditioning species and soil origin.

Aside from other possible explanations, it is very likely that results of this study, namely those on relatedness of conditioning species, were determined

by the power of the experimental design. First, at the individual species level, there were only three replicates per site category. However, this problem was mitigated when data were merged, increasing the total to nine replicates per site category. A much more problematic issue was the low number of replicates for each of three categories of conditioning species. Even after merging the data, each category was represented by only three species, which may be inadequate to prove or reject the naturalisation hypothesis.

Aside from the limitations of the rather low experimental power, there are other possible reasons that may explain the lack of a pattern of the effects of soil origin and relatedness of conditioning species. The lack of an effect of soil origin on plant growth during the first experimental phase could have occurred because the specific pathogens associated with congenics existed in densities that were too low to show any significant effect. In contrast to previous studies that obtained the soil microbial community directly from the plant rhizosphere (e.g., Callaway et al. 2004; van Grunsven et al. 2007), we applied a rather conservative approach to search for habitat effects. We used similar method as MacKay and Kotanen (2008) did and sampled the soil

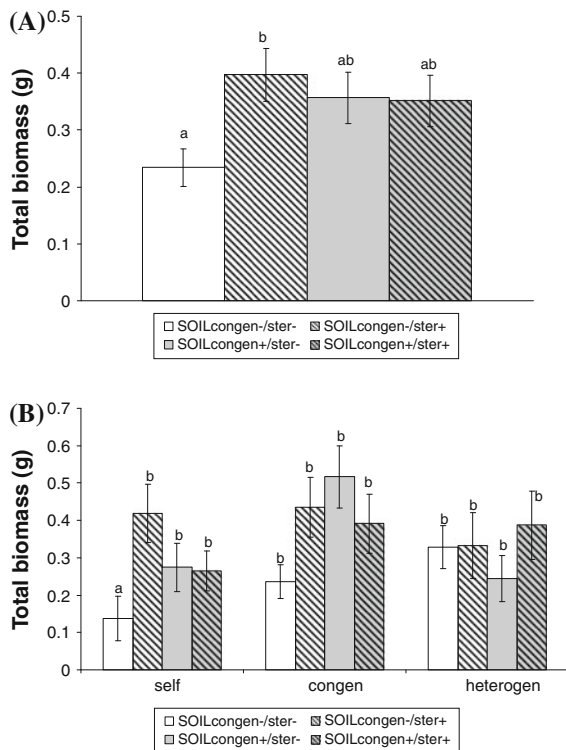


Fig. 3 a Total biomass (g; mean \pm SE) of *Stenactis annua* harvested after the second experimental phase. Plants were grown in pots with a soil inoculum originating from both site types, with a congeneric (SOILcongen+) and without (SOILcongen-). The inoculum was treated by sterilisation (ster+) or left untreated (ster-). In B), identical results are further structured according to conditioning species used in the first experimental phase. The soil was stimulated by itself (self), congeneric *Erigeron acer* (congen) and heterogeneric *Geum urbanum* (heterogen). Bars sharing the same letter are not significantly different ($P > 0.05$; S-N-K post-hoc test)

irrespective of the position of congeneric individuals at the site to more realistically reflect the distribution of the exotic individuals and their interaction with soil biota. Consequently, the abundance of specific soil biota could be low.

The effect of soil origin \times sterilisation appeared (marginally) significant in the second experimental phase because of the effect of this interaction on the total biomass of *S. annua* (Table 3; see also Fig. 3a). Therefore, we assume that microbes in this second phase were only abundant enough to influence the growth of plants of target species. Our findings are in accord with those of MacKay and Kotanen (2008), which showed that the impact of an inoculum on the growth of *Ambrosia artemisifolia* increased over

several generations in plant-soil feedback experiments, suggesting accumulation of soil biota over time.

Surprisingly, the effect of soil origin on the growth of *S. annua* was in contrast to what we expected; a greater negative effect was shown for unsterilised inocula sampled at congeneric-free sites than from sites where congenics were present. The positive impact of congeneric *Erigeron acer* on *S. annua* was further shown by the significant effect of a three-way interaction between sterilisation, soil origin and stimulating species. Growth of this exotic was most improved when cultivated in soil with an unsterilised inoculum from congeneric-sites and stimulated by *E. acer* in the first experimental phase (Table 3; Fig. 3b). Although the naturalisation hypothesis predicts that related native species will produce specialised enemies that decrease the performance of exotics, in fact, they may be the hosts of specialised symbionts with positive effects on newcomers. A good example comes from Brown et al. (2002), who studied the interaction of an exotic plant, its native congeneric and shared pollinators. They found that invasive *Lythrum salicaria* may draw pollinators away from its native congeneric *Lythrum alatum*, suggesting that *L. alatum* (at its own expense) improves the performance of this exotic species.

The category of the conditioning species showed no effect in the overall analysis. At the level of individual exotic species, however, the identity of conditioning species influenced exotics' growth. As discussed above, the performance of *S. annua* was improved by the congeneric *E. acer*. On the contrary, plants of *E. ciliatum*, performed worst when the soil was conditioned by the congeneric *E. hirsutum*. The response of *I. parviflora* was not influenced by conditioning species. Large variations of exotic species and their limited number, as well as a low number of conditioning species (see discussion above) precludes us from making a robust conclusion on the role of relatedness of native species on plant-soil feedback of exotics.

Nevertheless, significant differences of conditioning species on *E. ciliatum* and *S. annua* performance may indicate different levels of success of these exotics across invaded communities. Aside from differences in direct competition effects, variation in native species composition may change the proportions of soil mutualistic and pathogenic microorganisms and influence the performance of exotics

indirectly. For *I. parviflora*, we found no variation in the feedback to stimulating species, and therefore, its success probably depends less on the native plant composition of the colonised communities. The question is then whether this finding can be linked with the fact that *I. parviflora* has become one of the most widespread exotic species in Central Europe as documented by Sádlo et al. (2007).

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