

Help from under ground: soil biota facilitate knotweed invasion

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Abstract. Soil biota can be important drivers of plant community structure. Depending on the balance between antagonistic and mutualistic interactions, they can limit or promote the success of plant species. This is particularly important in the context of exotic plant invasions where soil biota can either increase the biotic resistance of habitats, or they can shift the balance between exotic and native plants towards the exotics and thereby greatly contribute to their dominance. Here, we explored the role of soil biota in the invasion success of exotic knotweed (*Fallopia × bohemica*), one of the world's most noxious invasive plants. We created artificial native plant communities that were experimentally invaded by knotweed, using a range of substrates where we manipulated different fractions of soil biota. We found that invasive knotweed benefited more from the overall presence of soil biota than any of the six native species. In particular the presence of the full natural soil biota strongly shifted the competitive balance in favor of knotweed. Soil biota promoted both regeneration and growth of the invader, which suggests that soil organisms may be important both in the early establishment of knotweed and possibly its later dominance of native communities. Addition of activated carbon to the soil made the advantage of knotweed disappear, which suggests that the mechanisms underlying the positive soil biota effects are chemically mediated. Our study demonstrates that soil organisms play a key role in the invasion success of exotic knotweed.

Key words: belowground interactions; biological invasions; *Fallopia*; invasibility; invasiveness; mycorrhiza; plant-soil interactions.

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INTRODUCTION

During the last centuries, global trade has been breaking biogeographic barriers and moving plants from their original areas of distribution to new areas, where they are sometimes able to establish in natural habitats (Vitousek et al. 1996, Williamson and Fitter 1996, Hulme et al. 2008). A few of these naturalized species become very abundant in their new habitats; they successfully outcompete native species (Mack et al. 2000, Richardson et al. 2000a), and cause substantial ecological and economic damage (Pimentel et al.

2001, Ricciardi 2007). Dominant plant invaders that suppress native plant species are often believed to possess a superior ability to compete for resources (Richards et al. 2006, Grotkopp and Rejmanek 2007, van Kleunen et al. 2010). This ability might be inherent (Theoharides and Dukes 2007, Pysek et al. 2009, van Kleunen et al. 2011), or it might have evolved in the new range, possibly because escape from natural enemies (Maron and Vila 2001, Keane and Crawley 2002) allowed species to reallocate resources from defenses to growth and reproduction (Blossey and Notzold 1995, Bossdorf et

al. 2005).

Plant-plant interactions and plant resource uptake are influenced by interactions with soil biota. Below ground, plants not only interact with other plants, but also with a wide range of soil organisms, including bacteria, fungi, nematodes, and various kinds of arthropods. These soil biota can influence plant success, and drive plant community composition and dynamics (Packer and Clay 2000, Bever 2002, Wardle 2002). The net impact of soil biota on the performance of a plant is the sum of all antagonistic (herbivores, parasites and pathogens) and mutualistic plant-soil interactions (e.g., mycorrhiza, nitrogen fixers) this plant is experiencing (van der Putten et al. 2007, van der Heijden et al. 2008). As this balance is often highly species-specific, soil biota can shift the competitive balance between different plant species.

Interactions between plants and soil biota, and the associated changes in plant-plant interactions and community composition can be influenced by chemicals exudated from plant roots, which may stimulate or inhibit the growth of particular soil biota (Callaway et al. 2008). If such changes in soil biota have negative effects on other plants, they can be regarded as cases of indirect allelopathy (Stinson et al. 2006).

Exotic plants are particularly interesting in this context because in their introduced range they often lack many of their original soil mutualists and antagonists, and at the same time they encounter novel soil biota they may not be adapted to. The outcome of these altered or novel interactions is likely to influence invasion success (Klironomos 2002, Callaway et al. 2004, Levine et al. 2004, Wolfe and Klironomos 2005, Pringle et al. 2009), by promoting the invader, or by enhancing community resistance to invasion (Stampe and Daehler 2003). Invasion success will be promoted if exotic plants profit more from the presence of mutualists than native plants (mutualism facilitation hypothesis; Richardson et al. 2000b, Mitchell et al. 2006), or if they suffer less from soil pathogens than the natives (Reinhart et al. 2010, Callaway et al. 2011). Recent research has shown that for several highly invasive exotic plants such effects are chemically mediated (e.g., Callaway et al. 2008, Mangla et al. 2008).

A particularly aggressive group of plant

invaders in Europe and North America are the Asian knotweeds *Fallopia japonica* (Japanese knotweed), *Fallopia sachalinensis* (Giant knotweed) and their hybrid *Fallopia × bohemica* (Bohemian knotweed) (Bailey and Connolly 2000). In their introduced range, the species grow extremely fast and vigorously, and they frequently outcompete native vegetation and form monospecific stands (Gerber et al. 2008, Aguilera et al. 2010). Invasive knotweeds also cause significant structural damage in urban habitats, which, together with the high costs of their removal, makes them the most problematic invasive plants in temperate ecosystems in Europe (Williams et al. 2010). Previous studies show that invasive knotweeds are indeed superior resource competitors (Parepa et al. 2013); they can exert allelopathic effects on native plants (Siemens and Blossey 2007, Murrell et al. 2010, Parepa et al. 2012) and may disrupt soil mutualisms (Urgenson et al. 2012). However, the mechanisms underlying their competitive ability and allelopathic potential, in particular the potential role of soil biota in mediating them, are still not well understood. Moreover, most previous studies focused on established knotweed stands and therefore examined mechanisms of dominance, whereas we know less about the factors that contribute to the colonization and early success of invasive knotweeds in novel habitats.

Here, we explored the role of soil biota in the colonization of exotic knotweed into native plant communities. Using experimental invasion of artificial native plant communities, together with a manipulation of soil biota, we asked (1) whether soil biota affect knotweed performance and its success in native communities, and if yes, (2) which fractions of the soil biota are most important, and (3) whether the effects are chemically mediated.

METHODS

Plant material

For the native plant communities, we selected six native plant species: two grasses (*Lolium perenne*, *Poa trivialis*) and four forbs (*Geranium robertianum*, *Silene dioica*, *Symphytum officinale* and *Urtica dioica*). All species commonly occur in habitats invaded by knotweed (Gerber et al.

2008). We used seed material from a regional supplier of wild-collected seeds (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany).

As invader, we chose *Fallopia* × *bohemica*, a hybrid of *F. japonica* and *F. sachalinensis*, two tall, perennial members of the Polygonaceae family that were introduced to Europe from Eastern Asia in the 19th century as ornamentals. Both parent species are well known for their vigorous growth and clonal spread, but the hybrid appears to even surpass the vigor and rate of spread of its parents (Mandak et al. 2004), and it is expected to eventually become the most abundant and problematic of the invasive knotweeds (Bailey et al. 2009). We used rhizomes from an invasive population of *F. × bohemica* located at the river Birs close to Delémont, Switzerland (47.37 N, 7.36 E). The population has served as a source of plant material in previous studies (Murrell et al. 2010, Parepa et al. 2012), and its hybrid identity has been verified through molecular methods (Krebs et al. 2010).

Soil biota inoculates

To create soil biota inoculates, we collected soil from ecologically similar but uninvaded riparian habitats close (<1 km distance) to the location where we collected the *Fallopia* rhizomes. From each of ten locations we collected a 25 by 25 cm square of 10 cm top soil, a total of 60 L soil from all locations, which we then mixed with an equal volume of water. Following Klironomos (2002), we separated 50L of the resulting liquid and sieved the supernatant through a 200 µm sieve to create a coarse filtrate of bacteria, fungi and other small soil organisms such as protozoa, small acari, rotifers and nematodes (Bardgett 2005). Half of the coarse filtrate was filtered again through another sieve of 20 µm mesh size to obtain a fine filtrate containing microbes only. This filtrate was expected to be dominated by bacteria. In addition to these inoculates from field soil, we also obtained a commercial multi-strain arbuscular mycorrhizal fungal inoculate (Symbi-vit; Symbiom, Lanskroun, Czech Republic), consisting of spores and hyphae of five common AMF species (*Glomus. claroideum*, *G. etunicatum*, *G. geosporum*, *G. intraradices* and *G. mosseae*). These AMF species are very widespread and expected to be present in the habitats invaded by

knotweed in Central Europe.

Experimental design

In July 2009 we set-up artificial native plant communities that consisted of one seedling of each of the six native species, planted in a circle with randomized species order in four-liter flower pots. In the center of each pot we buried one 8–10 cm *Fallopia* rhizome with two intact nodes. To surface-sterilize rhizomes, we immersed them in 2% bleach solution for five minutes prior to planting. As substrate, we used a 1:1 mixture of sand and a local agricultural field soil (0.2% N, 4.3% C, 35 mg P/kg, pH = 7.5) (RICOTER Erdaufbereitung AG, Aarberg, Switzerland). In all soil biota treatments (see below), the soil was initially sterilized by gamma irradiation (≥25 kGray). To avoid any effects of soil sterilization on nutrient levels, as well as nutrient depletion towards the end of the experiment, we applied liquid NPK (7:5:6) fertilizer equivalent to 30 kg N/ha once at the beginning of the experiment, and a second time at the start of the second growing season in April 2010. The pots were placed in an experimental garden in a fully randomized order.

We inoculated the sterilized substrate with the soil biota filtrates and the AMF in a factorial manner, with three levels of soil filtrate treatments (control, fine filtrate, coarse filtrate), and two levels of AMF (with or without AMF inoculate) (Fig. 1). For the soil filtrates we added 300 ml of filtrate, in the control treatment 300 ml tap water. The AMF inoculate was mixed into the substrate at a concentration of 25 g/L. In addition to these six treatment combinations based on sterilized soil, we set up an additional treatment with unsterilized substrate, which allowed us to examine the overall effect of the full natural soil biota. To all treatments which did not receive AMF we added the same, but sterilized, amount of the growing substrate of the AMF inoculate.

To half of the pots in each treatment we added activated carbon (Charcoal Activated, Merck KGA, Darmstadt, Germany) at a concentration of 20 mL/L. Activated carbon (referred to as “AC” in the remainder of this paper) has a high capacity of adsorbing organic compounds and can therefore be used to test for chemically-based belowground interactions, including allelopathy (e.g., Prati and Bossdorf 2004, Bossdorf et al.

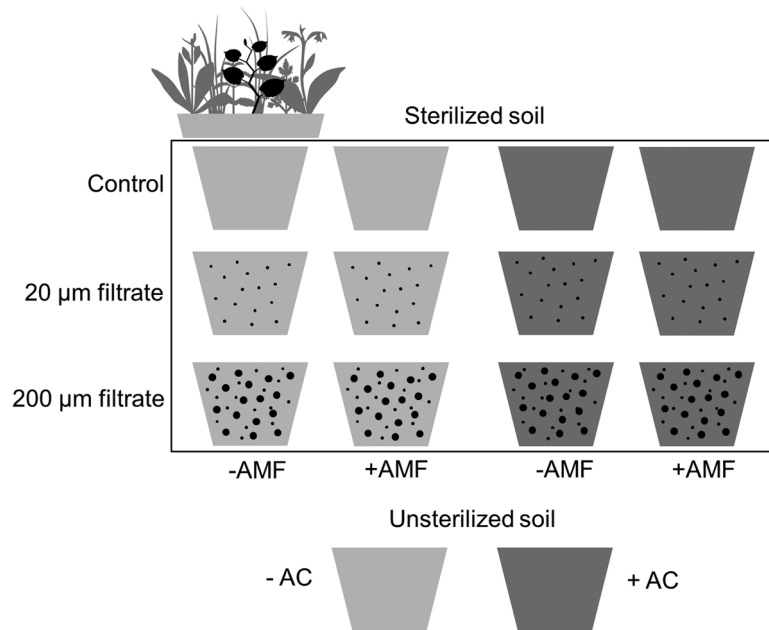


Fig. 1. Schematic of the 14 different experimental treatments of our study, in which we tested for the effects of soil biota on the success of invasive knotweed in native plant communities. 20 μm filtrate = microbes only, dominated by bacteria; 200 μm filtrate = bacteria, fungi and other small soil organisms; AMF = mix of five common arbuscular mycorrhizal fungi; AC = activated carbon.

2009). AC does not appear to have any direct effects on the native plants used here (Parepa et al. 2012). Moreover, a soil analysis of the fresh substrates indicated that AC did not have a direct effect on soil nutrient contents (data not shown). Each of the 14 treatment combinations—six soil biota treatments plus unsterilized soil, each with or without AC—was replicated 16 times, with a total of 224 pots and 1344 planted native plant seedlings in the experiment (Fig. 1).

The experiment was set up in July 2009. During the first four months we regularly recorded the regeneration success of knotweed rhizomes. In August 2010 we harvested all aboveground biomass, separated it by species, dried it to constant weight at 80°C for two days, and weighed it.

Statistical analyses

To test for differences in knotweed performance among soil biota treatments, as well as effects of AC, we analyzed the regeneration success and biomass of *Fallopia* with generalized linear models that included the effects of soil treatment (seven levels), activated carbon (two levels), as well as

their interaction. For the regeneration data, we fitted a GLM with binomial error and for the log-transformed biomass data (from the pots where rhizomes successfully regenerated) we fitted a GLM with Gaussian error.

To quantify the (relative) invasion success of knotweed in native plant communities, we calculated the ratio between knotweed and total native community or total native forbs biomass. We then explored soil biota effects on these two ratios as well as on the biomasses of individual native species by calculating log response ratios for each treatment compared to the sterile soil. We used *t*-tests to test whether individual log-response ratios were significantly different from zero. All analyses were done in R 2.8.0 (R Development Core Team 2008).

RESULTS

At the time of harvest, all experimental plant communities were very dense and almost certainly constituted highly competitive environments both above and below ground. The most dominant native plants were the two grasses,

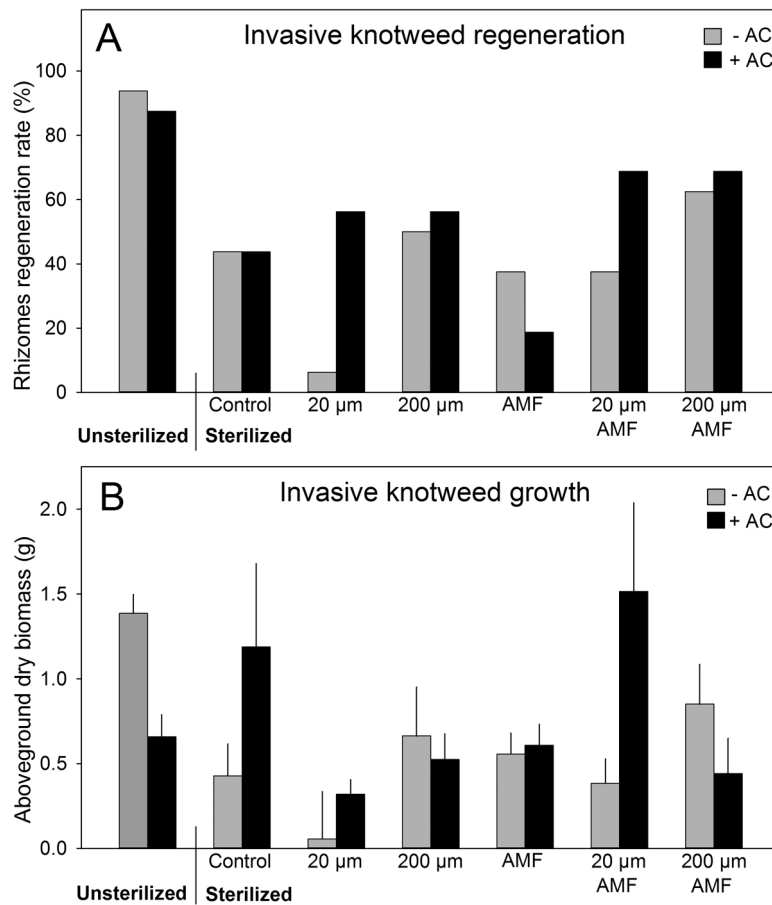


Fig. 2. The effects of soil sterilization and addition of different soil biota on the regeneration (A) and growth (B) of invasive knotweed, with or without activated carbon (AC) added to the substrate. 20 µm = fine soil filtrate containing microbes only, dominated by bacteria; 200 µm = coarse soil filtrate containing bacteria, fungi and other small soil organisms; AMF = mix of five common arbuscular mycorrhizal fungi. Error bars in panel B are 1 SE.

which accounted for some 80% of the total native biomass. Among the forbs, *Symphytum officinale* was the most dominant species, followed by, in order of their average biomass, *Silene dioica*, *Urtica dioica* and *Geranium robertianum*. About 50% of the planted knotweed rhizomes regenerated during the time of our experiment (between 6% and 93%, depending on the treatment; Fig. 2A), usually during the first weeks after planting. On average, knotweed achieved 13% of the total community biomass (between 5% and 20%, depending on the treatment; Fig. 2B).

Knotweed regeneration and growth

The regeneration of knotweed rhizomes was significantly affected by soil biota ($\chi^2 = 38.9$, $P <$

0.001), and there was also a significant interaction between soil biota and activated carbon ($\chi^2 = 13.0$, $P = 0.043$), but no main effect of AC ($\chi^2 = 2.6$, $P = 0.108$). In unsterilized, natural soil, almost all *Fallopia* rhizomes regenerated, whereas in most other treatments based on sterilized soil the regeneration rate was 40–60% (Fig. 2A). Knotweed regeneration was drastically reduced (to less than 10%) in the fine filtrate treatment, and this detrimental effect disappeared when AC was added to the substrate (Fig. 2A). Addition of AC also substantially increased regeneration in the fine filtrate + AMF treatment, and it decreased regeneration in the AMF only treatment (Fig. 2A).

Soil biota also significantly affected the bio-

Effects of Soil Biota on species biomass and knotweed success

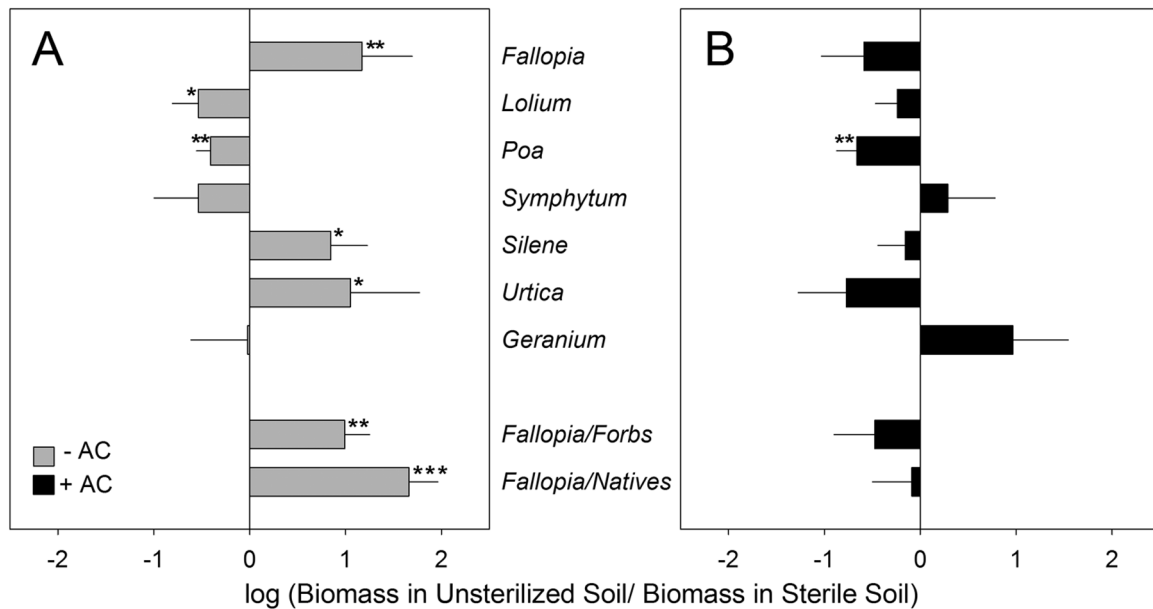


Fig. 3. The effects of the presence of the full natural soil biota on knotweed biomass, native species biomasses, and knotweed invasion success, without (A) or with (B) activated carbon (AC) added to the soil. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

mass of knotweed ($F = 6.14$, $P < 0.001$). Again, there was no main effect of AC ($F = 0.99$, $P = 0.321$), but a significant soil biota by AC interaction ($F = 2.68$, $P < 0.016$). Without AC, *Fallopia* achieved almost three times more biomass in the unsterilized soil than in the sterilized control soil (Fig. 2B). Intriguingly, this difference disappeared and even reversed if AC was added. Knotweed biomass was generally lower in treatments based on sterilized soil than in unsterilized soil, and the lowest values were found in the fine filtrate only treatment (Fig. 2B). As in the regeneration data, *Fallopia* tended to do better where AMF and filtrates were added in combination, and there was a strong positive effect of AC addition in the fine filtrate and AMF + fine filtrate treatments (Fig. 2B).

Responses of native species

Among the native plants, *Silene dioica* and *Urtica dioica* benefited most from the presence of the full natural soil biota, whereas the two grass species showed a negative response, and the other two forbs did not respond significantly at all (Fig. 3A). Except for the negative effect on one

of the two grasses, *Poa trivialis*, addition of AC eliminated all significant soil biota effects. The responses of the native species to the different soil biota fractions were species-dependent (Table 1): *Urtica dioica* benefited from the addition of AMF, or AMF in combination with fine filtrate, whereas *Silene dioica* showed a positive response only to the combination of AMF and coarse filtrate. Addition of AC eliminated two of these three effects, but resulted in several new, mostly negative, effects of different soil biota fractions.

Knotweed invasion success

As knotweed benefited most from the overall presence of soil biota, the ratio between knotweed biomass and the biomass of native forbs or all native plants strongly increased in the presence of soil biota (Fig. 3A). The effect disappeared and even reversed if AC was added to the soil (Fig. 3B). While the pattern was strongest for the comparison of unsterilized natural soil with sterilized soil, it was also found in treatments where the coarse 200 μm filtrate, alone or in combination with AMF, was added to the soil (Table 1). In contrast, addition of the fine

Table 1. Responses of knotweed and native species biomasses, and knotweed invasion success, to the addition of different fractions of soil biota, or their combinations, with or without activated carbon (AC).

Species	20 µm filtrate		200 µm filtrate		AMF		20 µm + AMF		200 µm + AMF	
	-AC	+AC	-AC	+AC	-AC	+AC	-AC	+AC	-AC	+AC
<i>Fallopia × bohemica</i>	-2.03	-1.31	0.44	-0.82	0.11	-0.67	-0.11	0.24	0.69	-0.99
<i>Lolium perenne</i>	0.07	0.51	0.20	0.10	0.10	0.20	-0.02	0.31	0.02	0.34
<i>Poa trivialis</i>	-0.30	-0.18	-0.09	-0.11	0.03	-0.11	-0.25	-0.07	-0.19	-0.19
<i>Symphytum officinale</i>	-0.52	-0.27	-0.40	-0.14	-0.18	0.02	-0.33	0.14	0.13	0.51
<i>Silene dioica</i>	0.38	-0.82	-0.20	-0.36	0.38	-0.84	0.41	-0.81	1.10	-0.65
<i>Urtica dioica</i>	-0.42	-2.77	1.10	0.81	1.92	-0.82	2.88	1.89	1.04	0.70
<i>Geranium robertianum</i>	-1.40	0.01	-1.17	-0.93	-0.28	0.15	-0.20	0.97	-0.97	1.43
<i>Fallopia</i> /Native forbs	-2.18	-1.18	1.02	-0.72	0.56	-0.07	0.54	1.04	1.36	-1.24
<i>Fallopia</i> /All natives	-1.91	-1.24	0.70	-0.70	0.69	-0.21	0.35	0.23	1.06	-1.01

Notes: The values are log response ratios of biomasses or biomass ratios. Positive/negative values represent a positive/negative effect of soil biota relative to the sterilized soil. Significant values ($P < 0.05$) are in bold. The 20 µm filtrate contains only microbes and is dominated by bacteria. The 200 µm filtrate contains bacteria, fungi and other small soil organisms such as protozoa, small acari, rotifers and nematodes.

20 µm filtrate alone to the sterilized soil had a negative effect on the knotweed invasion success, and addition of AC weakened this effect.

DISCUSSION

Soil biota can play an important role in plant invasions. At the one extreme end, they may act as a biotic filter and prevent exotic plant establishment (Knevel et al. 2004). At the other end, they may drive invasion success, either because invaders strongly benefit from mutualistic interactions (van der Putten et al. 2007) or because native plants are more strongly suppressed by pathogens and herbivores than the exotics (Rekah et al. 2001, Keane and Crawley 2002, Mangla et al. 2008).

Here, we investigated the effects of soil biota on the invasion success of exotic knotweed, one of the world's most noxious invasive plants. We found that the presence of soil biota strongly shifted the competitive balance between knotweed and native plants towards the invader. Our results thus indicate that soil biota play a key role in knotweed invasion.

Our experiment differed from most previous invasive-native plant interaction studies in that we worked with mixtures of native species that were invaded by knotweed, rather than the pairs of plant individuals commonly used in plant-plant interactions research. To further increase the ecological realism of our set-up, we used native species that co-occur in natural communities invaded by knotweed, as well as a natural soil of local origin. Although our community

approach did not allow us to disentangle plant-plant and plant-soil interactions at the level of individual species, we chose it for two reasons: First, it was ecologically realistic and thus, hopefully, its results transferable to real communities. Second, compensatory effects among different native species are likely to increase community resistance to invasion (van der Putten et al. 2007), and thus we avoided overestimating invader dominance.

Overall effects of soil biota

The comparison of plant performances in unsterilized versus sterilized soil allowed us to evaluate the overall effects of the full soil biota. We found that invasive knotweed was the species that benefited most from soil biota, more so than any of the native forbs which also showed positive responses. As a result, the presence of soil biota strongly shifted the competitive balance in favor of knotweed. Our results thus indicate that soil organisms from the invaded range do not exert biotic resistance against knotweed invasion, but that instead they facilitate knotweed establishment. In contrast to their positive effects on knotweed and two other native forbs, soil biota had negative effects on the two grasses. Since these grasses constituted the majority of the native community biomass, it is possible that part of the observed positive effects of soil biota on knotweed result from negative soil biota effects on the dominant grasses, and a release of knotweed from grass competition. Taken together, our data support the mutualism facilitation hypothesis (Richardson et al. 2000b,

Mitchell et al. 2006) for plant invasiveness (Reinhart and Callaway 2006; see also Sun and He 2010, Aschehoug et al. 2012), and they are consistent with Klironomos (2002) who showed that many native, rare plants are negatively influenced by their soil biota, whereas the opposite is true for exotic, dominant plants.

Intriguingly, the addition of activated carbon eliminated the positive effects of soil biota on knotweed, along with several other soil biota effects on native plants. This indicates that the specific mechanisms underlying these soil biota effects may be chemically mediated. In light of previous studies that demonstrated allelopathic potential of knotweed (Siemens and Blossey 2007, Murrell et al. 2010), the results of our experiment thus support the idea that knotweed affects native plants through an indirect kind of allelopathy where its negative effects are mediated by soil organisms. This could be either chemically-mediated suppression of the mutualists of native plants, e.g., if root exudates alter the homing abilities of mutualists, or a chemical stimulation of pathogens that have stronger negative effects of natives than on the invader (accumulation of local pathogens hypothesis; Eppinga et al. 2006). Related effects have been found for other plant invaders (e.g., Stinson et al. 2006, Callaway et al. 2008).

Effects of different fractions of soil biota

Comparison of the effects of different fractions of soil biota, or their combinations, can give us an idea of the mechanisms that underly the observed overall effects of soil biota. There are two main results: the fine (20 μm) soil filtrate has a negative effect on knotweed growth and invasion success, whereas the opposite is true for the coarse (200 μm) filtrate.

The fine soil filtrate, which is expected to be dominated by soil bacteria, strongly suppresses knotweed and inhibits both the regeneration of rhizomes, which is the primary mechanism of knotweed spread, and the growth of knotweed. As knotweed is the species most negatively affected by the fine filtrate, some of the microbial organisms in this filtrate have the potential to limit knotweed spread and thus in principle to be candidates for biocontrol agents (Stubbs and Kennedy 2012). More generally, our study suggests that, in contrast to what has been found

for other aggressive plant invaders (e.g., Reinhart et al. 2010, Callaway et al. 2011), soil pathogens from the introduced range can at least sometimes have strong negative effects on exotic species, even though they do not share a coevolutionary history (see also Nijjer et al. 2007, Parker and Gilbert 2007).

Adding the coarse soil filtrate, which likely also contained a certain amount of native mycorrhizal fungi, promoted knotweed regeneration and in particular growth, and as a consequence shifted the competitive balance in favor of knotweed. The effect was not as strong as for the full natural soil biota, but it nevertheless suggests that at least part of the positive overall effects of soil biota on knotweed must be caused by soil organisms $>20 \mu\text{m}$, e.g., fungi or protozoa. These could be either mutualists of knotweed, or antagonists, such as root grazers, of the dominant native species.

Adding AMF to the coarse filtrate further increased knotweed success. This is surprising because invasive knotweeds are usually considered to be non- or at least rarely mycorrhizal (Wu et al. 2004). Therefore, soil biota from the coarse filtrate must interact with AMF in a complex fashion to promote knotweed growth (Artursson et al. 2006). Another explanation could be that knotweed is able to connect to the mycorrhizal network and thus to indirectly parasitize on neighboring species, as has been found e.g., for another highly successful plant invader, spotted knapweed (Marler et al. 1999, Zabinsky et al. 2002, Carey et al. 2004).

As for the overall effects of the full soil biota, addition of activated carbon to the different soil biota fractions eliminated most of their effects. The positive effects of the coarse filtrate are eliminated or even reversed, and the negative effects of the fine filtrate are strongly reduced. Thus, both positive and negative effects must be to some extent chemically mediated and appear to concern activated carbon disrupting plant-microbe interactions.

Conclusions

Our study suggests that soil biota may play an important role in the invasion success of exotic knotweed, particularly during its initial establishment. Knotweed greatly benefits from the overall presence of soil biota, more so than any of

the six native plant species studied. At least part of this overall positive effect appears to be caused by soil biota in the size range of 20–200 μm , whereas soil microbes below 20 μm appear to exert biotic resistance against knotweed. However, the negative effects are more than outweighed by the positive effects of other soil biota, and future research should now aim to identify the specific soil organisms and mechanisms of interaction that are underlying these observed effects of soil biota on knotweed invasion.

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