From plant traits to invasion success: Impacts of the alien Fallopia japonica (Houtt.) Ronse Decraene on two native grassland species

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From plant traits to invasion success: Impacts of the alien Fallopia japonica (Houtt.) Ronse Decraene on two native grassland species

T. Mincheva, E. Barni & C. Siniscalco

Abstract

Alien invasive plants threaten biodiversity, productivity and ecosystem functioning throughout the world. We examined the effect of Fallopia japonica on two native grassland species (Trifolium repens, Lolium perenne). We hypothesized that its negative effects on the native species are dependent on three mechanisms: (i) allelochemicals released and accumulated in soil with a history of invasion, (ii) altered soil biota and (iii) direct resource competition. We measured the response of the native species as the difference in their functional traits when grown under the three conditions. Our results demonstrate that neither allelochemicals nor soil biota from soil with history of F. japonica invasion had measurable effects on either species. Competition with the invader strongly reduced height, biomass and specific leaf area (SLA) of T. repens, while it had a lower effect on L. perenne. Furthermore, our results reveal that F. japonica took advantage of a positive plant–soil and plant–plant interaction. The results show that the prominent mechanism underpinning the invasion success of F. japonica in the grassland was the direct resource competition. This prominent role is also confirmed by the significant interactions between competition, allelochemicals and soil biota from soils with history of invasion of F. japonica on SLA of the native species.

Keywords

Reynoutria japonica, competition, specific leaf area, allelopathy, soil biota, Lolium perenne, Trifolium repens

Introduction

Fallopia japonica (Houtt.) Ronse Decraene (Japanese knotweed, syn. Reynoutria japonica), originally from Asia, is a particularly aggressive invader in Europe and other parts of the world. The invasion by F. japonica is the second most intensively studied plant invasion globally (Hulme et al. 2013). The species is a rhizomatous herbaceous perennial geophyte. In the native range, F. japonica is a common pioneer species on bare grounds and facilitates later succession of native species (Adachi et al. 1996). On the contrary, in the invaded range, the species is particularly competitive; furthermore, it causes multiple negative impacts on biodiversity, i.e. native flora (e.g. Bimová et al. 2004; Aguiler et al. 2010), invertebrate assemblages (Gerber et al. 2008), on soil nutrient cycling (Dassonville et al. 2007) and on economy (Reinhard et al. 2003). The species is considered one of the world’s worst invasive alien species (Lowe et al. 2000; Webster 2003).

Invasive knotweeds (F. japonica, F. sachalinensis and their hybrid Fallopia x bohemica) fit various theories proposed to explain the exceptional success of some alien species. For example, one leading hypothesis that explains the performance of successful invaders is the enemy release hypothesis (Keane & Crawley 2002) which proposes that alien plants are introduced in their novel range without their natural enemies resulting in lessened top-down regulation. Furthermore, the evolution of increased competitive ability hypothesis (Blossey & Notzold 1995) postulates that because of the absence of specialized herbivores and pathogens in the new range, invasive plants shift their resource allocation from herbivore defence to increased growth or reproduction. In a
biogeographic comparison of F. japonica populations in Europe and Japan, Maurel et al. (2013) found “quasi-absence of herbivores, low leaf damage and increased plant vigour and growth” in the non-native range and explained that this was the reason why invasive F. japonica had competitive advantage over the native coexisting species.

Several studies report that competition for light (i.e. shading) by invasive knotweeds modifies or excludes native vegetation (Hathaway 1999; Verloove 2002; Tokarska-Guzik et al. 2005; Siemens & Blossey 2007; Moravkova et al. 2011) or retards germination of seeds and seedling establishment of woody species (Lohmeyer 1969; Urgenson et al. 2009). Moreover, Moravkova et al. (2011) demonstrated that poor germination and establishment of native species in Fallopia-invaded stands is not dependent on the effect of shading alone and suggested that invasive Fallopia taxa attain competitive superiority over native species through allelopathy, in accordance to the novel weapon hypothesis (Callaway & Aschehoug 2000). According to the latter, invasive plants release novel allelochemicals with phytotoxic or at least fitness-reducing effects on resident plants that have not coevolved, which may result susceptible to novel phytochemicals compared to adapted competitors in the native range. Allelopathic compounds are released from the plant by leaching, root exudation, volatilization or residue of decomposition (Inderjit 2005). Fallopia species have been reported to contain large amounts of phenolic compounds, known to inhibit germination, establishment and growth of other species in the community (Inoue et al. 1992; Fan et al. 2009, 2010). In fact, there is increasing evidence for negative phytotoxic effects of either invasive Fallopia living plants, litter, leaf and rhizome extracts or soil contaminated with leaves or rhizomes, on native plant species development (Vrchotova & Sera 2008; Moravkova et al. 2011; Murrell et al. 2011; Parepa et al. 2012; Sera 2012; Dommanget et al. 2014). In this last research, the knotweed allelopathic effect resulted to be mediated by the polyphenol concentration in the soil and/or by changes in soil properties.

It has been reported difficult to separate direct allelopathic effects (inhibition of germination, establishment and growth of co-occurring species) from indirect negative effects of exotic invasive plants mediated by soil biota including bacteria, fungi, nemathods and arthropods (Mangla et al. 2008). Soil biota, through plant–soil positive or negative feedbacks (Levine et al. 2003; Reinhart & Callaway 2006), may decide the fate of an invader and the native plant community (van der Putten et al. 2007). For example, invasion success can be enhanced either because invasive species encounter fewer soil-borne enemies in their introduced ranges, or because they encounter novel but strong soil mutualists. Besides, the presence of indigenous enemies in soils, or the absence of key soil mutualists, both acting on the invasive alien species, can help native communities to resist invasions. These feedback relationships, negative or positive, may alter plant–soil biota interactions in ways that may facilitate invasion and inhibit re-establishment by native species, or may prevent alien plant establishment on the other hand (reviews by Levine et al. 2003; Reinhart & Callaway 2006; van der Putten et al. 2007).

Concerning invasive knotweeds, it is still under debate whether direct allelopathy only (Murrell et al. 2011), or indirect allelopathy mediated by soil organisms (Parepa et al. 2013) is involved in the mechanisms of reduction of native species. There are studies evidencing that secondary compounds isolated from Fallopia taxa have antibacterial and antifungal effects (Daayf et al. 1995; Kumagai et al. 2005), but knowledge on the persistence of knotweed allelochemicals in the soil is lacking. In case allelopathic compounds do accumulate and persist in soil, then allelopathic residues will continue to affect the establishment of native plants, even after the complete removal of Fallopia from invaded sites, and such “legacy effects” may compromise the success of restoration attempts, as recently evidenced for other invasive plants (Hamman & Hawkes 2013).

The aims of this study were to explore the impacts of F. japonica on native co-occurring plants, by assessing the response of the native plants to F. japonica invasion. The response of the native species was evaluated by measuring the variation of their functional traits. We focused on plant
traits that have been repeatedly reported to be correlated with invasiveness (Davidson et al. 2011). In particular, we selected two whole-plant traits implicated in light capture and overall performance, vegetative height and above-ground biomass; and one leaf trait correlated with growth rate, nutrient use and competitive ability, specific leaf area (SLA). SLA is implicated in the regulation of nutrient assimilation and allocation and is positively related to higher growth rates; moreover, SLA is often reported as being the most significant factor in explaining differences in relative growth rate between species in laboratory studies (Knops & Reinhart 2000).

The study is based upon a greenhouse experiment with the purpose of disentangling the mechanisms used by F. japonica to displace the native plants through: (i) direct competition for resources and/or (ii) allelopathic compounds accumulated in the soil after long persistence of F. japonica and/or (iii) alteration of feedbacks with soil biota. Specifically, we hypothesized negative response of the native species limiting their performance when grown (1) in competition with F. japonica; and/or (2) on soil with history of invasion by F. japonica; and/or (3) in the absence of their own soil biota. Furthermore, we hypothesized neutral or positive response of F. japonica grown with native species, in its own soil, and/or in own biota. Response was determined by comparing the plant functional traits of a given species grown under the different conditions.

**Material and methods**

**Site description**

In the region of Piedmont (NW Italy), three Asian knotweed taxa are distributed: Fallopia japonica (Houtt.) Ronse Decraene, Fallopia sachalinensis (F. Schmidt) Ronse Decraene and their common hybrid, Fallopia x bohemica (Chrtk & Chrtkova) J.P. Bailey; however, F. japonica is the most widely distributed in the region, as referred in Bouvet (2013). On the base of morphological features (Beerling et al. 1994; Bouvet 2013), we identified that the species at our study site was F. japonica. The growth experiment was carried out using sprouts of F. japonica and soil collected from a grassland that was partially colonized by the invader, located in Valchiusella, north-western Italy (45°28′52″ N, 7°46′52″ E), at 680 m a.s.l. The site served as a source of materials in a previous work and is described in details in Mincheva et al. (2014). The site represented two clearly separated adjacent plant communities: (i) a stand of native vegetation, a typical lowland grassland (Arrhenatherion elatioris), dominated by Dactylis glomerata, Festuca arundinacea, Trifolium pratense, Trifolium repens, Taraxacum gr. officinale and Achillea millefolium (G stand); (ii) a dense monospecific stand of F. japonica (F stand). The alien plant has been present in the study site for the last 30 years (personal communication by the landowner).

**Selection of target species**

As representatives of the native plant communities, we selected two target species: Lolium perenne L. and Trifolium repens L. Both species grow in temperate grasslands that are often invaded by Fallopia taxa (e.g. Gerber et al. 2008) and they represent the two dominant functional types, i.e. grasses and legumes. Furthermore, representatives of these two functional groups have been included in previous competition studies with Fallopia taxa (e.g. Murrell et al. 2011; Moravková et al. 2011). Both species are known to be mycorrhizal. Only two target species were selected because of the exceedingly high number of treatments and replicates. Seed material was provided by Gran Paradiso National Park, located nearby the study area. At the beginning of the experiment (May 2011), seeds of the native species were sowed in trays on sterile peat substrate and seedlings were grown in a greenhouse with natural light for 11 weeks.

At the peak of growth of F. japonica (August 2011), we collected young F. japonica sprouts from the F stand. Plants not exceeding 50 cm height, with similar weight and size, were selected and their rhizomes were excavated with a bulk of soil to keep the root system as intact as possible. We
transported the plants in sealed plastic bags to the laboratory where we carefully washed off the soil from the rhizomes with tap water and transplanted them within the same day.

Soil collection and preparation

Soil samples were collected from the two stands (F = invaded, G = native grassland) in June 2011. To test for the effect of allelopathic compounds released by F. japonica living plants and litter, and accumulated in the soil after a long period of invasion, soil was collected from the invaded (F) and uninvaded stand (G). Five plots sized 0.5 × 0.5 m were randomly placed in each stand. Plots were positioned at a minimum distance of 6–10 m from the border between stands in order to avoid any shading or underground effects of F. japonica on the native grassland (Aguilera et al. 2010), and one soil sample was taken from each plot. Each sample was collected from 30 × 30 cm square and 30 cm depth. Soils were sieved using a 0.5-cm mesh and placed in plastic bags for transportation to the laboratory. Each sample was split into two subsamples: one, for general chemical analysis, was stored at 4 °C; the other one, for the growth experiment, was kept in paper bag at room temperature until further proceeded. We mixed the five samples to obtain soil from invaded stand (F soil) and from native stand (G soil) which were used in the growth experiment to test their allelopathic properties.

To test the effect of soil biota, one half of the soils were sterilized at 120 °C for 20 min. The sterilized and non-sterilized soils were kept in sealed plastic bags until the growth experiment began.

The two stands had similar topography and water availability and, presumably, similar soil conditions before the arrival of the invader. Soil from the invaded stand, and from the native stand were similar in their physical and chemical properties, except for sand and silt content (76 and 22% in G, 89 and 10% in F, respectively) as well as total N content and C/N ratio (0.32% and 11.8 in G, 0.16% and 18.7 in F, respectively; Mincheva et al. 2014).

Experimental design

We set up a three-factorial growth experiment design with 20 treatment combinations: native soil vs. soil with history of F. japonica invasion (G, F); sterilized soil vs. non-sterilized soil (S, NS); no-competition growth (monoculture of L. perenne, T. repens, F. japonica) vs. competition growth (L. perenne + F. japonica, T. repens + F. japonica) (Table I). For each treatment, we had 8 replicates, resulting in a total number of 160 experimental units. We used the four soil types as substrate to fill in 3-L pots. In the case of no-competition treatments, we planted 10 seedlings of each native species and one sprout of F. japonica per pot, respectively. In the case of competition treatments, we planted one F. japonica sprout in the centre of the pot and 10 seedlings of one of the native species surrounding it. After transplanting, F. japonica stems were cut to a height of ca. 20 cm, leaving 5–6 nodes. Pots were watered every three days with tap water and were not fertilized.

**Table I. Representation of the experimental design.**

<table>
<thead>
<tr>
<th>Soil origin</th>
<th>Fallopia japonica</th>
<th>Native grassland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil sterilization</td>
<td>S</td>
<td>NS</td>
</tr>
<tr>
<td>Cultivated species</td>
<td>F, L, P, T, FT</td>
<td>F, L, P, T, FT</td>
</tr>
</tbody>
</table>

Notes: S = sterilized soil; NS = non-sterilized soil; F = F. japonica; L = Lolium perenne; T = Trifolium repens; FL = F. japonica + Lolium perenne; FT = F. japonica + T. repens.
**Functional traits assessment**

Plants were grown for 12 weeks, after which functional traits were measured following the protocols proposed by Cornelissen et al. (2003). We recorded the vegetative height of each plant, afterwards we harvested all above-ground biomass, separated it by species, oven dried at 60 °C until constant weight was reached and weighed it. For the leaf measurements, we considered the petiole and leaflet part of the leaf. Ten randomly selected leaves per species were collected. They were immediately wrapped in moist absorbent paper, brought in the lab, and their leaf area was measured using the open-source software Leaf Area, on leaf images taken with a professional scanner. Leaf samples were oven dried at 60 °C until constant weight was reached and weighed. SLA was calculated as the ratio of leaf area to dry weight (mm$^2$/mg).

**Statistical analysis**

To address the research questions in this study, the performance of the native species (L. perenne, T. repens) and F. japonica was compared among treatments with the analysis of variance, running general linear models including the treatments “soil origin” (two levels), “soil sterilization” (two levels) and “competition” (two levels) as fixed factors, as well as their interactions. The dependent variables were vegetative height, total above-ground biomass and SLA of the three species. The data was log transformed. Differences among treatments and blocks were analysed using one-way ANOVA. In addition, Tukey's HSD test was performed for “competition” treatment for the dependent variables of F. japonica.

All statistical tests were performed at the 0.05 level of statistical significance, using SPSS Statistics 20.0.

**Results**

*F. japonica, L. perenne* and *T. repens* plants deriving from 160 treatment units were analysed, their vegetative height and above-ground biomass measured, and SLA calculated. Results are presented in Table II.
Table II. Plant performance of *Fallopia japonica* and of the two native species, measured with three functional traits, according to soil conditioning, soil biota, resource competition and their interactions.

<table>
<thead>
<tr>
<th>Treatments</th>
<th><em>Fallopia japonica</em></th>
<th><em>Lolium perenne</em></th>
<th><em>Trifolium repens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vegetative height (cm)</td>
<td>Above-ground biomass (mg)</td>
<td>SLA (mm²/mg)</td>
</tr>
<tr>
<td>Origin of soil (A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>50.25</td>
<td>1.34</td>
<td>30.63</td>
</tr>
<tr>
<td>G</td>
<td>46.94</td>
<td>1.32</td>
<td>29.54</td>
</tr>
<tr>
<td>p value</td>
<td>0.260</td>
<td>0.795</td>
<td>0.462</td>
</tr>
<tr>
<td>Sterilization (B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>46.24</td>
<td>0.99</td>
<td>31.58</td>
</tr>
<tr>
<td>S</td>
<td>50.91</td>
<td>1.67</td>
<td>28.58</td>
</tr>
<tr>
<td>p value</td>
<td>0.221</td>
<td>0.002</td>
<td>0.040</td>
</tr>
<tr>
<td>Competition (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-competition (F)</td>
<td>49.39</td>
<td>1.58</td>
<td>23.58</td>
</tr>
<tr>
<td>No-competition (LP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-competition (TR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competition (F+LP)</td>
<td>44.69</td>
<td>0.99</td>
<td>33.60b</td>
</tr>
<tr>
<td>Competition (F+TR)</td>
<td>51.66</td>
<td>1.41</td>
<td>33.08b</td>
</tr>
<tr>
<td>p value</td>
<td>0.248</td>
<td>0.099</td>
<td>0.000</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A×B</td>
<td>0.235</td>
<td>0.252</td>
<td>0.613</td>
</tr>
<tr>
<td>A×C</td>
<td>0.435</td>
<td>0.140</td>
<td>0.040</td>
</tr>
<tr>
<td>B×C</td>
<td>0.117</td>
<td>0.110</td>
<td>0.000</td>
</tr>
<tr>
<td>A×B×C</td>
<td>0.945</td>
<td>0.550</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Abbreviations: F – soil from invaded *F. japonica* stand; G – soil from native grassland vegetation stand; NS – non-sterilized soil; S – sterilized soil; F – no-competition treatment, monoculture *F. japonica*; LP – no-competition treatment, monoculture of *L. perenne*; TR – no-competition treatment, monoculture of *T. repens*; F+LP – competition treatment, co-growth of *F. japonica* and *L. perenne*; F+TR – competition treatment, co-growth of *F. japonica* and *T. repens*.

Significant p values are in bold.

Table II. Plant performance of *Fallopia japonica* and of the two native species, measured with three functional traits, according to soil conditioning, soil biota, resource competition and their interactions.

**Native species**

The cultivation of *T. repens* and *L. perenne* on soils from the F or from the G stand (“soil origin” treatment), as well as on sterilized or non-sterilized soils (“soil sterilization”), did not cause any effect on the traits of both native species, except for the vegetative height of *T. repens* which showed a 15% increase when the species was grown in soil from the F stand (Table II). Competition with *F. japonica* induced negative effects in both native species. However, this effect was stronger in *T. repens* than in *L. perenne*. In fact, competition with the invader caused a significant decrease in all traits of *T. repens*: vegetative height diminished by 20%, above-ground biomass by 68% and SLA by 13%, compared to the no-competition treatments (Figure 1).
Figure 1. Effects of competition with F. japonica on T. repens plant traits: (a) height, (b) total above-ground biomass and (c) SLA. Boxplots display the media (line) and the median (dot) with the first and third quartiles. Statistical results are shown (ns non-significant; *p-value < 0.1; ***p-value < 0.001).

On the other hand, vegetative height and above-ground biomass of L. perenne remained unaffected by competition with the invader (Figure 2), while SLA decreased by 22% compared to the no-competition treatments.
There was also a significant interaction between treatments “soil origin” and “competition”, and “soil sterilization” and “competition” for SLA of *L. perenne*; and between “soil origin” and “soil sterilization” among all treatments for SLA of *T. repens*; with the main effect of “competition” in both species.

**Fallopia japonica**
Cultivating *F. japonica* on soils from F or G stand (“soil origin”) had no effect on any of the measured traits of the invader (Table II). Sterilization of soil (had a negative effect on SLA, which diminished by 9% in sterilized soil compared to non-sterilized soil (Figure 3). Vegetative height and above-ground biomass were not affected by any of the treatments.

Figure 3. Effects of soil biota on *F. japonica* plant traits: (a) height, (b) total above-ground biomass and (c) SLA. Boxplots display the media (line) and the median (dot) with the first and third quartiles. Statistical results are shown (ns non-significant; *p*-value < 0.1; ***p*-value < 0.001).
Figure 3. Effects of soil biota on *F. japonica* plant traits: (a) height, (b) total above-ground biomass and (c) SLA. Boxplots display the media (line) and the median (dot) with the first and third quartiles. Statistical results are shown (ns non-significant; *p*-value < 0.1; ***p*-value < 0.001).

Competition with both the native species had a positive effect on the SLA of *F. japonica*. In particular, SLA had a 30% higher value in monoculture than in competition treatment with *L. perenne* (Figure 4).
Interactions between “soil origin” and “competition”, “soil sterilization” and “competition” and among all treatments were significant for SLA of the invader.

**Discussion**

Our results suggest no significant effects of the soil historically invaded by *F. japonica* on the two investigated native species, while significant negative effects of direct competition were detected on both native species. Furthermore, SLA of *F. japonica* increased, while SLA of the native species decreased in the competition treatment, compared with no-competition treatment. These results clearly show that the prominent mechanism underpinning the invasion success of *F. japonica* in the grassland is the direct resource competition. However, species-specific responses of the native species to competition by the invader have been demonstrated.
We found strong support for our first hypothesis that direct competition with F. japonica reduces native species performance. However, while competition with F. japonica caused a considerable decrease of the legume’s above-ground biomass and height, it had no effect on the same traits of the grass. These results are consistent with other studies that found a negative relationship between knotweed occurrence and native forbs biomass production (Aguilera et al. 2010; Murrell et al. 2011; Parepa et al. 2013), while biomass of grasses remained unaffected (Murrell et al. 2011).

As for the leaf trait, competition with F. japonica had negative effect on SLA of both native species. SLA responds more rapidly to resource-limiting conditions compared to the whole-plant traits (Munier-Jolain et al. 2013). Therefore, these results indicate that L. perenne possesses a certain phenotypic plasticity to tolerate the decreased resource availability due to competition, at least by adjusting leaf characteristics, as demonstrated in recent studies (Roscher et al. 2010). The growth experiment may have run not long enough to detect effects on the whole-plant traits of the grass, but only to express a significant decrease in leaf traits, particularly SLA. In fact, despite this prompt response to lower resource availability, both native plant species underwent a decreasing performance, leading to their exclusion in the F. japonica invaded stand. Since a decrease in transmitted light due to the increased shading is usually correlated with increasing SLA (Roscher et al. 2010, 2011), the observed SLA reduction may be rather a response to soil nutrient limitations (Nassiri & Elgersma 2002).

F. japonica had significantly higher values of SLA compared to the monoculture growth, when grown together with native species. The trend was in the same direction for the other two traits measured, height and above-ground biomass. This observation allows to make conclusions that F. japonica takes advantage of a positive plant–plant interaction. Positive plant–plant interactions (i.e. facilitation) occur when one species enhances the survival, growth or fitness of another. This phenomenon has been described and discussed for many plant species (DeAngelis et al. 1986; Hunter & Aarssen 1988; Bertness & Callaway 1994; Callaway 1995; Callaway & Walker 1997), but no investigations on the mechanisms of facilitation of knotweed by native plants have been conducted up to date. Parepa and colleagues (2013) hypothesize that invasive knotweed is able to connect to the mycorrhizal network and indirectly parasitize on neighbouring species, but further experiments are needed to elucidate the proper role of this factor in knotweed invasion success.

We found no support for our second hypothesis that long persistence of F. japonica in the soil leads to suppressed native species performance. The soil with history of invasion had no effect on any of the traits of the native species, apart from the vegetative height of T. repens, which increased in soil from the stand invaded by F. japonica. Similarly, Parepa et al. (2012) reported that neither F. x bohemica living plants, nor litter leachates or trained soil, have any negative impact on germination, biomass and diversity of the native community. On the contrary, Murrell et al. (2011) found strong allelopathic effects of F. x bohemica living plants on native community biomass; besides, Sera (2012) reported considerably high phytotoxicity for crop plants cultivated in soil contaminated with F. japonica leaves. Dommanget et al. (2014) demonstrated the negative impact of F. japonica leachates released by living rhizomes during the growing period. The leachates contained soluble polyphenol compounds which inhibited the growth of Salicaceae species cuttings. Our findings did not considered the leachates, but only the effects of the soil with history of invasion wanting to test the persistence of the allelopathic effect in the soil. These results did not corroborate the results obtained by Parepa et al. (2012) bringing no evidence for the hypothesis that soils with legacy of invasive Fallopia taxa exert allelopathic effects on the native species. One explanation may be that allelopathic compounds liberated by invasive Fallopia taxa living plants and litter do not persist in soil or may leach in depth (Moravková et al. 2011). Therefore, soil legacies by F. japonica do not appear to last over long time and thus should not impose efforts for soil restoration of habitats once the living biomass of the invader has been removed.
We found no support for our third hypothesis that native species suffer in the absence of their own soil biota, or in the presence of F. japonica soil biota. However, we found strong evidence that full soil biota, alone or in combination with other factors, may contribute to F. japonica invasion. The presence of soil biota, regardless of its origin (whether collected from stand with history of F. japonica invasion or from native grassland stand), positively affected the invader’s performance, suggesting that F. japonica benefits from the presence of active soil biota. Studying plant–soil microbe feedbacks in F. japonica and in other invasive species, Klironomos (2002) provided evidence that the latter had escaped relatively host-specific pathogens in their own soil, but had connected with non-host-specific mutualists in their newly invaded soil. Similarly, Parepa et al. (2013) found that the presence of soil biota strongly shifted the competitive balance between knotweed and native plants towards the invader thus suggesting that the success of the invader may be not directly determined by allelopathy but mediated by the soil biota. Furthermore, as for the native species, we observed a trend of increased grass performance in sterilized soils. On the contrary, the legume did not show a clear response depending on soil biota presence. Our observations are consistent with Parepa et al. (2013) that report a negative response of grasses, and no response at all of the forbs to soil biota. Our experiment establishes that no single mechanism appears to be responsible for the displacement of native grassland species in the dense F. japonica stands. Our experiment also demonstrates species-specific responses of the native species to competition by the invader, which seems to be the main mechanism of displacement of the native species. Furthermore, our study contributes to the knowledge that soil biota may be one of the drivers of plant invasion. Invasive knotweeds, once established, can modify soil biotic and abiotic components (Dassonville et al. 2007; Dassonville 2008; Aguilera et al. 2010; Maurel et al. 2010; Dassonville et al. 2011). Maurel et al. (2010) explains the poor success of the attempts to remove F. japonica from invaded communities with the lack of “restoration” of the soil characteristics. Where aliens rely on plant–soil feedback or soil legacies, native plant restoration may require soil-based management. In these cases, changing mycorrhizal fungal abundance, increasing soil pathogen loading and slowing nutrient cycling rates may help restore native plants to invaded fields (Kulmatiski et al. 2006).

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