In the presence of specialist root and shoot herbivory, invasive-range *Brassica nigra* populations have stronger competitive effects than native-range populations

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SUMMARY

1. The evolution of increased competitive ability (EICA) hypothesis predicts that release from specialist herbivores enables invasive plants to evolve increased growth. The most powerful tests of EICA hypothesis are provided by approaches that simultaneously assess the effects of specialist herbivory and competitive interactions. However, such approaches are extremely rare, and hence how simultaneous release from root and shoot herbivory influence competitive ability of invasive plants remains little understood.

2. Here, we tested whether invasive-range *Brassica nigra* plants have evolved increased competitive ability, and whether expression of competitive ability depends on separate and simultaneous effects of specialist root and shoot herbivory. To do this, we grew *B. nigra* plants from eight invasive-range and eight native-range populations in the presence vs. absence of competition with a community of native plant species, and in the absence vs. presence of separate and simultaneous damage by a specialist root herbivore (*Delia radicum*) and a specialist shoot herbivore (*Plutella xylostella*). *Brassica nigra* performance was assessed by measuring biomass production and flowering of individual *B. nigra* plants.

3. In partial support of the EICA hypothesis, invasive-range *B. nigra* had greater flowering than native-range conspecifics in the absence of competition. However, contrary to a prediction of the EICA hypothesis, invasive-range *B. nigra* produced less above-ground biomass than native-range *B. nigra* in the absence of shoot herbivory and competition. Moreover, with simultaneous root and shoot herbivory, invasive-range *B. nigra* suppressed a competing community more strongly than native-range *B. nigra* did.

4. *Synthesis*. Our results suggest that invasiveness may be driven by mechanisms other than increased individual size. Simultaneous root and shoot herbivory in the invasive range may enhance suppressive effects of introduced plant species that have not completely escaped herbivore damage in the introduced range.

Introduction

A popular hypothesis to explain invasiveness of exotic plant species is the escape from natural enemies such as specialist herbivores (enemy release hypothesis) (Keane & Crawley 2002; Müller-Schärer, Schaffner & Steinger 2004; Joshi & Vrieling 2005), and the subsequent evolution of increased competitive ability (EICA hypothesis) (Blossey & Nötzold 1995). Indeed, introduced plants often encounter fewer enemies in their introduced range (Mitchell & Power 2003; Liu & Stiling 2006; van Kleunen & Fischer 2009), although there

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is no consistent evidence that this translates into a reduction in damage, nor leads to an increase in performance (Chun, van Kleunen & Dawson 2010). Furthermore, there is also little evidence for the EICA in invaders (Felker-Quinn, Schweitzer & Bailey 2013). However, the limited number of studies and heterogeneity in approaches could have jeopardized the results of these meta-analyses.

The heterogeneity among studies may stem from the way competitive ability has been inferred. Support for EICA in invaders often comes from common-garden experiments that inferred competitive ability indirectly from differences in size between invasive-range and conspecific native-range plants in the absence of competition (Buschmann, Edwards & Dietz

2005; Stastny, Schaffner & Elle 2005; Oduor *et al.* 2011; also see Felker-Quinn, Schweitzer & Bailey 2013). On the other hand, direct approaches that assessed effects of pairwise inter-specific competition between invasive-range and conspecific native-range populations vs. individual members of the invaded community have provided mixed support for the EICA hypothesis (support: Zou, Rogers & Siemann 2007; no support: Leger & Rice 2003; Blair & Wolfe 2004; Bossdorf *et al.* 2004; Gruntman *et al.* 2013). However, as plants typically compete in multispecies plant communities (Callaway & Aschehoug 2000; Catford, Jansson & Nilsson 2009; Heard & Sax 2013; Oduor 2013; Metlen & Callaway 2015), it may be more realistic to evaluate competitive ability in a community context (Oduor, Stift & van Kleunen 2015).

Although escape from herbivory is at the core of the predictions of the EICA hypothesis, the interaction between herbivory and competitive ability of invasive and conspecific native-range plants has rarely been considered (Felker-Quinn, Schweitzer & Bailey 2013; Oduor, Stift & van Kleunen 2015; Zheng *et al.* 2015). When damaged by a community of shoot herbivores, invasive-range *B. nigra* plants were little affected by pairwise competition with legumes, grasses and conspecifics, whereas their native-range conspecifics became more competitive (Oduor *et al.* 2013). Such a pattern did not emerge under competition in a community setting, when *B. nigra* plants were damaged by the specialist root herbivore *Delia radicum* (Oduor, Stift & van Kleunen 2015). Clearly, there is a need for studies that aim to understand the interplay between herbivory and competitive ability and its consequences for post-introduction evolution.

Such studies should consider both root and shoot herbivory because both processes may interactively influence plant fitness (Maron 1998; Johnson, Erb & Hartley 2016). Root herbivory may limit water and nutrient uptake, disrupt water and nutrient transport within the plant, and reduce storage of photo-assimilates (Bazzaz *et al.* 1987; Knapp & Rice 1998; Blossey & Hunt-Joshi 2003; Zvereva & Kozlov 2012). Shoot herbivory may reduce photosynthetic tissues (Hambäck 2001) and reproductive output (Karban & Strauss 1993; Zangerl *et al.* 2002). Hence, the joint negative effects of root and shoot herbivory may be more detrimental to individual plant fitness than the sum of their separate effects (Zvereva & Kozlov 2012). Synergistic effects of root and shoot herbivory were not found with regard to competitive ability of native- and invasive-range populations of *Chromolaena odorata* (Zheng *et al.* 2015), but we are unaware of any other such studies. Since root and shoot herbivory often act simultaneously (Blossey & Hunt-Joshi 2003; Bezemer & van Dam 2005; Coverdale, Altieri & Bertness 2012), it is essential that more studies jointly test the effects of root and shoot herbivory on competitive abilities of invasive and conspecific native-range populations.

Here, we tested whether potential escape from specialist root and shoot herbivory in the invasive range of *B. nigra* has led to increased competitive ability within a community setting. Specifically, we manipulated competition and specialist above-ground and below-ground herbivory on *B. nigra* plants

from eight native-range and eight invasive-range populations to address the following questions: (i) Do competitive responses (i.e. sensitivity to the impacts of neigbours) of invasive and native-range *B. nigra* populations differ, and does this difference depend on the presence of specialist root and shoot herbivores? (ii) Do the competitive effects (i.e. suppressive effects on neighbours) on the competing community depend on the origin of *B. nigra* (invasive or native) and on the presence of specialist root and shoot herbivores of *B. nigra*?

Materials and methods

STUDY SPECIES AND SEED SOURCES

Brassica nigra (Brassicaceae) (L.) W. D. J. Koch is a self-incompatible annual herb native to Europe, Asia and North Africa. It was introduced to North America approximately 200 years ago (Bell & Muller 1973; Feeny & Rosenberry 1982; Westman & Kresovich 1999), likely from multiple populations in the native range (Oduor *et al.* 2015). *Brassica nigra* has become invasive in certain regions of North America where it often forms dense monospecific stands (Lankau & Strauss 2008).

We used bulked seed samples collected from several maternal plants in eight invasive-range and eight native-range populations of *B. nigra*. Seeds from five competitor species that co-occur with *B. nigra* (grasses – *Elymus glaucus* and *Nassella pulchra*; forbs: *Medicago lupulina*, *Sonchus oleraceus* and *Achillea millefolium*) were obtained directly from the field or from seed germplasm collections (see Tables S1 and S2, Supporting Information). To avoid maternal carry-over effects, we raised 10 *B. nigra* plants from each of the 16 populations in a common greenhouse environment to produce bulked seeds for each plant fathered by multiple plants from the same population. These seeds were used for the experiment.

As a root herbivore, we used *D. radicum* (L.) (Diptera: Anthomyiidae), a Brassicaceae-specialist native to Europe (van Dam *et al.* 2012). Although reported as a serious introduced pest of Brassicaceae crops along the north-eastern coast of North America (Newfoundland, Canada) (Biron *et al.* 2003; Dixon *et al.* 2004), there have been no reports of *D. radicum* in natural invasive-range populations of *B. nigra*. Invasive *B. nigra* has thus likely escaped *D. radicum*. As a shoot herbivore, we used *Plutella xylostella* (Linnaeus) (Insecta: Lepidoptera: Plutellidae), a Brassicaceae specialist (Sarfraz, Dosdall & Keddie 2007) native to Europe but with a world-wide distribution (Talekar & Shelton 1993; Sarfraz, Keddie & Dosdall 2005). Although *P. xylostella* has been found in parts of the invasive range of *B. nigra* in California, USA (Oduor *et al.* 2013), we used it as a surrogate for many of the other specialist shoot herbivores that *B. nigra* may have escaped from.

EXPERIMENTAL DESIGN

We performed a common-garden experiment in the botanical garden of the University of Konstanz (Germany) comparing performance of *B. nigra* plants from the eight invasive- and eight native-range populations. To test whether invasive plants differed in their response to competition, we grew plants without competition (one *B. nigra* plant alone) and with competition from a community (*B. nigra* surrounded by *A. millefolium*, *E. glaucus*, *N. pulchra*, *M. lupulina* and *S. oleraceus*, randomly assigned to one of five positions at equal distances).

These two competition levels were crossed with two levels of root herbivory (with and without the specialist root herbivore *D. radicum*) and crossed with two levels of shoot herbivory (with and without the specialist shoot herbivore *P. xylostella*). Thus, our design was fully crossed, with 3 two-level factors, resulting in eight treatment combinations. We had six replicates for each of these eight treatment combinations, which resulted in 768 experimental pots (i.e. 8 treatment combinations × 16 populations × 6 replicates). In addition, we grew 48 replicates of the community of five competitor species (five individual plants per pot) without competition from *B. nigra*. The 816 (i.e. 768 + 48) experimental pots were assigned to 24 cages (2·5 × 2·5 × 2·0 m, L × W × H). The 24 cages were outside in the botanical garden of the University of Konstanz (Germany) and arranged in three rows of eight separated by 0·3 m. Each of the eight treatment combinations was randomly assigned to one of the eight cages per row (i.e. three cages per treatment combination). To prevent the ingress of insects and keep the deliberately released insect herbivores within the cages, cage-sides were lined with a white nylon fabric (mesh size: 0·2 × 0·2 mm; McStoff. de), cage-tops with a netting material (mesh size: 0·8 × 0·8 mm; FA.BIO 02, Hartmann-Brockhaus, Egenburg, Germany), cage-floors with plastic sheeting and pots were placed in hard plastic trays. Light levels in the cages were ~80% of those outside.

Within each cage, there were 32 pots with *B. nigra* (each *B. nigra* population was represented twice within each cage), plus 2 of the 48 pots in which the community was grown alone without *B. nigra*. The 34 pots were randomly assigned to positions spaced 0·4 m apart. Plants were watered once a day in April and May, and thereafter twice a day (morning and afternoon) until harvest time in July. Watering was standardized by filling the trays placed beneath each pot using a hose pipe until saturation.

PRE-CULTIVATION OF *B. NIGRA* AND COMPETITOR PLANTS AND INITIAL RELEASE OF HERBIVORES

On 17 April 2014, seeds of the five competitor species *A. millefolium*, *E. glaucus*, *N. pulchra*, *M. lupulina* and *S. oleraceus* were sown in plastic plug-trays filled with a commercial potting soil (Profi-Classic CL-T, Gebr. Patzer GmbH & Co. KG, Sinntal, Germany) in a phytochamber (12 h-21 °C/12 h-17 °C day/night cycle, 90% humidity). Because *B. nigra* seeds germinate 5 days earlier than the competitors (Oduor, Stift & van Kleunen 2015), *B. nigra* seeds were sown a week later. On 11 and 12 May 2014, we transplanted seedlings to 2·5-L plastic round pots with potting soil with 10 g of a slow-release fertilizer (Osmocote Classic 14 % N, 14 % P_2O_5, 14 % K_2O; Scotts, Geldermalsen, The Netherlands).

To impose the two levels of specialist root herbivory (with or without), we obtained eggs of the specialist root herbivore *D. radicum* from a laboratory culture and subsequently infested the *B. nigra* plants (4 weeks old) that had been assigned to the 'with root herbivory' treatment level. This was done by placing eight *D. radicum* eggs around the root collar of the *B. nigra* plant (see Oduor, Stift & van Kleunen 2015; for details), which is the usual place for oviposition, from where emerged larvae burrow into the soil to feed on roots until they pupate (van Dam *et al.* 2012). A pilot experiment confirmed that the root herbivore indeed caused feeding damage in our experiment (Fig. S1).

To impose the two levels of specialist shoot herbivory (with and without), we obtained *P. xylostella* larvae from a laboratory culture and subsequently infested the *B. nigra* plants (4 weeks old) that had been assigned to the 'with shoot herbivory' treatment level (note that

half of these plants had also been infested with the specialist root herbivore). We achieved infestation by placing four 1st-instar and four 2nd-instar larvae of *P. xylostella* on the leaves. Damage by *P. xylostella* larvae was visually confirmed (Fig. S2).

To simulate herbivory as it would occur under natural field conditions, the *P. xylostella* and *D. radicum* larvae were not controlled after their initial release, so that the experimental plants that were assigned to the treatment combinations with herbivores (i.e. root herbivory alone, shoot herbivory alone and simultaneous root and shoot herbivory) were exposed to multiple generations of the herbivores.

MEASUREMENT OF *B. NIGRA* PERFORMANCE AND COMMUNITY PRODUCTIVITY

At harvest (after 3·5 months of growth), we assessed the performance of individual *B. nigra* plants. For each plant, we assessed whether it flowered. Furthermore, for plants that flowered, we counted how many flowers (and fruits) had been produced until harvest. We determined shoot biomass, which is a good predictor of seed yield in annual plants in general (Shipley & Dion 1992) and in *B. nigra* (Oduor *et al.* 2011, 2013). We also assessed the community productivity (i.e. shoot biomass of the five competitor species, not including that of *B. nigra*). All plant tissue was dried to a constant weight at 70 °C for 72 h prior to weighing.

STATISTICAL ANALYSIS

To test whether specialist root and shoot herbivory (separately and simultaneously), competition, and *B. nigra* range of origin (native or invasive), and their interactions had significant effects on performance of *B. nigra* plants, we used generalized linear mixed-effects models in R v3.3.2 (R Development Core Team 2013) through R-Studio v0.99.484 (RStudio Team 2016). To analyse the above-ground biomass (for all 743 *B. nigra* plants that survived, out of 768), we fit the models using maximum likelihood with the *lme* function in the *nlme* package (Pinheiro *et al.* 2007) with a Gaussian distribution (data were square-root transformed [$Y^{0.5}$] to assure normality of residuals and homogeneity of variance). To analyse the number of flowers and siliques (for the 467 plants that had flowered by the end of the experiment), we also used a Gaussian distribution (data were power-transformed [$Y^{0.3}$] to assure normality of residuals and homogeneity of variance). To analyse the proportion of *B. nigra* plants that had flowered at the end of the experiment (743 plants), we used the *glmer* function in the *lme4* package (Bates 2011) with a binomial (link = 'cloglog') distribution using the *bobyqa* optimizer. The fixed part of all models included four two-level factors, reflecting the fully crossed design of our experiment: *B. nigra* range (invasive vs. native), competition (with vs. without a community), root herbivory (absence vs. presence of a specialist root herbivore) and shoot herbivory (absence vs. presence of a specialist shoot herbivore) and all possible interactions. The random part of the model included *B. nigra* population and cage.

To test whether specialist root and shoot herbivory, competition and range of origin, and their interactions had a significant effect on the competitive effects of *B. nigra* on the community of five competitor species, we used a linear mixed-effect model with community biomass (*n* = 384, excluding *B. nigra*) as the dependent variable and a Gaussian distribution. This analysis was done for the subset of pots in which *B. nigra* was grown with the community. Apart from excluding the fixed factor competition, the models were the same as above.

To test the general effect of the presence of *B. nigra* on community biomass, we selected the subset of cases where the community was grown alone and with *B. nigra* but without herbivory (*n* = 144), and used a linear mixed-effects model. The model fixed part included *B. nigra* presence (absent vs. present) and *B. nigra* range (invasive vs. native; fitted sequentially after presence of *B. nigra*), while cage was treated as the random part of the model.

For all models, we assessed the significance of the interactions and main effects by sequentially removing them from the full model starting with the highest order interactions (four-way) and using likelihood ratio tests to compare the model with the effect removed to their counterparts including the effect. In cases where there were significant interactions between main effects, we used the *glht* command in the *multcomp* package (Hothorn, Bretz & Westfall 2008) to perform *post hoc* least squares means comparisons between factor-level combinations.

Results

ABOVE-GROUND BIOMASS OF *B. NIGRA*

Both root and shoot herbivory negatively affected *B. nigra* above-ground biomass, and the combined effect of root and shoot herbivory was more severe than the sum of the separate effects (significant root herbivory × shoot herbivory [RH × SH] interaction; Fig. 1a and Table S3). Competition also had a negative effect on *B. nigra* biomass, which was stronger without root herbivory than with root herbivory (−50% vs. −43%; significant competition [C] × RH interaction; Fig. 1b). Without shoot herbivory, the effect of competition was stronger in native plants, because native plants performed better (produced more biomass) than invasive plants when grown alone, but not when competing in a community (significant R × C × SH interaction; Fig. 1c). With shoot herbivory, invasive- and native-range plants produced similar biomass without competition, and the two groups of

plants had a similar reduction in biomass due to competition (Fig. 1c).

FLOWERING AND FLOWER PRODUCTION BY *B. NIGRA*

Both types of herbivory had a significant negative effect on the proportion of plants that flowered, and the effect of shoot herbivory was much stronger than that of root herbivory (94% without vs. 30% with shoot herbivory; 65% without vs. 60% with root herbivory; Fig. 2a,b; Table S3). However, root and shoot herbivory did not interact (Table S3). Competition also had a negative effect on the proportion of plants that flowered. This effect was slightly stronger for invasive plants (71% without competition vs. 60% with competition) than for native plants (61% without competition vs. 59% with competition; significant R × C interaction; Fig. 2c). The effect of competition on the proportion of plants flowering did not depend on specialist shoot and root herbivory (Table S3).

Within the subset of flowering plants (*n* = 467), the effect of competition on the number of produced flowers and fruits per individual depended on shoot herbivory (significant C × SH interaction; Fig. 2d). Without shoot herbivory, competition reduced mean flower production by 40% (456 flowers and fruits without vs. 271 with competition). With shoot herbivory, however, competition increased flower production by 42% (74 flowers and fruits without vs. 105 flowers and fruits with competition; Fig. 2d). There were no differences between *B. nigra* plants from the native vs. invasive range, and none of the other factors interacted with range (Table S3).
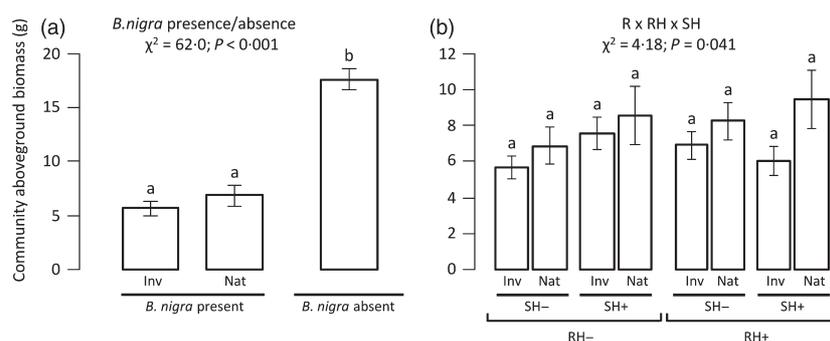
COMMUNITY BIOMASS

In the subset of plants grown without herbivory on *B. nigra* (*n* = 144), the community produced significantly less biomass
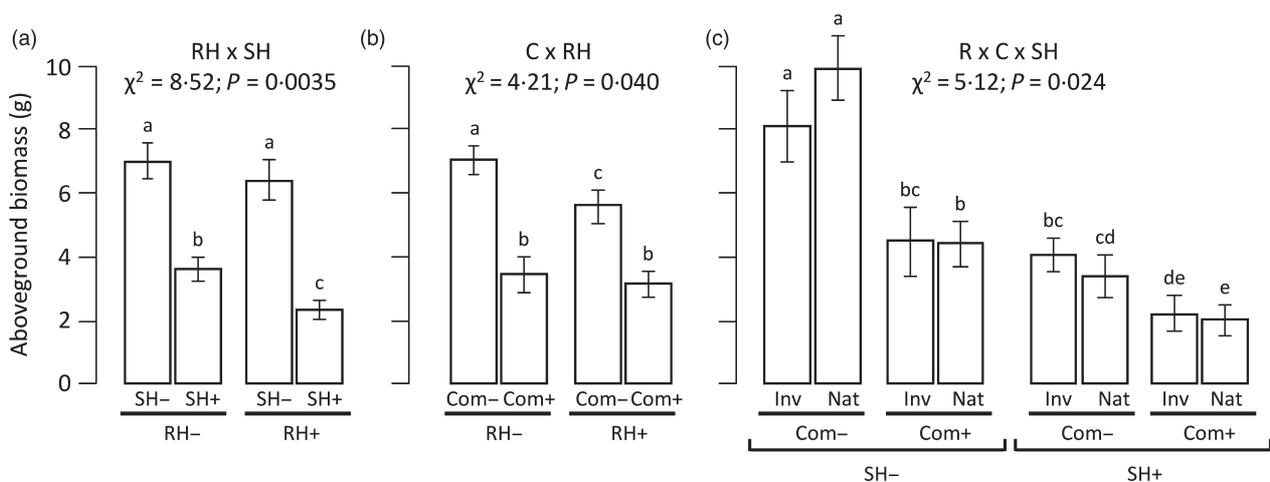
Fig. 1. Mean (±1SE) *Brassica nigra* above-ground biomass illustrating significant effects of the: (a) interaction between root herbivory by *Delia radicum* (absence [RH−] or presence [RH+]) and shoot herbivory by *Plutella xylostella* (absence [SH−] or presence [SH+]); (b) interaction between competition (absence [Com−] or presence [Com+] of *Elymus glaucus*, *Nassella pulchra*, *Medicago lupulina*, *Sonchus oleraceus* and *Achillea millefolium*) and root herbivory; (c) interaction between *B. nigra* range (R: invasive/native), competition and shoot herbivory. For each given combination of factor levels, the means and SEs are based on *B. nigra* population means calculated across all the levels of other factors. Significance of all main and interactive effects was determined by log-likelihood ratio tests (cf. Table S3). Letters above bars indicate the results of *post hoc* least squares means comparisons (bars that do not share a letter are significantly different).
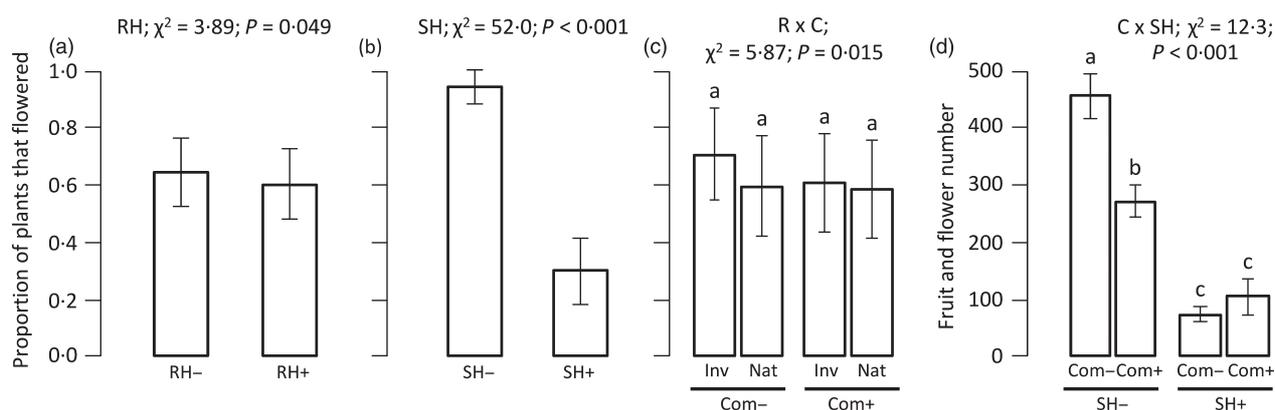
Fig. 2. Mean (±1SE) proportion of *Brassica nigra* plants that flowered (a–c) and total fruit and flower count per individual *B. nigra* plant (d). For proportion of plants that flowered, the panels show significant effects of: (a) root herbivory by *Delia radicum* (absence [RH−] or presence [RH+]); (b) shoot herbivory by *Plutella xylostella* (absence [SH−] or presence [SH+]); (c) the interaction between *B. nigra* range (R: invasive/native) and competition (absence [Com−] or presence [Com+] of *Elymus glaucus, Nassella pulchra, Medicago lupulina, Sonchus oleraceus* and *Achillea millefolium*). For fruit and flower number (d), the panel shows significant effects of the interaction between competition and shoot herbivory. For each given (combination of) factor level(s), the means and SEs are based on *B. nigra* population means calculated across all the levels of other factors. Significance of all main and interactive effects was determined by log-likelihood ratio tests (cf. Table S3). Letters above bars indicate the results of *post hoc* least squares means comparisons (bars that do not share a letter are significantly different).

Fig. 3. Mean (±1SE) above-ground biomass yield of a community of five species (*Elymus glaucus, Nassella pulchra, Medicago lupulina, Sonchus oleraceus* and *Achillea millefolium*) illustrating significant effects of: (a) the absence vs. presence of invasive-range and native-range *Brassica nigra* plants; (b) the three-way interaction between *B. nigra* range (R: invasive/native), root herbivory by *Delia radicum* (absence [RH−] or presence [RH+]) and shoot herbivory by *Plutella xylostella* (absence [SH−] or presence [SH+]). Herbivores only attacked *B. nigra*, as they are Brassicaceae specialists. For each given combination of factor levels, the means and SEs are based on population means calculated across all the levels of other factors. Significance of all main and interactive effects was determined by log-likelihood ratio tests (cf. Table S4). Letters above bars indicate the results of *post hoc* least squares means comparisons (bars that do not share a letter are significantly different).

when competing with *B. nigra* (−64%; log-likelihood ratio test: $\chi^2 = 62 \cdot 0$, d.f. = 1, $P < 0 \cdot 001$; Fig. 3a). The origin of *B. nigra* (invasive or native) had no significant effect ($\chi^2 = 2 \cdot 70$, d.f. = 1, $P = 0 \cdot 10$, Fig. 3a).

In the subset where the community competed with *B. nigra* while manipulating shoot and root herbivory on *B. nigra* ($n = 384$), the community biomass depended on the interaction of shoot and root herbivory, and the range of origin of *B. nigra* (significant R × RH × SH interaction; Fig. 3b; Table S4). When neither or only one of the herbivore species was present, the community biomass was little affected by the origin of the *B. nigra* plants (Fig. 3b). However, when competing with *B. nigra* under attack by root and shoot herbivores simultaneously, the community produced 36% less biomass when competing with invasive-range *B. nigra* than when competing with native-range *B. nigra* (Fig. 3b).

Discussion

The EICA hypothesis poses that, due to release from specialist enemies, plants from invasive-range populations of a species should evolve a higher growth potential and thus competitive ability at the cost of defences against specialist herbivores (Blossey & Nötzold 1995; Keane & Crawley 2002; Joshi & Vrieling 2005). Therefore, the EICA hypothesis predicts that in the absence of specialist herbivores, plants from the invasive range should outperform conspecific plants from the native range, whereas in the presence of specialist herbivores the reverse should be true. However, we did not find such a difference in performance between native- and invasive-range *B. nigra* populations. Furthermore, opposing the predictions of the EICA hypothesis, when *B. nigra* competed with a community, the negative

effect of specialist shoot herbivory by *P. xylostella* did not differ in magnitude between native- and invasive-range *B. nigra* plants. Without competition, specialist shoot herbivory even had a stronger negative effect on native- than on invasive-range *B. nigra* plants (Fig. 1c). Although *P. xylostella* is considered a specialist herbivore (Sarfraz, Dosdall & Keddie 2007), it has a world-wide distribution (Talekar & Shelton 1993). Therefore, despite lower numbers of specialist herbivores on *B. nigra* in North America than in Europe (Oduor *et al.* 2013), a possible explanation for our finding is that *B. nigra* might not have escaped *P. xylostella* in a large part of its invasive range, and might even be exposed to it more frequently.

The differences in above-ground biomass production and flowering between invasive- and native-range plants of *B. nigra* disappeared when they were grown in a community of five competitors (Figs 1c and 2c). Although this means that the invasive-range plants were more responsive to the presence–absence of competition than native-range plants, their equal performance in the presence of competitors indicates that the invasive-range plants did not have a higher competitive ability. The few other studies that have examined the EICA prediction of increased competitive ability in invasive-range plants by manipulating competitive environments have produced mixed results, with some finding support for the prediction and others not (reviewed in Oduor, Stift & van Kleunen 2015). However, those studies grew invasive-range and conspecific native-range plants only in pairwise competition and without manipulating herbivory (but see Oduor, Stift & van Kleunen 2015).

Our experiment shows that the negative response to competition with a community was not as strong when invasive and native-range *B. nigra* were attacked by the specialist root herbivore *D. radicum* (Fig. 1b, confirming earlier findings in the same study system (Oduor, Stift & van Kleunen 2015) and by the specialist shoot herbivore *P. xylostella* (Fig. 2d). Overall, the damage done by the shoot herbivore *P. xylostella* appeared to be stronger than that of the root herbivore *D. radicum* (Fig. 1a; also compare Fig. 2a with b). This appears to contradict suggestions that root herbivory generally has a stronger impact on plant growth and fitness than shoot herbivory (Brown & Gange 1990; Maron 1998; Wardle *et al.* 2004; Zvereva & Kozlov 2012), but may simply be a consequence of the fact that we only assessed above-ground performance measures, which are obviously more directly affected by shoot herbivory than by root herbivory. Although it is inherently difficult to physically disentangle roots in communities (Rewald *et al.* 2012), it would be of interest for future studies to also examine below-ground measures of performance (root biomass).

When grown in a community with five other species, invasive-range *B. nigra* plants had stronger suppressive effects on the community than native-range *B. nigra* plants, but only significantly in the presence of both root and shoot herbivory (Fig. 3b). This difference in competitive effect provides further evidence against the EICA hypothesis, which rather predicts increased suppressive effects of invasive plants on other species in the absence of herbivory (Blossey & Nötzold 1995). A possible explanation could be that invasive populations have adapted to new enemies by producing chemicals for plant defence that also affect competitive interactions with other plant species (Siemens *et al.* 2002; Thelen *et al.* 2005; Zheng *et al.* 2015). Like many Brassicaceae, *B. nigra* deters herbivores using glucosinolates, a class of secondary compounds (Feeny & Rosenberry 1982). In *B. nigra*, sinigrin (allyl-glucosinolate) accounts for 90–99% of the total glucosinolate concentration in plant tissues (Feeny & Rosenberry 1982; Traw 2002). Sinigrin also mediates competitive interactions between *B. nigra* and heterospecific neighbours through allelopathy; high sinigrin-expressing *B. nigra* genotypes suppress heterospecific neighbours more than low sinigrin-expressing *B. nigra* genotypes (Lankau & Strauss 2007; Lankau & Kliebenstein 2009). Sinigrin is expressed constitutively in tissues of Brassicaceae, but herbivory can induce higher sinigrin expression (Traw 2002; Soler *et al.* 2005). Invasive-range *B. nigra* populations have been shown to express significantly higher concentrations of leaf sinigrin than native-range *B. nigra* populations (Oduor *et al.* 2011). Increased expression of root sinigrin may therefore be a plausible explanation of the increased suppressive effects of invasive-range *B. nigra* plants after induction by the joint effects of root and shoot herbivory, but future studies should test this hypothesis.

Conclusions

In this study, we tested whether the EICA hypothesis holds for *B. nigra*. Unlike previous work, we assessed competitive responses and effects in an experimental multispecies community, while manipulating specialist root and shoot herbivory. Opposite to EICA predictions, invasive- and native-range *B. nigra* plants hardly differed in performance when grown in a community with five competitors. However, in a competition-free environment, invasive-range plants produced more biomass than native-range ones when attacked by the specialist shoot herbivore *P. xylostella*, whereas the reverse was true in the absence of shoot herbivores. Despite the absence of a difference in above-ground biomass between invasive- and native-range *B. nigra* when grown with competitors, the competitors themselves were more negatively affected by the invasive-range *B. nigra* than by native-range *B. nigra*, particularly when both shoot and root herbivores were present. Although these differences in biomass production and flowering under common environmental conditions do suggest that the invasive-range plants have undergone evolutionary change, most of the differences between native- and invasive-range plants were not in line with the predictions of the EICA hypothesis.

Authors' contributions

Acknowledgements

Data accessibility

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.gt0m3 (Oduor, van Kleunen & Stift 2017).

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Supporting Information

Details of electronic Supporting Information are provided below.

Fig. S1. Roots of individual *Brassica nigra* plants damaged (left) or undamaged (right) by larvae of a specialist root herbivore (*Delia radicum*).

Fig. S2. Individual *Brassica nigra* plants (4 weeks old) damaged (left) or undamaged (right) by larvae of a specialist shoot herbivore (*Plutella xylostella*). *Brassica nigra* was grown with competition (top panel) or alone without competition (bottom panel).

Table S1. *Brassica nigra* seed sources for the current experiment. Populations marked by '†' were obtained from United States Department of Agriculture (USDA) GRIN germplasm collection.

Table S2. Seed sources of five competitor species used in the current experiment.

Table S3. Results of likelihood ratio model comparisons of linear and generalized linear mixed-effects models to test whether *Brassica nigra* range (invasive vs. native), competition with a community (absence vs. presence), specialist root and shoot herbivory on *B. nigra* (absence vs. presence) and their interactions had a significant effect on above-ground biomass yield of *B. nigra*, the proportion of *B. nigra* plants that flowered, and flower and fruit number per *B. nigra* plant.

Table S4. Likelihood ratio model comparisons of linear mixed-effects models to test whether range of *Brassica nigra* (invasive or native), root herbivory and shoot herbivory on *B. nigra* and their interactions had a significant influence on above-ground biomass yield of a community of five competitor species (*Elymus glaucus*, *Nasella pulchra*, *Medicago lupulina*, *Sonchus oleraceus* and *Achillea millefolium*).