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Research

Relatedness with plant species in native community influences ecological consequences of range expansions

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Global warming is enabling many plant species to expand their range to higher latitudes and altitudes, where they may suffer less from natural aboveground and belowground enemies. Reduced control by natural enemies can enable climate warming-induced range expanders to gain an advantage in competition with natives and become disproportionately abundant in their new range. However, so far studies have only examined individual growth of range expanders, which have common congeneric plant species in their new range. Thus it is not known how general is this reduced effect of above- and belowground enemies and how it operates in communities, where multiple plant species also interact with each other. Here we show that range-expanding plant species with and without congenics in the invaded habitats differ in their ecological interactions in the new range. In a community-level experiment, range-expanding plant species, both with and without congenics, suppressed the growth of a herbivore. However, only range expanders without congenics reduced biomass production of the native plant species. In the present study, range expanders without congenics allocated more biomass aboveground compared to native plant species, which can explain their competitive advantage. Competitive interaction and also biomass allocation of native plants and their congeneric range expanders were similar. Our results highlight that information about species phylogenetic relatedness with native flora can be crucial for improving predictions about the consequences of climate warming-induced range expansions.

Keywords: climate change, range shifts, phylogenetic relatedness, competition, plant–herbivore interaction, plant–soil interaction

Introduction

Current rapid climate change is expected to strongly influence the composition and functioning of natural ecosystems (Pecl et al. 2017). However, the complex nature of

species responses to climate change complicates predictions (Tylianakis et al. 2008), and hampers adaptation and mitigation measures. Plant, animal and microbial species respond to climate change by a variety of mechanisms, including plasticity, genetic adaptation, or range expansion to higher altitudes and latitudes where climate conditions used to be cooler (Walther et al. 2002, Parmesan and Yohe 2003, Lenoir et al. 2008). Species range expansion is widely occurring and has been documented for numerous organisms (Walther et al. 2002, Parmesan and Yohe 2003). Recent study has highlighted that range expanding species can change ecosystem functioning in their new range, which can influence human well-being and, ultimately, can feed back to the course of climate change (Pecl et al. 2017). However, our understanding about the ecological consequences of range expansions is still limited.

Intracontinentally range expanding plant species can, similar to intercontinentally introduced exotics, benefit from escaping co-evolved above- and belowground enemies (Engelkes et al. 2008, Dostálek et al. 2016), which are expected to be absent in their new range (conceptualized by Bardgett and van der Putten 2014). This similarity between range expanders and introduced exotics enables the testing of mutually developed concepts. For example, the success of introduced exotic plant species may depend on the relatedness between the invader and native flora (Strauss et al. 2006, Schaefer et al. 2011, Bezeng et al. 2015, Li et al. 2015). So far, however, it is unknown whether relatedness with the invaded community may also influence the success and impact of plant species that expand their range within a geographic region because of climate warming.

Darwin (1859) already highlighted the importance of relatedness between exotic species and native species that are present in the invaded communities; however, the increasing empirical evidence is still not conclusive. Several studies have recorded that introduced exotic species with only distant relatives in the native flora have higher ecological impact (Strauss et al. 2006, Schaefer et al. 2011, Bezeng et al. 2015). The characteristics of distantly related exotics are expected to be distinctive from native species, which enables them to occupy new niches, host fewer natural enemies and become successful in their new range (Darwin 1859, Diez et al. 2008). At the same time, some studies have also recorded higher success of exotics that are closely related to species in the native flora of the invaded range (Duncan and Williams 2002, Park and Potter 2013, Li et al. 2015). Exotics with close relatives in their new range can benefit from pre-adaptation to the local abiotic conditions (Darwin 1859, Duncan and Williams 2002) when it outweighs the disadvantage of possible control by natural enemies from closely related native species (Gilbert and Webb 2007).

It is increasingly recognized that the growth of plants is tightly linked with soil organisms (Wardle et al. 2004, van der Heijden et al. 2008, Bardgett and Wardle 2010). Soil communities are highly diverse, where some organisms can suppress (e.g. root-feeding nematodes and pathogens) and

others promote plant growth (e.g. mycorrhizal fungi). Thus, soil organisms can influence the abundance of plant species (Klironomos 2002), as well as the interactions between plants and their aboveground herbivores (Bezemer and van Dam 2005, van Dam and Heil 2011). Such well-established aboveground–belowground interactions can change when a new species, especially one with distinctive physiological traits from the native flora, invades an ecosystem (Wardle et al. 2004).

The success of plant species can be strongly influenced by competitors (Strauss and Irwin 2004), especially in the case of range-expanding plants, who face novel neighbours in their new range (Alexander et al. 2015). However, with a few exceptions (Meisner et al. 2013, Engelkes et al. 2016), most current studies that have examined biotic interactions of range-expanding plant species have been performed by exposing congeneric range-expanding and native plant species individually to aboveground and belowground biota (Engelkes et al. 2008, Morriën and van der Putten 2013, Dostálek et al. 2016). In the present study we tested the hypothesis that range expanders without congeneric natives in invaded habitat in their new range (hereafter: unrelated range expanders) have stronger ecological impact than range expanders which face congeneric species in invaded habitat (hereafter: related range expanders). Additionally we examined if soil communities from the original and new range of range expanders influence the ecological impact of these plants. We performed a multi-species community experiment and estimated the ecological impact of range expanders by measuring the growth of four native plant species and one herbivorous insect species growing with unrelated and related range expanders and with soil communities from different ranges.

Methods

Test species

We tested the impact of range-expanding plant species using twelve plant species that all co-occur in north-western Europe in riverine habitats along the river Rhine-Waal. We used presence or absence of a native species from the same genus in north-western Europe as an indication for close and distant relatedness between range expanding plant species and native flora in the new range. *Bunias orientalis*, *Dittrichia graveolens*, *Lactuca serriola* and *Rapistrum rugosum* are range expanders that do not have common congeneric plant species invaded communities in their new range (named: ‘unrelated range expanders’). *Centaurea stoebe*, *Geranium pyrenaicum*, *Tragopogon dubius* and *Rorippa austriaca* are range expanders, which can encounter closely related species in invaded communities in their new range (named: ‘related range expanders’). The native plant species are *Centaurea jacea*, *Geranium molle*, *Tragopogon pratensis* subsp. *pratensis* and *Rorippa sylvestris* (named: ‘natives’). The range of the native plant species stretches from south-eastern Europe, where the range expanders originate from, to north-western Europe.

All range-expanding plant species have been present in the Netherlands at least since 2009 (Sparrius 2014). Seeds of all plant species were collected from the Netherlands, either by ourselves or by the company Cruydt-Hoeck Wildebloemenzaden (Nijeberkoop, the Netherlands; Supplementary material Appendix 1 Table A1).

We selected a generalist leaf-chewing insect, *Mamestra brassicae* as an herbivore, because this migratory butterfly is native throughout Europe and its larvae feed on a wide variety of food plants (Theunissen et al. 1985, Macel et al. 2014). Eggs of *M. brassicae* were obtained from an insect culture of the Laboratory of Entomology at Wageningen University and Research (the Netherlands). Larvae of *M. brassicae* were reared at NIOO-KNAW on artificial food in a climate-controlled room at $22 \pm 2^\circ\text{C}$, with a light regime of 16:8 L/D.

Soil collection

We collected soil from both the original and new ranges of the range-expanding plant species. As the original range is larger than the new range, we selected two areas, one in Slovenia and one in Austria, to represent this range. The riparian area along the Rhine/Waal in the Netherlands served as the new range. In both ranges, we randomly selected three locations of approximately 30 ha riverine area with sandy loam soil. Within each location we randomly chose three sub-locations of 2 ha each, where the soil was collected from the top 3–15 cm layer in July 2014. Soil from each location was separately stored cool at $4\text{--}8^\circ\text{C}$, and transported to the laboratory where we sieved all soil samples using a 4 mm mesh to remove roots, stones, macroinvertebrates and earthworms.

We used six kg of soil from each of the 18 sub-locations from Slovenia and Austria and 12 kg from each of the nine sub-locations from the Netherlands as soil inoculum. The soil inoculum of each sub-location was carefully homogenized (while kept separated from the others) and thereafter split into two halves. One-half of homogenized inoculum from each sub-location was stored at 4°C until the start of the experiment. The other half was sterilized by gamma irradiation (>25 kGray) at Isotron (Ede, the Netherlands). From each sub-location in the Netherlands, we collected an additional amount of 95 kg of soil to serve as background soil to which the soil inocula were added. This background soil (855 kg in total) was sieved, pooled, homogenized and sterilized as indicated above before being inoculated.

Experimental setup

To test our hypothesis, we established five types of plant communities: 1) a mixture of natives and related range expanders, 2) a mixture of natives and unrelated range expanders, 3) only natives, 4) only related range expanders, 5) only unrelated range expanders. We grew plant communities in sterilized soil inoculated with three different live soil communities: 1) from the original range of the range expanders, 2) from the new range of the range expanders, 3) a mixture

of both original and new range, and 4) a control inoculum from sterilized soil. To be able to make broad and non-location-specific conclusions, we created nine soil mixtures, each composed of a mixture of two randomly selected sub-locations from different locations per range (Supplementary material Appendix 1 Table A2). These nine soil mixtures served as independent replicates in our experiment. In order to keep the variation only in the biotic environment, the substrate always consisted of 80% background soil, 10% of live inocula and 10% of sterilized inocula (Supplementary material Appendix 1 Table A3). Before adding the soil, we placed 1.5 kg of gravel (4–8 mm fraction) on the bottom of each 7-l pot with 25 cm diameter to improve drainage.

We created five mesocosms (one for each plant community) for each soil inoculation treatment, which was replicated nine times, leading to 180 pots in total. Fungal and bacterial community composition was analyzed at the start of the experiment, as described in the Supplementary material Appendix 1 Supplementary methods and in Fig. A1.

Seeds of plant species were pre-treated if needed to enhance germination rate (Supplementary material Appendix 1 Table A1), surface-sterilized with a 0.5% hypochlorite solution and germinated under sterile conditions. Pots filled with soil were randomly allocated to one of five plant community treatments described. Eight seedlings were transplanted into each pot in a circular pattern, at pre-determined positions. Mesocosms with one type of plants had four plant species and two seedlings per plant species, whereas the other mesocosms had one seedling from each of the eight plant species. Competition between plants from the same genus was minimized by not planting individuals from the same genus next to each other. Seedlings that died during first two weeks of the experiment were replaced with new ones. Mesocosms were randomly located in a greenhouse at $21/16^\circ\text{C}$ day/night, 16 h photoperiod. Natural daylight was supplemented by 400 W metal halide lamps ($235 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR). The soil moisture level in mesocosms was maintained at 60% of water holding capacity by supplying water three times per week. Once a week, the positions of mesocosms within the greenhouse were randomly re-arranged.

Twelve weeks after transplantation, all mesocosms were individually placed into a fine-meshed cylindrical insect cage (70 cm height, 25 cm diameter). We selected five out of nine replicates of each treatment combination for herbivore treatment (See Supplementary material Appendix 1 Table A4 for schematic description of experimental setup). Two third-instar larvae of *M. brassicae* were introduced in each of the 100 mesocosms selected for exposure to herbivory after the initial weight of larvae was recorded. Thereafter larvae were placed in the middle of each mesocosm in an open petri dish to enable them to freely choose the plants to feed on. Due to the lack of visible damage by *M. brassicae* larvae after four days, two additional third-instar larvae were weighed and placed in the insect mesocosms as described above. All larvae were kept in the mesocosms until harvesting.

In week fourteen all larvae were collected, weighed on a microbalance and their instar stage was recorded. To estimate the performance of *M. brassicae*, we calculated the mean weight gain of larvae per mesocosm (mean weight at the harvest – mean initial weight). Further on, the second and third youngest leaf from each plant was clipped, dried at 70°C until constant weight, weighed and stored in separate paper bag in order to analyze plant chemistry. The rest of the aboveground plant parts in all mesocosms were also clipped, separated by plant individuals, dried as indicated above and weighed. It was not possible to separate the roots of the different plant species. Therefore, all roots present in each mesocosm were carefully removed from the soil, rinsed with water, dried to a constant weight as indicated above, and weighed.

Chemical analysis of plant samples

In all communities larvae of *M. brassicae* preferred to feed on *Brassicaceae* plants, we determined leaf carbon (C) and nitrogen (N) concentrations of only *Brassicaceae* plants. We analyzed the nutrient content of *R. sylvestris*, *R. austriaca* and *B. orientalis* in each pot using a CN-analyzer. As *R. rugosum* only had leaves at the base of the shoots, this species was excluded from the analysis.

Data analysis

All analyses were performed using the R statistical language, packages ‘lmerTest’ (Kuznetsova et al. 2014) and ‘pbkrtest’ (Halekoh and Højsgaard 2014), ver. 3.0.1 (< www.r-project.org >). Prior to analyses at species level, the average biomass of each species in a mesocosm was calculated, plant biomass was log-transformed and the insect weight data were square root-transformed to fulfil the assumptions of normality and homogeneity of variances. To estimate differences in plant growth, we used plant biomass from mesocosms where herbivores had not been introduced (n = 4).

First, we tested the hypothesis that community level growth of range expanding plant species, and especially that of unrelated range expanders, is influenced by soil biota, differing in soil from the original and new range. To test this hypothesis, we analysed above- and belowground community biomass with factorial analyses of variance (ANOVA) including plant community type, soil inocula from the original range, soil inocula from the new range and their interactions as fixed factors. To clarify if the plant community biomass is influenced only by the presence and not the origin of soil biota, we fitted also reduced model with plant community type and inoculation with live soil (with two levels: yes and no, Supplementary material Appendix 1 Table A4) as fixed factors. second, we tested the hypothesis that when considering the biomass of individual species, range expanders, and specifically unrelated range expanders, have more biomass in communities with native plant species. We expected this pattern to be stronger in the presence of soil biota from their new range. To test this hypothesis, we analyzed the average biomass of plant species with similar history in the new range (native, related

expanders or unrelated expanders) separately. For each group, we fitted full mixed-effects models with plant community type, soil inocula from the original range, soil inocula from the new range, plant species identity and their interaction as fixed factors while using pot identity as random factor. To clarify the importance of the presence of soil biota, we fitted a reduced model with plant community type, presence of live soil inoculum and species identity as fixed factors with mesocosm identity as a random factor. In case the effect of treatment combinations depended on species identity (significant three-way interaction: Plant community × Soil biota × Species), we fitted a minimum adequate model separately for each plant species. Third, we tested the hypotheses that herbivores that feed on communities of range expanders, and especially on unrelated range expanders, gain less weight than when they feed on communities of natives. We expected this difference to be stronger when range expanders are growing with soil biota from the original range. To test this, we used ANOVA with same full and reduced models for analyzing herbivore weight gain as we used for analyzing community level plant biomass. For each variable, the explanatory power of full and reduced models was tested by ANOVA, and the results of the minimal adequate model are presented. A Tukey HSD post hoc test was used to estimate differences between the treatments.

To visualize how the cumulative aboveground biomass of native, related range expanders and unrelated range expanders changed with plant community type, we calculated the ratio of biomass of these plants in communities of only same type of plants and in mixtures of natives and range expanders.

Data deposition

Data available from the Dryad Digital Repository: < <http://dx.doi.org/10.5061/dryad.2hn31> > (Koorem et al. 2017).

Results

Plant communities

The minimal adequate model for the response of plant communities included plant community type and presence of live soil biota, suggesting similar effect of soil communities from different ranges. Aboveground biomass was significantly different between plant community types (Table 1), being highest in communities with only unrelated expanders and intermediate in communities with unrelated expanders and natives; aboveground biomass was equally low in communities with only natives, only related expanders and in communities with natives and related expanders (Fig. 1a). Belowground biomass was also significantly different between plant community types (Table 1) but showed exactly the opposite pattern. Belowground biomass was lowest in communities with only unrelated expanders, intermediate in communities with unrelated expanders and natives and equally high in communities with only natives, only related expanders and in communities with natives and related expanders (Fig. 1b).

Table 1. Communities of native plant species, related range expanders and unrelated range expanders differ in their biomass but not in their reaction to soil biota. The results of analyses of variance are given, testing the response of aboveground and belowground biomass of mesocosm to community type (Plant community) and inoculation with live soil communities (Soil biota). Numerator degrees of freedom (Num df), denominator degrees of freedom (Den df), F- and p-values are reported.

Variable	df		Aboveground biomass		Belowground biomass	
	Num	Den	F	p	F	p
Plant community	4	70	75.1	<0.001	65.2	<0.001
Soil biota	1	70	6.50	0.01	10.3	0.002
Plant community \times Soil biota	4	70	0.26	0.90	1.11	0.36

Both above- and belowground biomass of plant communities were significantly influenced by the addition of live soil biota (Table 1), which decreased the biomass.

Individual plant species

The minimal adequate model for the response of the biomass of individual plant species for unrelated expanders, related expanders and natives included mesocosm identity as a random factor and plant community type, species identity and presence of live soil biota as fixed factors. This suggests that there is no significant effect of the origin of soil biota for the growth of individual plant species in this study.

The biomass of the four unrelated expanding plant species differed significantly from each other (Table 2), with *Rapistrum rugosum* having the highest biomass, followed by *Lactuca serriola*, *Bunias orientalis* and *Dittrichia graveolens*. The aboveground biomass of all unrelated range expanders was influenced by plant community type (Table 2), being significantly higher in mixtures with native plants than in communities with only unrelated range expanders (Fig. 2). Inoculation with soil communities did not influence the aboveground biomass of unrelated expanders (Table 2, Supplementary material Appendix 1 Fig. A2).

The aboveground biomass of related range expanders (*Genanium pyrenaicum*, *Centaurea stoebe*, *Tragopogon dubius* and *Rorippa austriaca*) depended on the interaction between plant species identity, plant community type and the presence of live soil biota (Table 2). The biomass of *G. pyrenaicum* was not influenced by plant community type ($F_{1,28}=0.06$, $p=0.82$, Fig. 2b). Presence of soil biota significantly increased aboveground biomass of *G. pyrenaicum* ($F_{1,28}=11.7$, $p=0.002$, Supplementary material Appendix 1 Fig. A2). There was a significant interaction between plant community type and presence of soil biota ($F_{1,28}=9.88$, $p=0.004$). Presence of soil biota increased biomass of *G. pyrenaicum* in communities with related range expanders, but not in the communities with native plant species (Tukey HSD test). Biomass of *C. stoebe* and *T. dubius* was not influenced by plant community type ($F_{1,28}=0.09$, $p=0.76$ and $F_{1,28}=0.74$, $p=0.40$, respectively, Fig. 2b). Presence of soil biota significantly increased aboveground biomass of both *C. stoebe* and *T. dubius* ($F_{1,28}=19.1$, $p < 0.001$ and $F_{1,28}=104$, $p < 0.001$, respectively, Supplementary material Appendix 1 Fig. A2) and there was no significant interaction between plant community type and presence of soil biota ($F_{1,28}=0.05$, $p=0.82$

and $F_{1,28}=0.86$, $p=0.36$, respectively). The biomass of *R. austriaca* was not influenced by plant community type ($F_{1,28}=0.57$, $p=0.46$, Fig. 2b), presence of soil biota ($F_{1,28}=3.43$, $p=0.07$) or the interaction between plant community type and presence of soil biota ($F_{1,28}=2.97$, $p=0.10$).

The aboveground biomass of native plant species differed in their response to plant community type with the negative effect of unrelated range expanders being significant for *Centaurea jacea*, *Geranium molle* and *Rorippa sylvestris* but although same trend was evident, the difference was not significant for *Tragopogon pratensis* (Table 3, Fig. 2b). Native plant species differed also in their response to soil inoculation with live biota (Table 3). *Rorippa sylvestris* was the only native plant species that had significantly less aboveground biomass when soil biota was alive, whereas the three other plant species showed an opposite pattern (Supplementary material Appendix 1 Fig. A2).

Herbivore weight gain

The initial weight of *Mamestra brassicae* larvae did not differ among plant communities (plant community type: $F_{4,80}=0.39$, $p=0.82$) and soil communities (original range: $F_{1,80}=0.13$, $p=0.72$; new range: $F_{1,80}=0.29$, $p=0.59$; data not shown). Mean weight gain of *M. brassicae* larvae was negatively correlated to the community shoot biomass ($r=-0.23$, $p=0.03$). Herbivores preferred to feed on Brassicaceae plants and gained most weight in communities where the biomass of plants from this family was high ($r=0.25$, $p=0.02$).

The minimal adequate model, describing the relative weight gains of the generalist herbivore, included plant community type and inoculation with live soil biota as fixed factors, suggesting similar effect of soil communities with different origin. The results indicated that *M. brassicae* gained less weight in communities consisting of unrelated or related range expanders than in communities consisting of native plant species (Plant community: $F_{4,80}=6.27$, $p=0.0002$, Fig. 3). Inoculation with live soil communities decreased the performance of herbivores, estimated as weight gain of *M. brassicae* larvae: (Soil biota: $F_{1,80}=19.92$, $p < 0.0001$; mean \pm SE 57.7 ± 5.33 and 103 ± 12.6 in live and sterilized soil communities, respectively). The effect of inoculated live soil on insects did not differ among plant communities (plant community \times Soil biota: $F_{4,80}=2.18$, $p=0.08$).

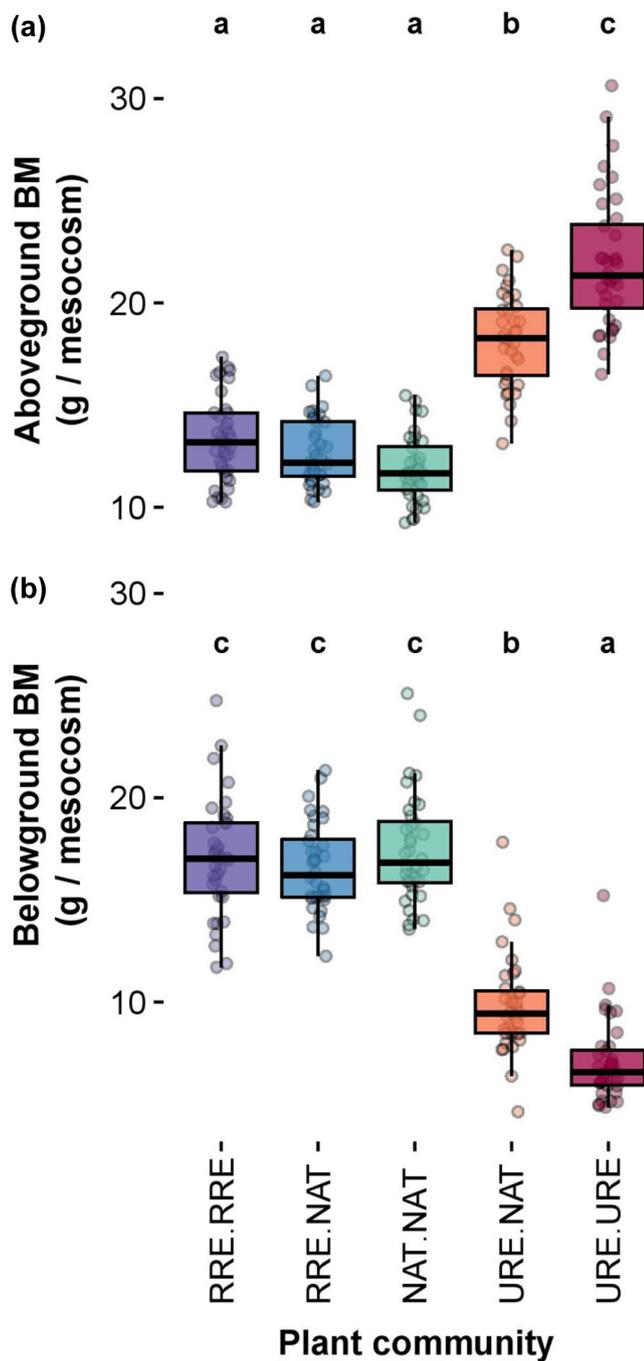


Figure 1. Communities of unrelated range expanders have different above- and belowground biomass allocation than natives and related range expanders. (a) Plant community aboveground and (b) belowground biomass (BM) in grams per mesocosm. Median (central black line), quartile (box), maximum and minimum (whiskers) and outlying values (points) per mesocosm are given. Abbreviations for plant community types: only natives (NAT.NAT), only related range expanders (RRE.RRE), only unrelated range expanders (URE.URE), a mixture of related range expanders and native plant species (RRE.NAT), a mixture of unrelated range expanders and native plant species (URE.NAT). Letters indicate significant difference in species aboveground biomass according to Tukey HSD test.

Plant chemistry

The minimal adequate model, describing the leaf nitrogen concentration of Brassicaceae plants included plant community type, soil from original range and soil from the new range and their interactions as fixed factors. This suggests that soil communities from original and new range differ in their effect on the nitrogen acquisition of these plants. The results showed that leaf nitrogen concentration in Brassicaceae plants was negatively influenced by the presence of soil communities from the original range ($F_{1,40} = 16.6$, $p = 0.0002$), but was not significantly affected by the inoculation of soil communities from the new range ($F_{1,40} = 0.03$, $p = 0.86$) or by interaction between the soil communities from the original and new range ($F_{1,40} = 0.01$, $p = 0.93$). Leaf nitrogen concentration of Brassicaceae plants was dependent on plant community type ($F_{4,40} = 8.84$, $p < 0.001$), being highest for Brassicaceae plants in communities of unrelated range expanders, and lowest for Brassicaceae plants in communities of only native plant species and mixtures of native and related range expanding plant species (Supplementary material Appendix 1 Fig. A3). Plant nitrogen content was neither significantly influenced by the interaction between plant community type and soil communities from the original range ($F_{4,40} = 1.43$, $p = 0.24$), nor by the interaction between plant community type and soil communities from the new range ($F_{4,40} = 1.68$, $p = 0.17$). The three-way interaction between plant community type, soil communities from original range and soil communities from the new range had a marginally significant influence on leaf nitrogen content ($F_{4,40} = 2.66$, $p = 0.05$) and this was not explored further.

The minimal adequate model, describing the leaf carbon concentration of Brassicaceae plants included plant community type and inoculation with live soil biota as fixed factors, suggesting similar effect of soil communities with different origin. Leaf carbon concentration of Brassicaceae plants was significantly lower in the communities with native plants alone than in the communities with only related expanders and mixed communities of natives with related expanders ($F_{4,50} = 3.48$, $p = 0.01$). Leaf carbon concentration in Brassicaceae plants was not influenced by the inoculation of live soil biota ($F_{1,50} = 1.40$, $p = 0.24$). Leaf carbon concentration in Brassicaceae plants was not significantly affected by interaction between plant community type and inoculation with live soil communities ($F_{4,50} = 2.31$, $p = 0.07$).

Discussion

Our results provide the first evidence that range-expanding plant species without congeneric natives in their invaded communities can have a higher ecological impact than range expanders that encounter congeneric natives. Studies on introduced exotic species have suggested both benefits and disadvantages of being related to species that are native in the new range, which is known as Darwin's naturalization conundrum (Thuiller et al. 2010, Bezeng et al. 2015). In our

Table 2. Aboveground biomass response of four range-expanding plant species without and with common congeneric plants in the Netherlands (Unrelated range expanders and Related range expanders, respectively). The results of linear mixed-effects model are given, testing the effect of plant community type (Plant community), inoculation with live soil biota (Soil biota) and species identity (Species) as fixed factors with mesocosm identity as a random factor. The degrees of freedom for numerator (Num df) and denominator (Den df), F- and p-values are given.

Variable	Num df	Den df	F	p
Unrelated range expanders				
Plant community	1	28.52	12.2	0.002
Soil biota	1	28.52	2.5	0.13
Species	3	83.9	224	<0.001
Plant community × Species	3	83.39	0.28	0.84
Soil biota × Species	3	83.39	1.38	0.25
Plant community × Soil biota	1	28.52	0.68	0.42
Plant community × Soil biota × Species	3	83.39	0.03	0.99
Related range expanders				
Plant community	1	28	0.07	0.80
Soil biota	1	28	27.2	<0.001
Species	3	84	161	<0.001
Plant community × Species	3	84	2.09	0.11
Soil biota × Species	3	84	10.9	<0.001
Plant community × Soil biota	1	28	1.28	0.27
Plant community × Soil biota × Species	3	84	5.96	<0.001

study, range expanders that are closely related to natives did not have a competitive advantage in the community of their common congeneric species, but they reduced performance of the generalist herbivorous insect. Interestingly, the unrelated range expanders that were used in our study had both benefits: they were more competitive in the native community and reduced performance of the generalist herbivorous insects. Therefore, our results demonstrate an enhanced benefit of being unrelated. We are aware that these results may strongly depend on the traits of both the range expanders and the natives and in current study, these patterns might be influenced by the dominance of annual plant species among unrelated expanders and the dominance of perennial plant species among related expanders and natives. Nevertheless, our results are an important proof of concept and suggest that Darwin's naturalization conundrum also needs to be tested in the case of climate warming-induced range expansions.

The results of our study indicate that range-expanding plant species, independent of their relatedness with native flora, suppress the growth of a native generalist insect. This pattern may be due to range-expanding plant species having higher induction of toxic defensive chemistry (Engelkes et al. 2008), or more unique defensive compounds (Macel et al. 2014). The production of defence compounds of range expanding and native plant species seems to be influenced by soil biota as herbivorous insects grew worse when plants were inoculated with live soil biota. However, apparently, general defences that were activated, in our case did not require specialized co-evolved plant-soil organism interactions as the origin of soil communities did not change the interactions between range expanding plant species and herbivorous insect.

Belowground communities can increase plant resistance to aboveground herbivores in numerous ways (reviewed by

van Dam and Heil 2011). *Mamestra brassicae* is a generalist chewing insect, which can be influenced by plant-symbiotic micro-organisms such as arbuscular mycorrhizal fungi (Pozo and Azcón-Aguilar 2007). However, most likely these fungi are not the main drivers of observed pattern in this study as *M. brassicae* preferred to feed on plants from Brassicaceae and plants from this family are not frequently associated with arbuscular mycorrhizal fungi (Hempel et al. 2013). Instead, Brassicaceae plants might have benefited from induced defence, triggered by root herbivores or pathogens. This mechanism was demonstrated by Soler et al. (2009), who recorded higher level of chemical defence compounds, glucosinolates, in the aerial parts of *Brassica nigra* which were infested by herbivorous larvae of the cabbage root fly *Delia radicum*.

Plant species interactions with aboveground and belowground biotic environment (involving pathogenic, symbiotic, and decomposing organisms) play an important role in determining plant community structure (Bardgett and Wardle 2010). Reduced impact of natural enemies is thus predicted to enable exotic plant species to have an advantage in competition with natives (Keane and Crawley 2002), resulting in high abundance of exotics. Range expanders have repeatedly shown to experience less negative effect from soil communities in their new range (Engelkes et al. 2008, Dostálek et al. 2016). In the present study, the inoculated soil communities did not influence interactions between range-expanding plant species and natives. However, we tested the effects of field-origin soil communities, which are usually weaker than the effects of specifically conditioned communities (Zhang et al. 2016). In addition, community-level approach of this study can further weaken the influence of specific soil biota on specific plants (Maron et al. 2011). Thus, we cannot rule out the possibility of soil communities to change the interactions

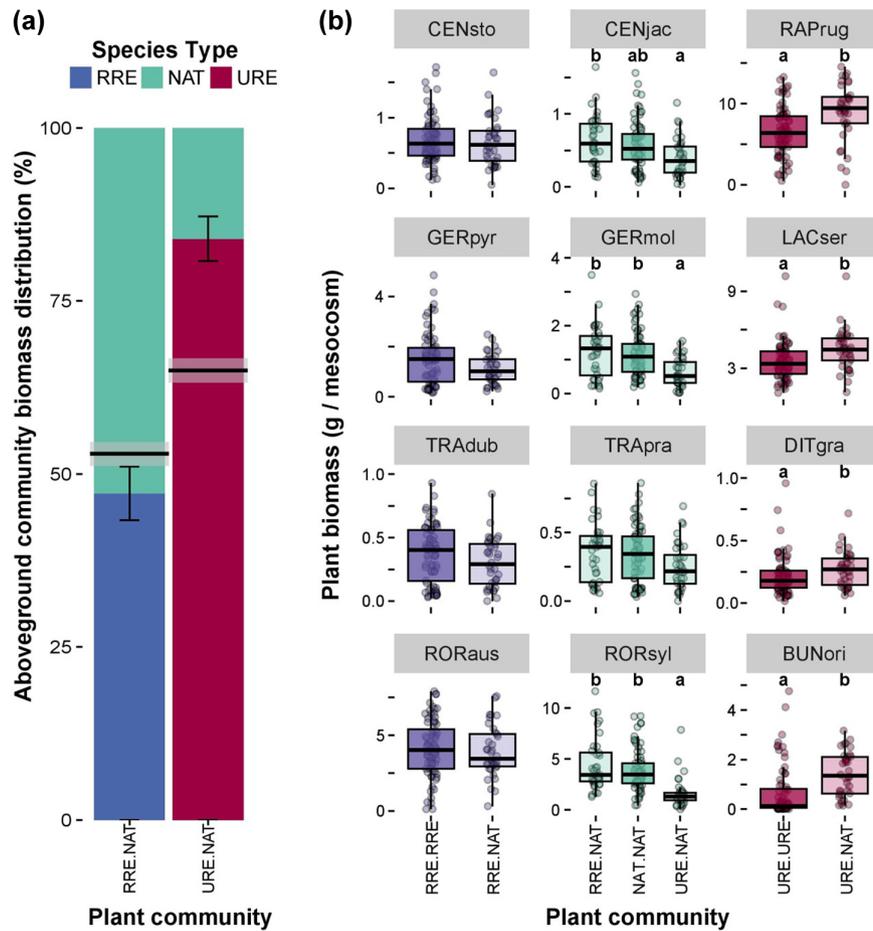


Figure 2. Unrelated range expanders but not related range expanders reduce aboveground biomass of natives. (a) The proportional contribution of natives (NAT, green) and related range expanders (RRE, blue) as well as natives and unrelated range expanders (URE, red) to aboveground plant community biomass. The observed mean (+ SE indicated as error bars) and expected mean (solid line and \pm SE indicated as white shading), calculated by using cumulative average biomass of each species in communities with same type of plants, is presented. (b) Aboveground biomass of all of the test species, growing in different plant community types. Median (central black line), quartile (box), maximum and minimum (whiskers) and outlying values (points) per mesocosm are given. Abbreviations for plant community types are as in Figure 1. Plant species names: natives – *Centaurea jacea* (CENjac), *Geranium molle* (GERmol), *Tragopogon pratensis* (TRApra), *Rorippa sylvestris* (RORSyl); related range expanders – *Centaurea stoebe* (CENsto), *Geranium pyrenaicum* (GERpyr), *Tragopogon dubius* (TRAdub), *Rorippa austriaca* (RORaus); unrelated range expanders – *Bunias orientalis* (BUNori), *Dittrichia graveolens* (DITgra), *Lactuca serriola* (LACser), *Rapistrum rugosum* (RAPrug). Letters indicate significant difference in aboveground biomass of each species, grown in different types of plant communities, estimated by Tukey HSD-test. Note that the scale of y-axes in (b) varies among plant species.

between plant species when same species are growing in same locations for generations or when environmental conditions become stressful.

Our study focused on possible ecological impacts of range-expanding plant species in a specific (riverine) habitat. This enabled us to test ecologically meaningful interactions, but limited the number and the identity of plant species. In our study system, unrelated range expanders are mostly annuals while native and related range expanders are mostly perennials (Supplementary material Appendix 1 Table A1). Dissimilarity in plant characteristics (e.g. plant height, leaf size) has been argued to be an important factor in structuring the competitive interactions in case of latitudinal range expansions (Alexander et al. 2015). Future studies need to disentangle

the influence of life-history traits, plasticity and phylogenetic distance in determining the competitive interactions between range expanding and native plant species in both altitudinal and latitudinal range expansions. In addition, longer-term studies are needed to examine if the competitive advantage of unrelated range expanders persists in time, or that this advantage may disappear as a result of evolutionary dynamics. For example, the nature of competition can change in time as the importance of belowground competition is expected to increase (Wilson 1988), or when exposure to belowground enemies increases when time since introduction proceeds (Diez et al. 2010, Dostál et al. 2013). Thus, it is possible that unrelated range expanders that have smaller root systems may lose some of their competitive advantage in time.

Table 3. Aboveground biomass allocation of native plant species is influenced by plant community type and presence of soil biota but also on species identity. The results of linear mixed-effects model are given, testing the response of aboveground biomass of native plant species on plant community type (Plant community), exposure to live soil biota (Soil biota) and plant species identity (Species) with mesocosm identity as a random factor. Numerator degrees of freedom (Num df), denominator degrees of freedom (Den df), F- and p-values are reported.

Variable	Num df	Den df	F	p
Plant community	2	42	21.2	<0.001
Soil biota	1	42	9.34	0.004
Species	3	126	181	<0.001
Plant community × Species	6	126	4.93	<0.001
Soil biota × Species	3	126	18.7	<0.001
Plant community × Soil biota	2	42	0.08	0.93
Plant community × Soil biota × Species	6	126	0.62	0.71

Current human-induced climate warming is already leading to changes in existing communities (Walther et al. 2002, Parmesan and Yohe 2003, Lenoir et al. 2008). However, predictions about future changes are still under development. Earlier studies on climate warming effects have suggested the extinction of many species that are unable to migrate at the same rate at which abiotic climate conditions shift across continents (Thomas et al. 2004). Recently, it has been shown that species that expand their range may influence the composition and functioning of existing communities (Alexander et al. 2015, Engelkes et al. 2016). We show that biotic interactions may depend on relatedness

of range-expanding plant species with invaded communities and differ between trophic levels. In addition, results of our study indicate that plant characteristics, such as biomass allocation pattern but also plant height (Feng and van Kleunen 2016), may be important in determining the ecological impacts of range-expanding plant species. Therefore, we conclude that predicting consequences of climate warming on natural communities will require a more complete understanding of how relatedness of the range-expanding species with the native community influences their performance both at the plant–plant interactions level and in interactions with an aboveground herbivore.

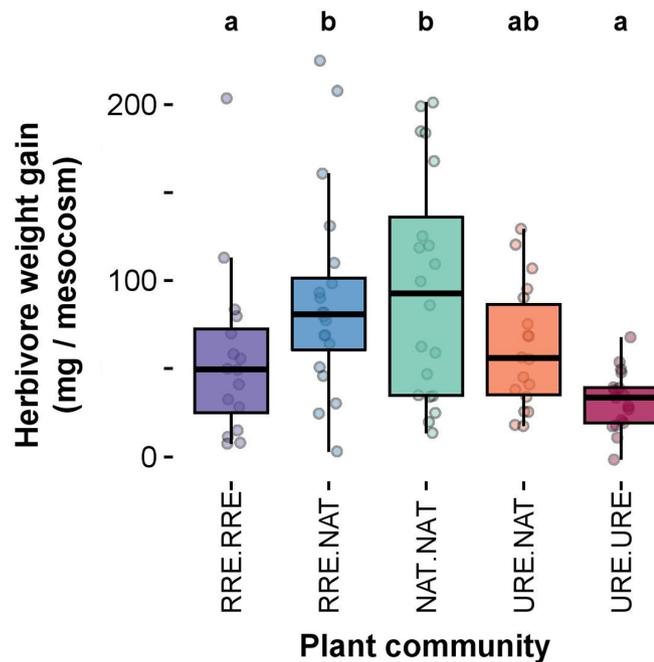


Figure 3. Unrelated and related range expanders are equally successful in suppressing the growth of aboveground herbivore compared to natives. Median (central black line), quartile (box), maximum and minimum (whiskers) and outlying values (points) of the weight gain of generalist herbivore, *Mamestra brassicae*, per mesocosm are given. Abbreviations for plant community types are as in Fig. 1. Letters indicate significant difference in herbivore weight gain according to Tukey HSD-test.

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Supplementary material (available online as Appendix oik-04817 at <www.oikosjournal.org/appendix/oik-04817>).
Appendix 1