Plant neighbours rather than soil biota determine impact of an alien plant invader

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Summary

1. Various factors have been shown contributing to the ecosystem impact of invasive alien plants, but their relative importance remains unclear. We focused on the effects of neighbouring plant community and soil biota as these biotic factors have been repeatedly put forward to explain invasion success (e.g. as components of the novel weapons and of the biotic release hypothesis).

2. To assess their relative importance in explaining the high impact of *Centaurea stoebe* during the invasion of new sites in the introduced range, we conducted a greenhouse experiment with both European (EU) and North American (NA) tetraploid *C. stoebe* competing with/without EU vs. NA neighbouring community and with the two neighbouring communities growing without *C. stoebe*. Plants were grown in sterilized commercial soil inoculated with naïve soil (from rhizosphere of plants other than *C. stoebe*) originating either from the home EU or the introduced NA range and half of which was sterilized to remove soil biota.

3. In the competition pots, relative competitive ability (difference between the relative growth rates of *C. stoebe* and neighbouring community) and impact level (biomass of the neighbouring community relative to that in non-competition pots) of *C. stoebe* was significantly higher when grown with the NA than with the EU neighbouring community, although growth rates of EU and NA neighbouring communities did not differ in the non-competition pots. Both soil origin and *C. stoebe* origin had no effect on these processes. Soil sterilization increased growth of both *C. stoebe* and neighbouring communities, but had only a moderate effect on impact level and type, and no effect on the relative competitive ability of *C. stoebe*.

4. These results suggest that during the colonization of new sites in North American grasslands, the impact of *C. stoebe* is strongly driven by reduced competitive ability of NA neighbours compared with EU neighbours, while altered biotic soil conditions in the introduced range and post-introduction evolutionary changes in the invader are of less importance. This differential impact appears to be due to inherently different mechanisms underlying the competitive interactions between EU and NA neighbouring communities when grown with *C. stoebe*.

Key-words: *Centaurea stoebe*, competition, invader impact, neighbour origin, plant invasion, soil origin, soil sterilization

Introduction

Invasive alien plant species (IAPs) are regarded as among the most important components of current global environmental change, threatening the integrity of agricultural and natural systems and causing enormous economic costs (Gurevitch *et al.* 2011). As it is the impact of invaders rather than their establishment *per se* that threatens native communities (Levine *et al.* 2003), it is important to examine the factors that regulate the competitive ability and impact of IAPs on the resident community once the invaders have successfully colonized a new site.

Competition for limiting resources is likely to play a role in the impact of IAPs (D'Antonio & Vitousek 1992; Levine *et al.* 2003). For instance, IAPs may have a higher compet-

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itive effect than native resident species by exploiting nutrients (Huenneke *et al*. 1990; Davis, Grime & Thompson 2000) or soil water (D'Antonio & Mahall 1991; Busch & Smith 1995; Enloe *et al*. 2004) that natives are not able to tap. Also, they may create a novel shade environment, which negatively impacts native seedling recruitment (Spellman & Wurtz 2011). Differences in resource acquisition resulting in competitive asymmetry between IAPs and native resident species may then explain the increased impact of a plant invader in the introduced range (Pattison, Goldstein & Ares 1998; Funk & Vitousek 2007), compared to the native range. Invasive alien plants may also negatively affect resident neighbours due to 'novel biochemical weapons' released by the invader (Callaway & Ridenour 2004). For example, Callaway & Aschehoug (2000) found that the invasive *Centaurea diffusa* L. had strong allelopathic negative effects on its naïve neighbours in the introduced range, but are relatively ineffective against their old neighbours at home, where IAPs share a co-evolutionary history (Callaway & Ridenour 2004).

In the past 10 years, below-ground processes have been identified as important factors for explaining plant invasions (Bever, Westover & Antonovics 1997). A common prediction relating soil biota to invasion is that IAPs may escape from soil-borne pathogens and macro-organisms that negatively affect plant growth and abundance at home via negative intraspecific plant–soil feedbacks (Klironomos 2002; Yang *et al*. 2013). Such a shift in interactions with soil organisms is in compliance with the commonly acknowledged enemy release hypothesis (Keane & Crawley 2002), though effects of enemy release may decline over time and/or as invaders spread, allowing alien and native plants to coexist (Diez *et al*. 2010). Furthermore, IAPs have been shown to cultivate soil biota with increasingly positive effects on their competitive ability in the introduced range (Reinhart *et al*. 2003; Callaway *et al*. 2004; Hierro *et al*. 2006). Differences in soil biota between the home and the introduced range may then explain the increased competitive ability and impact of IAPs on new neighbours compared to old neighbours. Invasive alien plants may also affect neighbours via negative interspecific plant–soil feedbacks (van der Putten *et al*. 2013), either due to accumulation of resident pathogens (Eppinga *et al*. 2006; Mangla, Inderjit & Callaway 2008) or, impairment of resident plant-mutualist networks (Marler, Zabinski & Callaway 1999; Stinson *et al*. 2006).

The strength and relative importance of mechanisms affecting the impact of IAPs on residents may shift over time. During the colonization of new sites in the introduced range, IAPs may immediately start competing with residents for limiting resources (Vilà & Weiner 2004). Plant–soil feedbacks in 'naïve' soil may also operate at an early stage of invasion when releases of allelopathic metabolites by IAPs lead to rapid negative interspecific plant–soil feedbacks (Callaway & Ridenour 2004), yet allelopathic effects on native resident species may decline with population age (Lankau *et al*. 2009). The build-up of negative plant–soil feedbacks via cultivation of soil microbial communities can, however, take months (Bever 1994; Kourtev, Ehrenfeld & Haggblom 2003) or years (Bezemer *et al*. 2006), and the temporal dynamics of IAPs effects on soil communities is often unknown (Ehrenfeld, Ravit & Elgersma 2005; Wolfe & Klironomos 2005). It is therefore likely that the whole set of possible plant–soil feedbacks affecting the impact of IAPs becomes fully effective only at a later stage of the invasion process.

Differences in the impact of plant invaders at home and away may result from different mechanisms dominating interspecific interactions in the two ranges. Based on a pairwise competition experiment using resident plants from both ranges, Sun *et al*. (2013) provided evidence that the impact of *C. stoebe* L. on its neighbours in the home range is driven by competition for limiting resources, but by other mechanisms in the introduced range (e.g. exploitation of resources that are not used by the new neighbours or interference competition). Clearly, there is a lack of studies that test simultaneously the importance of eco-evolutionary processes that have been put forward to explain the high impact of IAPs in the introduced range (Levine, Adler & Yelenik 2004; Wolfe & Klironomos 2005; van der Putten *et al*. 2013). Here, we set out to assess the relative importance of putative differences in neighbouring plant communities and soil biota between the home and the introduced range and of potential post-introduction evolutionary changes in the IAPs on the competitive ability and impact of *C. stoebe* (syn. *C. maculosa* Lam., Asteraceae), spotted knapweed, during the colonization of new sites in the introduced range (see Figs S1 and S2, Supporting Information). *Centaurea stoebe* is a widespread, short-lived herb native to Europe that was introduced into North America as a seed contaminant (Roché, Roché & Ben 1991). It has been the target of experimental studies documenting negative intraspecific plant–soil feedbacks in the home range (Callaway *et al*. 2004), negative intraspecific (Perry *et al*. 2005) and interspecific (Marler, Zabinski & Callaway 1999; Callaway *et al*. 2004) plant–soil feedbacks in the introduced range, and inherently higher competitive ability in the presence of new 'naïve' neighbours, compared to old neighbours from the home range (e.g. He *et al*. 2009; Sun *et al*. 2013). Evidence for post-introduction evolution in *C. stoebe* by genetic drift or selection is mixed. Ridenour *et al*. (2008) found that North American *C. stoebe* plants grew faster than plants from European populations, but this may not result in increased biomass of mature plants or increased reproductive output (Henery *et al*. 2010).

Following the lines of argumentation outlined above, we hypothesized that while neighbour origin (European vs. North American neighbours), soil origin (European vs. North American soil) and invader origin (European vs. North American *C. stoebe*) all affect the competitive ability and impact of *C. stoebe*, (putative) differences in neighbour origin play a more important role than differences in soil origin (which may become fully effective at a later

stage of the invasion) during the colonization of new sites in the introduced range. Based on earlier observations in pairwise competition studies between *C. stoebe* and individual EU vs. NA neighbours (cf. above; Sun *et al.* 2013), we further hypothesized that the impact of *C. stoebe* on its home neighbouring community is size-related, but the impact on its naïve neighbouring community from the invaded range is not.

Materials and Methods

PLANT SPECIES AND ORIGIN

In Europe, *C. stoebe* exists as two cytotypes, diploids ($2n = 2x = 18$) and tetraploids ($2n = 4x = 36$), but so far, only tetraploids have been recorded from its introduced North American range (Broz *et al.* 2009; Treier *et al.* 2009; Mráz *et al.* 2011). In our study, we only used tetraploid (4*x*) *C. stoebe* from both its home and introduced range. Seeds of 4*x C. stoebe* were collected from three European (Germany) and four North American (Montana, USA) populations (bulk samples of 10–20 mother plants; see Table S1).

To assess the competitive interactions with neighbouring communities from the home and the introduced range, seeds of five European (EU) and five North American (NA) perennial plant species were either collected from the field (bulk samples of 10–20 mother plants per population adjacent to a *C. stoebe* infested site) or purchased from commercial suppliers in Europe and the USA for EU and NA plants, respectively. Neighbour species were chosen among plants naturally occurring at 4*x C. stoebe* sites (Table 1) and representing different functional groups (i.e. grass, forb with spreading rhizomes and with woody root/taproot). We also selected the species within functional groups to achieve no significant different overall growth rates and total biomass between the two neighbouring communities, based on the study of Sun *et al.* (2013; $\chi^2 = 0$, $P = 1$ and $\chi^2 = 3{\cdot}39$, $P = 0{\cdot}07$ for growth rate and biomass respectively; cf. Supplemental material: Appendix B, C and E). Thus, while a co-evolutionary history of the EU plants with *C. stoebe* is most likely at the species level, none of the seed material used in the experiment had a direct experience with *C. stoebe*.

SOIL ORIGIN AND TREATMENTS

To assess the relative importance of soil-mediated mechanisms affecting the competitive ability and impact of *C. stoebe* during the initial stages of invasion, we collected 'naïve' soil in both ranges next to but outside existing *C. stoebe* populations. To single out the biotic effects of soil origin, the inoculated soil was sterilized in half of the pots.

We collected 12–15 L field soil (top 15 cm of the soil layer) from three separated spots (at least 10 m apart from each other) each from three different EU or NA grassland sites (i.e. a total of c. 40 L of each range) in autumn 2011. The sites were chosen among grasslands that are still pretty diverse where both native plant species and *C. stoebe* occur (see Table S2), but soil was only collected in an area where *C. stoebe* was not present to avoid negative plant–soil feedbacks due to plant–soil history. Stems and roots of plants, earthworms and rocks were removed and the soil was sieved with a 5-mm sieve. The soil samples collected within a site were pooled and stored in 10 °C.

Germinated seeds were grown in autoclaved potting soil (121 °C at 1·1 atm for 60 min; Trevors 1996) in seedling trays. After transplanting, the plant growth medium in the pots was a mix of autoclaved sand, vermiculite and a low nutrient commercial potting soil TKS-1 (Flora Gard, Berlin, Germany) in the ratio 1 : 1 : 1 by volume and 10% (to avoid significant nutrient differences) of total volume of field soil to represent the native grassland soil biota condition from the home or invaded range (see e.g. Mangan *et al.* 2010). Previous studies reported that autoclaved soil could safely be used in seedling trays for germination stage, but had large effects on the growth of plant (Williams-Linera & Ewel 1984). The commercial potting soil was therefore sterilized by γ-irradiation (max. 50 kGray, min. 29 kGray; LEONI Studer Hard AG, Däniken, Switzerland; cf. Zuppinger-Dingley *et al.* 2011). This procedure allowed keeping soil structure and nutrient levels similar among treatments. Thus, to single out differences in soil organisms, we eliminated soil organisms in the potting soil and in half of the EU and NA field soil samples by γ-irradiation (Petermann *et al.* 2008). The γ-irradiation that was used to sterilize soil can cause nutrient flushes released from dead soil organisms (Eno & Popenoe 1963). Therefore, soil sterilization can cause a positive effect on plant growth when the initial soil biota effects are driven by antagonists, either by killing the antagonists or indirectly by subsequent nutrient flushes. When the initial soil biota effects are driven by mutualists, soil sterilization can cause a positive (nutrient flush), negative (by killing the mutualists) or a neutral (combination of the two) effect on plant performance.

GERMINATION CONDITIONS AND TRANSPLANTING

Neighbour plants from the home range and from the introduced range as well as 4*x C. stoebe* from EU and NA were grown from seeds from December 2011 through May 2012. Seeds were germinated in petri-dishes, and geminated seeds were transplanted into seedling trays. Petri-dishes, filter papers and seedling trays were

Table 1. Native neighbour species of North America and Europe used in the experimental assemblages. The superscript numbers behind each species represent the source of seeds, collected from field where *Centaurea stoebe* occurred (1), UFA-Samen, Winterthur, Switzerland (2), B-and-T World Seeds, Paguignan, France (3)

Plant origin	Species name	Family	Function
Europe	*Koeleria pyramidata* (Lam.) Beauv.[2]	Poaceae	Grass
	Festuca rupicola Heuff.[2]	Poaceae	Grass
	Dianthus carthusicanorum L.[1,2]	Caryophyllaceae	Spreading rhizomes
	Artemisia campestris L.[2]	Asteraceae	Woody root
	Cichorium intybus L.[1,2]	Asteraceae	Taproot
North America	*Koeleria macrantha* (Ledeb.) Schultes[1]	Poaceae	Grass
	Poa sandbergii Vasey[1]	Poaceae	Grass
	Monarda fistulosa L.[1,3]	Lamiaceae	Spreading rhizomes
	Potentilla arguta Pursh[3]	Rosaceae	Woody root
	Penstemon wilcoxii Rydb.[1]	Scrophulariaceae	Woody root

sterilized with 70% ethanol. Before sowing, seeds were surface-sterilized by soaking them in a 7% sodium hypochlorite solution for 3 min and then rinsing for 2 min with autoclaved water to avoid microbial contamination (Bartelt-Ryser *et al*. 2005; Zuppinger-Dingley *et al*. 2011). Seeds were germinated in a 12-h day regime with 18 °C day and 12 °C night temperature in a climate chamber. At the end of December 2011, we put geminated seeds of all species into seedling trays with 150 (10 × 15) cell plugs of 15 ml volume filled with autoclaved potting soil (TKS-1). The seedling trays were placed on tables in a greenhouse at the University of Fribourg and exposed to natural light condition, which was supplemented by metal halide bulbs (18-h light, 6-h dark), and to a temperature of 15–22 °C. Seeds and seedlings were watered with autoclaved water.

On 10th February 2012, we established neighbouring communities by transplanting one seedling each of either five EU or five NA species into 2·5 L pots (18·2 cm surface diameter and 14·2 cm depth), at an average distance of 10–11 cm among seedlings. We then added or not five EU or five NA 4*x C. stoebe* plants between the neighbour plants, resulting in an average distance of 6–7 cm among plants in the competition pots. The pattern of the spatial arrangement of plants was the same in each pot (see Fig. S3). In addition, five EU or five NA 4*x C. stoebe* plants were also transplanted into pots without neighbours as control. The spacing among the plant individuals corresponds to a commonly observed neighbourhood distance in the field (Callaway *et al*. 2011; Mráz *et al*. 2012). In each pot with *C. stoebe* contained at least one individual from each of three/four populations of the same origin.

Each neighbouring community × *C. stoebe* origin combination (competition pots with ten individuals) as well as the two neighbouring communities and *C. stoebe* origins alone (non-competition (control) pots with five individuals) were grown on four soil types (sterile commercial soil mixed with sterile/non-sterile EU or NA soil) for a total of nine replicates (three sites of each soil origin × three replicates) in a complete factorial arrangement of treatments within a randomized complete block design. There were three blocks containing 96 pots of each set resulting in 288 pots in total. After transplanting, plants experienced natural light conditions supplemented by metal halide bulbs and a temperature of 15–22 °C in a greenhouse at the University of Fribourg. Plants were manually watered with 150 ml tap water per pot every three days to ensure that each pot was given an equal amount of water. In order to reduce position effects, pots in each block were re-randomized every fortnight.

DATA COLLECTION

The relative competitive ability of *C. stoebe* is defined here as its ability to accumulate biomass in mixture experiments relative to the accumulation of biomass by neighbouring plants (Connolly 1987). Impact level of *C. stoebe* is defined as the difference in biomass of neighbouring communities when grown alone and when grown in competition with *C. stoebe*, and impact type as the relationship between the biomass of *C. stoebe* and that of its neighbouring communities. In order to control for initial variation in size among plant species, we applied the Relative Efficiency Index (REI), proposed by Connolly (1987) to indicate the growth trajectories of *C. stoebe* and neighbour plants when grown in competition (cf. Sun *et al*. 2013). REI is an unbiased indicator of mixture dynamics during the early phases of competition, when traditional measures of competitive ability (such as relative yield) are inherently biased in favour of larger plants (Grace, Keough & Guntenspergen 1992). REI is the relative growth rates of *C. stoebe* compared to that of neighbouring community, and was calculated here as REI = $(\ln b_{1ci} - \ln b_{0ci}) - (\ln b_{1ic} - \ln b_{0ic})$, in each competition pot, where b_{1ci} refers to the biomass of *C. stoebe* in mixture with neighbouring community *i* at harvest, b_{0ci} to the biomass of *C. stoebe* in mixture with neighbouring community *i* at the beginning of the experiment, b_{1ic} to the biomass of neighbouring community *i* in mixture with *C. stoebe* at harvest, and b_{0ic} to the biomass of neighbouring community *i* in mixture with *C. stoebe* at the beginning of the experiment. Hence, the higher REI the stronger (higher relative competitive ability) is *C. stoebe* in dominating the neighbouring community. We used 30 surplus seedlings of each species to calculate the relationship between length of the longest leaf and biomass, and then estimated the biomass of each individual at the beginning of the experiment.

Reduction in biomass of neighbouring community and that of *C. stoebe* was assessed by calculating: $(b_- - b_+)/b_-$, where b_- is the biomass of the plants grown in control pots (absence of *C. stoebe* or neighbouring community competition), and b_+ is the biomass of the plants grown in the presence of competition pots.

The length of the longest fully expanded leaf of all seedlings was assessed 3 days after transplanting as a proxy for early biomass (cf. below). Plants were harvested on 17–19 May 2012 (days 98–100) and subsequently dried to a constant weight at 60 °C for 48 h and weighed to an accuracy of ±0·001 g.

STATISTICS

Linear mixed models were fit using the lmer/lme function obtained from the R package lme4/nlme that uses maximum likelihood to estimate the model parameters (Bates, Maechler & Bolker 2011). These two packages have similar syntax and can do similar things; lme4 allows general linear models and nlme has in-built correlation structures which makes it better suited for regression models (Pinheiro *et al*. 2012). General linear mixed models were calculated to assess effects of neighbour origin, soil origin, *C. stoebe* origin and sterilization treatments on biomass/reduction in biomass of neighbouring community, that of *C. stoebe* and REI. In all these models, neighbour origin, soil origin, *C. stoebe* origin and sterilization were included as fixed effects, and block and sites of soil nested within soil origin were treated as random factors. The experimental design did not allow testing *C. stoebe* origin against the number of *C. stoebe* populations. Thus, while our approach allowed a considerable statistical power despite the low number of populations within *C. stoebe*, a significant *C. stoebe* origin effect would have to be interpreted with caution since the statistical analysis does not distinguish between among-population and within-population effects.

Mixed-effects regression models were used to analyse the correlation between biomass of neighbouring communities and that of *C. stoebe* in the competition condition. Neighbour origin was also included as fixed effects in a combined analysis of data sets. As to the random structure, we compared a random intercept and slope model and a random intercept model using site factor, and we used the likelihood ratio test from restricted maximum likelihood fits for significance. They indicated no difference between the common slope and the slopes of each of the site ($P > 0{\cdot}1$). Eventually, model-II simple linear (geometric mean regression) regression using standard major axis method was used because both variables used for the regression were measurements, to compute the relationship between biomass of neighbouring communities and *C. stoebe* in the competition condition. All analyses were performed using R statistical software, version 2.15.1 (R Development Core Team, 2012).

Results

ORIGIN OF PLANT NEIGHBOURING COMMUNITIES

In the absence of *C. stoebe* competition, there was no difference between biomass of EU and of NA neighbouring

community in both un-sterilized EU and NA soils ($\chi^2 = 0{\cdot}87$, $P = 0{\cdot}35$).

In the competition pots, biomass of the EU neighbouring community was larger (on average 23%) than that of the NA neighbouring community ($\chi^2 = 18{\cdot}46$, $P < 0{\cdot}001$; Fig. 1a). Consequently, the impact of *C. stoebe* on the EU neighbouring community (reduction in biomass of neighbouring community) was smaller than that on the NA neighbouring community ($\chi^2 = 21{\cdot}35$, $P < 0{\cdot}001$; Fig. 1b) in both EU and NA soils. Soil origin had no effect on the biomass of neighbouring communities ($\chi^2 = 2{\cdot}23$, $P = 0{\cdot}14$; Fig. 1a) and their reduction ($\chi^2 = 2{\cdot}18$, $P = 0{\cdot}14$; Fig. 1b).

CENTAUREA STOEBE

Biomass of both EU and NA 4*x* *C. stoebe* was significantly larger (on average 25%) when they competed with NA than with EU neighbouring community in both EU and NA soils ($\chi^2 = 6{\cdot}44$, $P = 0{\cdot}01$; Fig. 2a). Similarly, the biomass of both EU and NA 4*x* *C. stoebe* was more strongly reduced when they competed with EU than NA neighbours in both EU and NA soils ($\chi^2 = 4{\cdot}44$, $P = 0{\cdot}03$; Fig. 2b). Interestingly, the biomass of *C. stoebe* was 25% higher when grown in NA soil than in EU soil ($\chi^2 = 4{\cdot}86$, $P = 0{\cdot}03$; Fig. 2a), but soil origin did not affect the reduction in biomass of *C. stoebe* ($\chi^2 = 0{\cdot}01$, $P = 0{\cdot}92$; Fig. 2b). Biomass and reduction in biomass did not significantly differ between EU and NA 4*x* *C. stoebe* ($\chi^2 < 2{\cdot}21$, $P > 0{\cdot}14$).

INTERACTIONS BETWEEN *C. STOEBE*, ITS NEIGHBOURING COMMUNITY AND SOIL ORIGIN

Relative Efficiency Index (REI) of *C. stoebe* competing with EU as compared with NA neighbouring communities was on average 60% lower in both EU and NA soils ($\chi^2 = 53{\cdot}52$, $P < 0{\cdot}001$; Fig. 3a). *Centaurea stoebe* origin did not significantly affect REI ($\chi^2 < 1{\cdot}57$, $P > 0{\cdot}21$).

Biomass of *C. stoebe* explained a highly significant amount of the variation in biomass of the EU neighbouring community in both EU and NA soils ($R^2 > 0{\cdot}25$, Δlog-likelihood $< -5{\cdot}63$, $P < 0{\cdot}04$; Fig. 4a,b), but only a minor and non-significant amount of the variation in biomass of the NA neighbouring community ($R^2 < 0{\cdot}15$, Δlog-likelihood $< -8{\cdot}21$, $P > 0{\cdot}11$; Fig. 4c,d).

SOIL STERILIZATION

In the competition pots, soil sterilization significantly increased the biomass of neighbouring communities (37%; $\chi^2 = 62{\cdot}74$, $P < 0{\cdot}001$; Fig. 1a,c). As in non-sterilized soil, biomass of the EU neighbouring community was significantly higher (on average 29%) than that of NA neighbouring community in both sterilized EU and NA soils ($\chi^2 = 38{\cdot}23$, $P < 0{\cdot}001$; Fig. 1c).

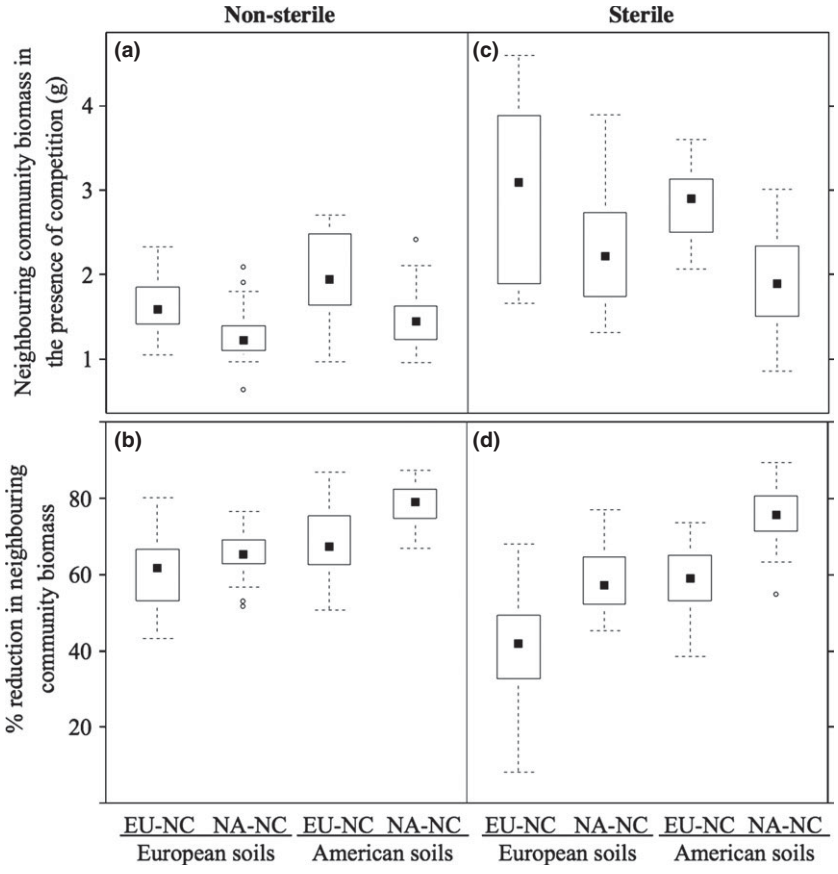

Fig. 1. Biomass (g dry weight) of European (EU) and North American (NA) neighbouring communities (NC) when grown in competition with EU or NA *Centaurea stoebe* in non-sterile (a) and sterile (c) soils, and reduction in biomass of EU and NA neighbouring communities in non-sterile (b) and sterile (d) soils, compared to control pots (neighbouring communities alone). Boxplots show median (IQR), whiskers show range excluding outliers (i.e. 1·5 × IQR), values greater/less than upper/lower limit are plotted with empty circles, (cf. Table S3).

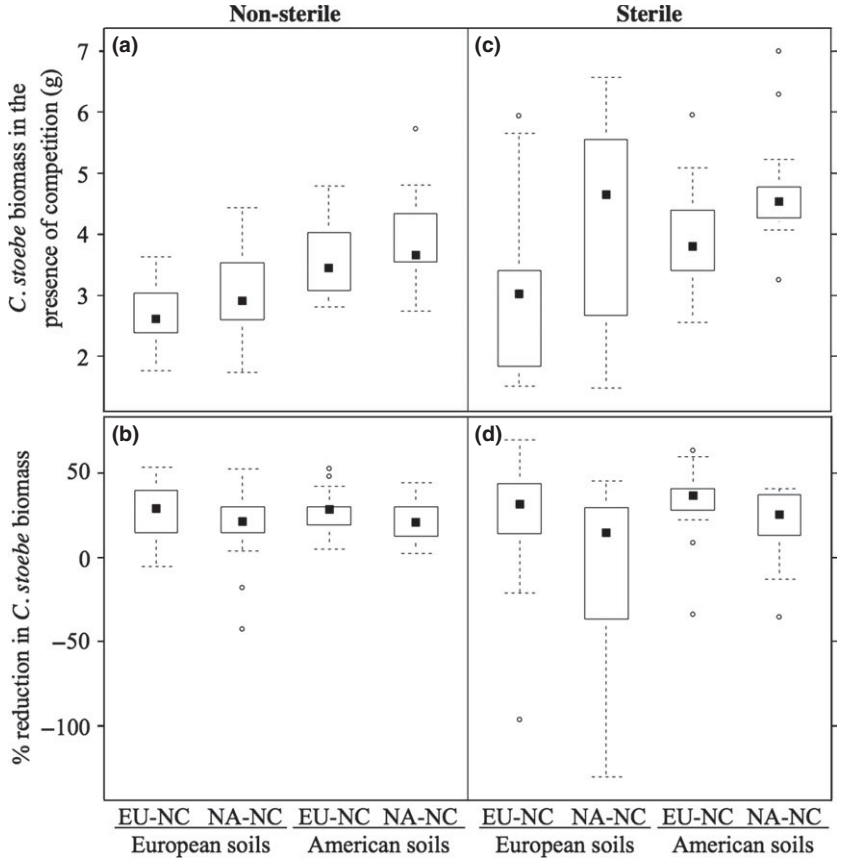

Fig. 2. Biomass (g dry weight) of tetraploid *Centaurea stoebe* when grown in competition with EU or NA neighbouring communities (NC) in non-sterile (a) and sterile (c) soils, and reduction in biomass of EU and NA *Centaurea stoebe* in non-sterile (b) and sterile (d) soils, compared to control pots (*C. stoebe* alone). Boxplots show median (IQR), whiskers show range excluding outliers (i.e. 1·5 × IQR), values greater/less than upper/lower limit are plotted with empty circles, (cf. Table S3).

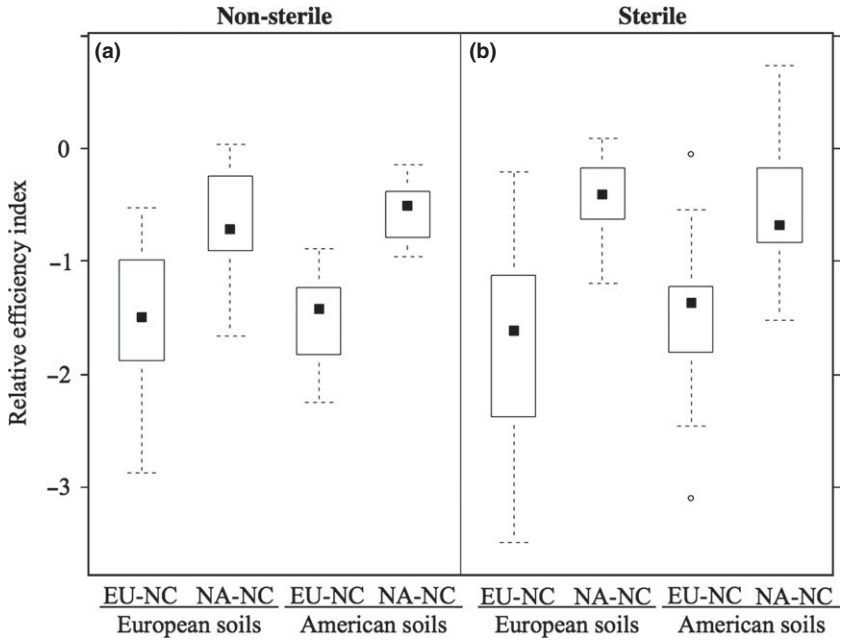

Fig. 3. Competitive ability, expressed by the relative efficiency index (REI), of tetraploid European (EU) and North American (NA) *Centaurea stoebe* when grown in competition with EU or NA neighbouring communities (NC) in a) non-sterile EU and NA soils and (b) sterile EU and NA soils. Boxplots show median (IQR), whiskers show range excluding outliers (i.e. 1·5 × IQR), values greater/less than upper/lower limit are plotted with empty circles, (cf. Table S3).

Soil sterilization also lead to an overall increase in biomass of EU and NA 4*x C. stoebe* (17%; $\chi^2 = 17{\cdot}14$, $P < 0{\cdot}001$; Fig. 2a,c). Yet, while the effect of soil sterilization on biomass of *C. stoebe* was significant when *C. stoebe* competed with NA neighbouring community in NA soil (17%; $\chi^2 = 18{\cdot}36$, $P < 0{\cdot}001$), it was non-significant when *C. stoebe* competed with EU neighbouring community in EU soil ($\chi^2 = 1{\cdot}94$, $P = 0{\cdot}16$; Fig. 2a,c). As in non-sterilized soil, the biomass of EU and NA 4*x C. stoebe* was significantly higher (on average 22%) when they competed with NA than with the EU neighbouring community in both sterilized EU and NA soils ($\chi^2 = 18{\cdot}36$, $P < 0{\cdot}001$;

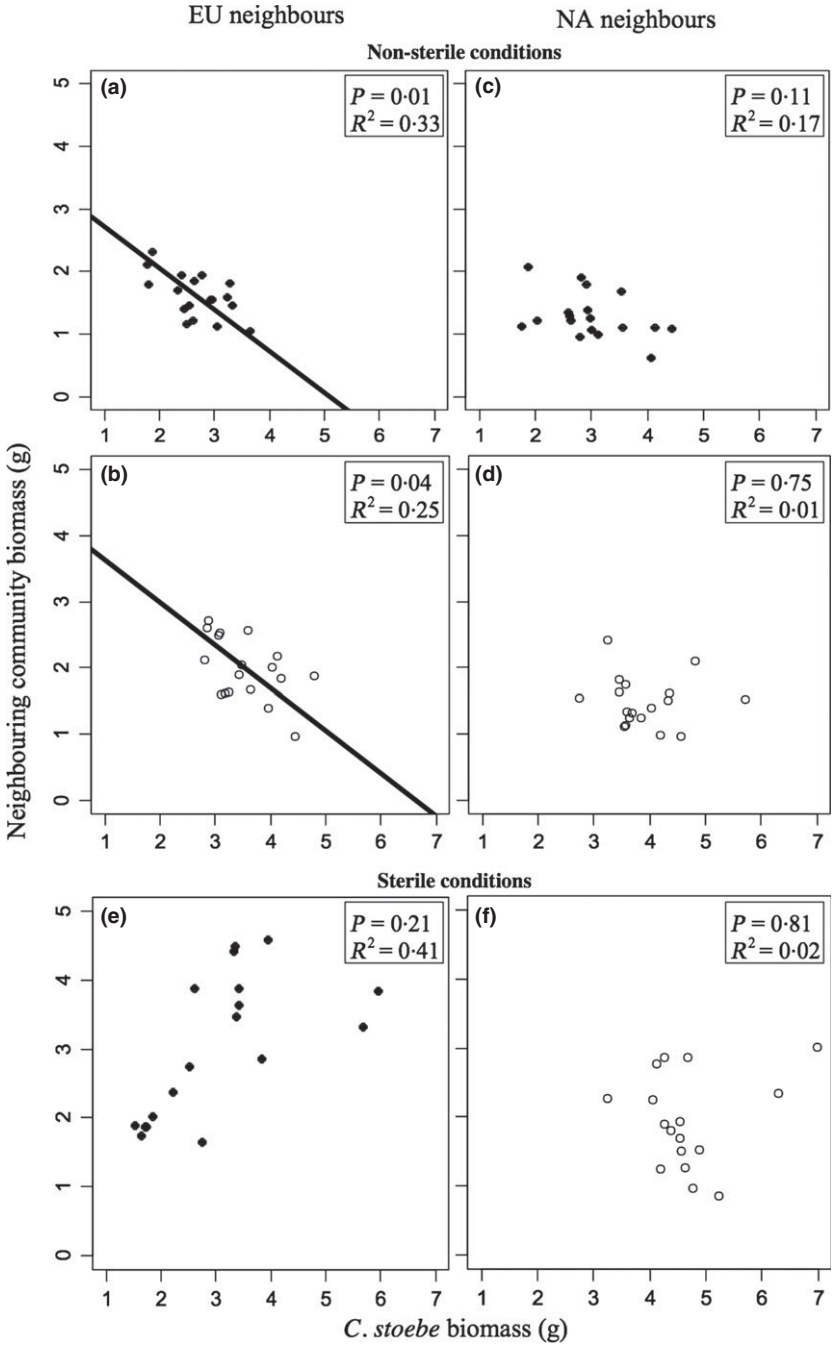

Fig. 4. Relationship between the biomass (g dry weight) of tetraploid *Centaurea stoebe* and that of EU neighbouring community in competition pots when grown in EU (a) and NA (b) non-sterilized soils and in EU sterilized soils (e), and the relationship between *C. stoebe* biomass and that of NA neighbouring community in competition pots when grown in EU (c) and NA (d) non-sterilized soils and in NA sterilized soils (f). Filled circles = results from pots with EU soil; open circles = results from pots with NA soil.

Fig. 2c). The origin of sterilized soil affected neither the biomass of neighbouring communities nor that of *C. stoebe* ($\chi^2 < 2{\cdot}63$, $P > 0{\cdot}11$).

The reduction in biomass of neighbouring communities was significantly lower in sterilized soil, compared to non-sterilized soil (18%, $\chi^2 = 25{\cdot}37$, $P < 0{\cdot}001$; Fig. 1b,d). As in non-sterilized soil, NA neighbours had stronger reduction in biomass than EU neighbours in both sterilized EU and NA soils when competing with *C. stoebe* ($\chi^2 = 38{\cdot}49$, $P < 0{\cdot}001$; Fig. 1d). Soil origin had marginally significant effects on the reduction in biomass of EU and NA neighbours ($\chi^2 = 3{\cdot}74$, $P = 0{\cdot}05$; Fig. 1d). The reduction in biomass of *C. stoebe* did not differ between sterilized and non-sterilized soil ($\chi^2 = 1{\cdot}99$, $P = 0{\cdot}16$; Fig. 2b,d). Like in non-sterilized soil, the reduction in biomass of both EU and NA 4*x C. stoebe* was higher when they competed with EU than with NA neighbouring community ($\chi^2 = 6{\cdot}37$, $P = 0{\cdot}01$; Fig. 2d).

Relative Efficiency Index (REI) of *C. stoebe* competing with neighbouring community did not differ between sterilized and non-sterilized soil ($\chi^2 = 0{\cdot}008$, $P = 0{\cdot}93$; Fig. 3a, b). As in non-sterilized soil, REI of *C. stoebe* competing with EU neighbouring community was approximately 58% lower than REI of *C. stoebe* competing with NA neighbouring community in both sterilized EU and NA soils ($\chi^2 = 41{\cdot}43$, $P < 0{\cdot}001$; Fig. 3b). REI did not differ

between pots with EU vs. NA 4*x* *C. stoebe* as competitors ($\chi^2 = 2{\cdot}63$, $P = 0{\cdot}11$), nor did it differ between soil origin ($\chi^2 = 0$, $P = 1$; Fig. 3a,b).

The biomass of *C. stoebe* and that of the EU neighbouring community were not correlated with each other in sterilized EU soil ($R^2 = 0{\cdot}40$, Δlog-likelihood = $-20{\cdot}71$, $P = 0{\cdot}21$; Fig. 4e), nor were the biomass of *C. stoebe* and that of NA neighbouring community in sterilized NA soil ($R^2 = 0{\cdot}02$, Δlog-likelihood = $-16{\cdot}29$, $P = 0{\cdot}81$; Fig. 4f).

Discussion

NEIGHBOUR ORIGIN DRIVES IMPACT OF *C. STOEBE* DURING COLONIZATION OF NEW SITES

In our competition pots, we found that *C. stoebe* produced larger biomass in NA than EU soil, which might indicate escape from soil biotic constraints. The results of our multi-factorial experiment, however, suggest that the high impact of *C. stoebe* during the colonization of new sites in North American grasslands strongly resulted from release from highly competitive plant neighbouring species at home, and altered biotic soil conditions in the introduced range and evolutionary changes in the plant invader were of less importance. REI, an index that provides information on the changes of the composition of plant associations over time (Connolly, Wayne & Bazzaz 2001), was used in our study to indicate the efficiency of *C. stoebe* relative to its neighbours in a mixture. In our study, we found that REI was substantially lower for *C. stoebe* when growing in competition with EU than with NA neighbours in both EU and NA soil, suggesting that *C. stoebe* is far more competitive in the presence of the NA than the EU neighbouring community regardless of soil origin. Since REI is not sensitive to variation in initial plant size and we did not observe a consistently different growth rate between EU and NA neighbouring communities in the non-competition pots, our results indicate an inherently different mechanism underlying the competitive interactions between EU and NA neighbouring communities when grown with *C. stoebe*. Ridenour *et al.* (2008) found that *C. stoebe* from North America were larger and demonstrated stronger competitive effects than plants from European populations (but see Henery *et al.* 2010). In contrast, in our greenhouse experiment we could not find evidence for post-introduction evolutionary change in relative competitive ability of *C. stoebe*. Therefore, our study provides evidence that the high impact of *C. stoebe* is not an inherent species trait, as proposed by Baker (1965), but is strongly driven by the origin of its neighbouring competitor(s). It should be noted though that we only tested a limited amount of populations of *C. stoebe* from the two ranges. Nevertheless, the increased vigour of *C. stoebe* in the presence of North American plant species as detected in our pot experiment and in a field experiment (Callaway *et al.* 2011) is unlikely to be driven by post-introduction selection for increased competitive ability of *C. stoebe*.

LARGER IMPACT ON THE NA NEIGHBOURING COMMUNITY

Our results indicate that the EU neighbouring community can more easily accumulate biomass in the presence of *C. stoebe* than NA neighbouring community. Moreover, the EU neighbouring community also impacted *C. stoebe* far more than the NA neighbouring community, providing additional support for their stronger competitive ability. These findings are consistent with the results of a field experiment, in which a strong impact of neighbouring vegetation on *C. stoebe* growth and reproduction was found in European but not in North American grasslands (Callaway *et al.* 2011).

Similar to the findings in unsterilized soil, *C. stoebe* had a lower impact on the EU neighbouring community than on the NA neighbouring community in both EU and NA sterilized soil. This further indicates that differences between soil biota in the home and the introduced range are not responsible for the shift in impact of *C. stoebe* when grown with its old vs. new neighbours.

EFFECT OF SOIL STERILIZATION ON THE COMPETITIVE ABILITY AND IMPACT OF *C. STOEBE*

The net effect of soil biota (strength and/or direction) can be explained by adding up potential negative effects of antagonists and potential positive effects of mutualists on plant growth (Richardson *et al.* 2000; Mitchell *et al.* 2006; Reinhart & Callaway 2006). In the competition pots, EU and NA neighbouring communities as well as *C. stoebe* showed a significant increase in biomass when soils were sterilized, suggesting that the plants generally benefited from a nutrient flush and/or a release from soil antagonists. However, since the soil collected in the field made up only 10% of the total soil in the pots, it is likely that the increased growth of plants grown on sterilized soil was due to a release from soil antagonists. Sterilization also resulted in reduced impact of *C. stoebe* on neighbouring communities, as suggested by the overall significant decrease in reduction in biomass of both EU and NA neighbours. These results provide some evidence that soil microbial biota contribute to increase the negative impact of *C. stoebe* on its neighbouring communities and are thus in line with the results of an interspecific competition experiment between *C. stoebe* and *Festuca idahoensis*, in which mycorrhizae increased *C. stoebe*'s negative effect on *F. idahoensis* (Marler, Zabinski & Callaway 1999). On the other hand, sterilization did not alter the effects of neighbouring communities on *C. stoebe*, or did it shift the competitive ability of *C. stoebe* against EU vs. NA neighbours.

RELATIVE IMPORTANCE OF FACTORS AFFECTING COMPETITION AND IMPACT OVER TIME

Our experiment was designed to assess the relative importance of neighbours, soil biota and post-introduction

evolution on the impact of *C. stoebe* during the early stages of invasion of North American grasslands, i.e. when both neighbours and soil are still 'naïve'. We acknowledge that the outcome of studies assessing the relative importance of different factors affecting plant invasions may considerably depend on the stage of the local plant invasion, e.g. whether the invader has just colonized a new site and is still rare, whether it has been present for a long time but at low densities (lag phase), or whether it has already built up high densities (Dietz & Edwards 2006). Based on a spatial simulation model of invasion and a meta-analysis on pairwise native-alien plant–soil feedbacks comparisons, Suding *et al.* (2013) predicted that the frequently observed positive plant–soil feedbacks for native species should result in invasion resistance when IAPs are rare. The benefits of encountering new neighbours that are poor competitors may, however, already be experienced at low densities during the initial invasion stages and may therefore explain why successful invaders such as *C. stoebe* are able to build up high densities. Characteristics of the soil biota (e.g. the lack of negative intraspecific plant–soil feedbacks due to the absence of co-evolved antagonists) may then explain why, over time, performance of *C. stoebe* does not decrease in the introduced range as significantly as in the native range (Callaway *et al.* 2004).

IMPACT TYPE OF *C. STOEBE*

Sun *et al.* (2013) proposed that the relationship between the biomass of the invader and that of the resident plants might provide insight into the mechanism underlying competitive interactions. A significant negative relationship between biomass produced by *C. stoebe* and that of its old neighbouring community suggests that they compete for limiting resources such as soil nutrients. In contrast, the biomass of *C. stoebe* explained very little of the variation in biomass of the NA neighbouring community, indicating that competition is driven by other forms of competition, such as by exploitation of resources that are not utilized by neighbours or by interference competition (Sun *et al.* 2013). These findings are in line with Ridenour & Callaway's (2001) suggestion that the exceptional competitive and invasive success of *C. stoebe* in North America is in part the result of allelopathic chemicals exuded from its roots. In addition, there is evidence that NA neighbours cannot be as efficient in exploiting soil moisture as EU neighbours nor *C. stoebe* (Ortega *et al.* 2012; Sun *et al.* 2013). These relationships did not differ between EU and NA soil origin, revealing that not only overall impact, but also impact type by *C. stoebe* is affected by neighbour origin rather than by soil origin.

Sterilization of EU soils alleviated the negative relationship between *C. stoebe* biomass and that of its EU neighbouring community, suggesting that the EU soil biota mediate resource competition. Sterilization did not change the relationships between *C. stoebe* biomass and that of its NA neighbouring community, thus providing additional support for the notion that soil biota do not substantially affect the impact of *C. stoebe* during the invasion of new sites in North American grasslands.

Conclusions

Over the past decades, much empirical and theoretical evidence has accumulated about mechanisms that enable some of the introduced plant species to displace resident species, such as an increased ability to exploit limiting resources, direct interference, lack of intraspecific plant–soil feedbacks or other forms of enemy release (Keane & Crawley 2002; Colautti *et al.* 2004; Wolfe & Klironomos 2005; van der Putten *et al.* 2013). To our knowledge, there are few studies experimentally assess the relative importance of neighbour origin, soil origin and post-introduction evolution during the initial stages of a plant invasion. Vivanco *et al.* (2004) found that experimental communities built from North American plant species are far more susceptible to invasion by *C. diffusa* than communities built from Eurasian species, regardless of the biogeographic origin of the soil biota. Similarly, in the case of *C. stoebe*, release from competition with co-evolved neighbours appears to be more important than release from soil biota or post-introduction evolution. It is likely, though, that other factors, especially escape from root herbivores (Blair *et al.* 2008), may also contribute to the high impact of *C. stoebe* in the introduced range, and that the relative importance of factors affecting the competitive ability of and impact by *C. stoebe* shifts during the invasion process. There is a clear need for more studies assessing multiple factors affecting the competitive ability and impact of IAPs also at later stages of the invasion process, which would require comparison of naïve soil with soil that has previously been 'trained' with IAPs (see e.g. Klironomos 2002). Nevertheless, combined evidence from such studies and well-designed field experiments (as the one by Callaway *et al.* 2011 in our case) will help to develop conceptual frameworks for a better understanding of the observed invasion success of some of the alien species.

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Data accessibility

Data associated with this study are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.3qn2r (Sun, Müller-Schärer & Schaffner 2014).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Graphical layout of the study concept.
Fig. S2. Graphical layout of our full factorial experimental design.
Fig. S3. The pattern of the spatial arrangement of plants in the pot.
Table S1. Origin of *Centaurea stoebe* populations.
Table S2. Soil collection sites.
Table S3. Statistical results of Figs 1, 2 and 3.