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ECOLOGICAL
EFFECTS
OF PLANT
INVASIONS

THOMAS VAN HENGSTUM

CURRICULUM VITAE

Thomas van Hengstum was born on 27 April 1983 in The Hague. When he was one year old he moved to the nearby city of Zoetermeer where he spent most of his time outside catching animals and climbing trees. He received his education at Erasmus College. After graduating in 2001 he moved to Amsterdam where he started his studies in Biological Sciences at the University of Amsterdam. His true passion for botany and ecology developed during a field course in Tenerife guided by Gerard Oostermeijer. Here, he performed a study on hybridization between three species from the genus *Pericallis*. During his Master's phase he returned to Tenerife to further investigate these hybridization events, of which the results have later been published. For his second Master's project he travelled to the Ecuadorian Andes where he investigated phenotypic plasticity and acclimation capacities of several alpine species of the genus *Draba*. After receiving his Master's degree (cum laude) in 2007 he travelled several months in Colombia. In 2008 Thomas once again returned to Tenerife where he, in cooperation with the University of Gran Canaria, investigated the reproductive biology of the highly endangered species, *Bencomia exstipulata*. Later that year he started a project at the University of Amsterdam where he investigated the escape of pollen grains from greenhouses. From 2008 until 2013 Thomas worked as a PhD student in the Experimental Plant Systematics group at the University of Amsterdam, where he worked on the ecological effects of plant invasions.

THOMAS VAN HENGSTUM

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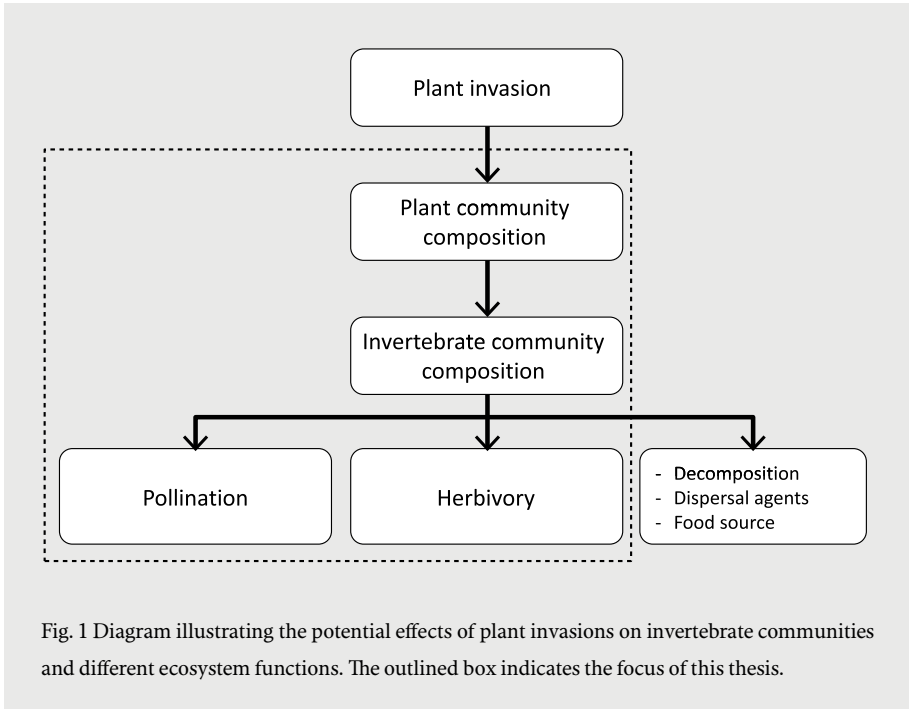
General introduction

Due to growing human mobility the number of species introductions outside their native range has increased dramatically over the past decades, leading to a steep rise in the number of plant invasions (Davis 2009, Hulme 2009). There is great concern over the ecological and economic effects of invasive species on their introduced environment. More recently, this concern has been further fuelled by the increased commercial cultivation of genetically modified organisms (GMOs), which are feared to exert even more harmful effects on the environment if they would become invasive (Ellstrand et al 2013; Andow and Zwahlen 2006). In this introduction I will explain how plant invasions may affect native biodiversity and ecosystem functioning. In particular, I will address the potential effects of invaders on plant-herbivore and plant-pollinator interactions, as well as on arthropod communities.

What are invasive species?

There is a lack of consensus on the definition of 'invasive species', and many different interpretations can be found in the literature (Colautti and MacIsaac 2004, Richardson and Pyšek 2006). All include rapid expansion as characteristic of invasive species, but many also take detrimental effects on economy, ecology and health into account. This can be problematic, because some effects of invasive species are temporal, spatially restricted or may not have been noticed yet (Colautti and MacIsaac 2004; Strayer et al 2006). Moreover, some effects may be dramatic while others may be negligible. Therefore, in this thesis I use a definition modified from (Richardson and Pyšek 2006), which only describes the expanding behaviour of invasive species: "exotic or native species that produce reproductive offspring, often in large numbers, at substantial distances from the parent plants and therefore have the potential to spread over a considerable area".

While most plant invasions are the result of a human-mediated introduction outside their native range, native species may also develop invasive behaviour following large-scale habitat modification, predator- or herbivore removal or climate change (Carey et al. 2012). In addition, native species may become invasive as a result of hybridisation and introgression of genes from a related (wild or crop) gene pool (Ellstrand 2003). An example of a native invader is *Lactuca serriola* in the Netherlands, which was considered rare before the 1950s, but is currently found in most of the country, as evidenced by its presence in 60% of all 5x5 km grid cells in recent surveys. Climate change, ruderalisation and hybridization with crop lettuce, *L. sativa*, were discussed as possible drivers for its rapid expansion (Hooftman et al. 2006).



Ecological effects of invasive plants

By definition, invasive species change the composition of the invaded plant communities, which may reduce the density of resident natives, and potentially drive some natives to local extinction. Besides a direct competitive effect on resident natives, invasive species may cause indirect effects that may in turn affect ecosystem functioning (Mack et al. 2000, White et al. 2006). A well-known example includes the invasion by a *Spartina* hybrid (*S. alterniflora* × *S. foliosa*) on the west coast of the United States, which dramatically increased sedimentation rates and caused the system to shift from an algae-based to a detritus-based food web (Levin et al. 2006). Another example of an invader that caused significant indirect effects is *Bromus tectorum* in the Western United States, which increased fire frequency by producing highly flammable biomass and by connecting previously separated patches of shrubs (Keeley 2006). Many of the indirect effects of invasions involve plant-animal interactions, including plant-pollinator and plant-herbivore relationships, which often play an important role in ecosystem functioning. In the next sections I will elaborate on some commonly occurring effects of invaders on plant-animal interactions (Fig. 1).

Arthropod communities and herbivory

Plant invasions can have significant impact on the composition, diversity and size of arthropod communities (French and Major 2001, Harris et al. 2004, Pearson 2009). It has often been hypothesized and sometimes been demonstrated that species richness and abundance of arthropods decreases following invasions (e.g. Greenwood et al 2004; Magoba and Samways 2011). At the same time, there are studies that found that arthropod communities increased in diversity or abundance (e.g. Ostoja et al. 2009, Pearson 2009). Thus far, it has remained unclear what are the actual factors influencing arthropod communities in response to invasions. Nevertheless, several possible drivers and mechanisms have been discussed in the literature: (i) exotic species are released from their natural enemies and therefore have fewer associated (specialist) herbivores than the natives they replace (Keane and Crawley 2002), (ii) the diversity of the plant community may be reduced (or enhanced) following invasion, providing local arthropod communities with less (or more) diverse resources and reduced (or enhanced) habitat complexity (Crooks 2002). Finally, (iii) arthropod community composition may be affected by environmental factors that are altered by the invader, such as water availability, irradiation and resource quality (Kimmins 2004; Levine et al. 2003).

In response to changes in arthropod community composition, plant-herbivore interactions may be affected considerably (White et al. 2006; Weiser and Siemann 2004). Invaders may affect the abundance or distribution of herbivores due to processes including resource concentration and spillover (Blitzer et al 2012; Rand and Louda 2004). As a result, invaders may indirectly alter the consumption rate and population dynamics of co-occurring natives (Holt 1977). This indirect effect, known as apparent competition, has been commonly observed in nature (Orrock et al. 2010; Meiners 2007). Thus, co-occurring native species may not only be affected by direct competition with invaders, but also by herbivore-mediated effects.

Plant-pollinator interactions

Besides through plant-herbivore interactions, invaders may also indirectly affect the fitness of native species through disruption of native plant-pollinator networks (Bjerknes et al. 2007, Memmott and Waser 2002, Morales and Traveset 2009). In the presence of invaders, co-flowering natives may experience increased competition for pollinators that are shared with the invader (Brown et al. 2002, Chittka and Schurkens 2001). Alternatively, the invader may act as a magnet-species for pollinators, attracting larger numbers of them and increasing rather than decreasing the visitation frequency to co-occurring native flowers (Ghazoul 2006, Johnson et al. 2003, Moeller 2004, Molina-Montenegro et al. 2008). It is not entirely clear when

native plants experience more intense competition for pollinators in the presence of invaders, or when pollination is facilitated (Morales and Traveset 2009). Nevertheless, some mechanisms have been identified that give clues about the interactions that can be expected. First of all, in order to have interactions between invaders and natives they need to have at least partly overlapping pollinator communities. High overlap between pollinator communities is expected when natives and invaders have similar flower morphology and overlapping phenology. Under these conditions strong native-invader interactions are to be expected (Memmott and Waser 2002). Other factors that may determine the strength of native-invader interactions include the flower attractiveness of the invader compared to the native plants, and the population size and density of the invader (Bjerknes et al 2007; Memmott and Waser 2002). As yet not much empirical evidence is available to test these expectations.

Risk assessment

Since invasions can cause considerable ecological and economic damage, effective management and mitigation of invasions is of major importance (Pimentel et al 2005; Gordon et al. 2008). Unfortunately, complete eradication of invading plant species is often unfeasible (Rejmanek 2000), and maximum priority is given to prevention and/or removal in very early stages of an invasion of potentially harmful plants. Risk assessment systems are designed to predict the species' invasive potential, as well as its potential to cause environmental harm (Andersen et al. 2004, Kolar and Lodge 2001, Pheloung et al. 1999). These systems serve two principal aims: (i) risk assessment of (potentially) invasive species prior to their introduction, leading to a decision to prohibit or authorize (with or without restrictions). (ii) Prioritization of control measures designed to halt both spread and harmful impact of established invasive species.

In current risk assessment systems, such as the Australian weed risk assessment system, (potential) invasive species are typically screened based on a set of criteria related to their reproductive system, biogeographical properties and other attributes, including growth form, toxicity and palatability (e.g. Pheloung et al. 1999; Daehler et al. 2004; Gassó et al. 2010). Although the implementation of risk assessment systems has proven successful in identifying potentially invasive species (Gordon et al. 2008), potential detrimental ecological effects are rarely taken into account. For instance, possible indirect effects of invaders, including effects on pollination and animal communities, are usually not included in risk assessment systems. In this thesis I will provide several examples of such indirect effects to illustrate the need to incorporate these factors in future risk assessment designs.

Transgene escape

Besides for 'conventional' species, risk assessment systems are widely implemented for the evaluation of genetically modified organisms (Andow and Zwahlen 2006). The rapid development of genetic engineering and increased cultivation of GMOs has led to concerns about transgene escape and their potential effects on natural ecosystems (Snow et al. 2005; Stewart et al. 2003; Andow and Zwahlen 2006). There are two pathways through which crop genes may be introduced into the wild. The first pathway is through spillage of propagules (e.g. seeds, tubers) during transport by wind, humans or other animals, which can result in the establishment of persistent feral populations. The second pathway is through hybridization of domesticated plants with wild relatives, which, after several hybrid generations, may lead to introgression of crop genes into wild populations (Ellstrand 2003).

Introgression of a fitness-enhancing crop gene could cause the receiving wild relative to become invasive. Especially introgression of transgenes that increase abiotic tolerance or herbivore resistance are thought to provide a competitive advantage in natural environments (Stewart et al. 1997). If such scenario would become reality, it is feared that they may cause similar or even more ecological damage than regular invaders (Warwick et al. 2009). In order to predict potential ecological damage following transgene escape we first need to consider three levels of escape, with an increasing impact on the recipient environment:

Diversity. Crop-wild hybridization leads to genetic erosion of the wild species, but the transgene does not affect plant density or distribution of the recipient wild species. Despite this fact, interacting species may still be negatively affected, for example due to a change in the plants chemical composition.

Density. Crop-wild hybridization locally enhances competitive ability and, as a result, increases plant density in the habitats of the recipient wild species. As a consequence, this could lead to the disappearance of competing species and quantitative changes in the (functional) diversity of the system. For instance, the abundance of pollinators and herbivores may be affected.

Distribution. At this third level, crop-wild hybridization increases the niche width of the wild recipient species, allowing it not only to increase in density, but also to invade previously unsuitable habitat. An increase in niche width may be caused by transgenes that enhance abiotic tolerance or herbivore resistance. When crop-wild hybrids invade new habitat, they will encounter new species to interact with and may introduce other species to the system, including herbivores, pollinators and pathogens. Thus, in addition to quantitative effects we may also expect qualitative

ecosystem effects. In this thesis we consider potential ecological effects under two scenarios: 'invaders' that increase in density and 'invaders' that colonize new habitat. Note though that although there have been many reports of gene flow from transgenic plants to wild relatives in the natural environment (Ellstrand 2013, Knispel et al. 2008, Lu and Yang 2009), thus far no negative ecological (community-wide) effects of transgene escape have been reported for the present range of genetically modified crops.

General aim of this thesis

The aim of this thesis is twofold: in the first place we aim to increase our understanding of ecological effects of plant invaders. Second, we aim to provide methodological insights that can be used for control prioritization and the development and improvement of ERA (environmental risk assessment) systems. Risk assessment of non-native species, including (transgenic) crops and ornamentals can reduce the risk of invasions and prevent subsequent ecological and economic damage.

Outline of this thesis

The research presented in this thesis focuses on the effect of plant invasions on arthropod communities, including the indirect effects on plant-pollinator and plant-herbivore interactions. In addition, we investigated the effect of plant introductions on native plant communities in the Netherlands.

Pollination and herbivory

The second chapter focuses on plant-pollinator and plant-herbivore interactions. For this purpose we experimentally introduced five native target species that were placed in the proximity of an 'invasive' model species, *Brassica rapa*. This allowed us to assess the potential effect of the invader on native flower visitation rates and seed set. We hypothesized that competition for pollinators with the invasive species would decrease the flower visitation frequency and seed set of co-flowering native species, provided that there is overlap in pollinator communities between the invasive- and native species. With regard to the potential effects on herbivory we hypothesized that co-occurring native plant species experience increased leaf and fruit consumption due to apparent competition, provided that the native and invasive species share herbivore species.

Invertebrate communities

The third chapter was dedicated to the effects of plant invaders on local invertebrate communities. Two invasive model species, *Brassica napus* and *Lactuca serriola*,

were experimentally introduced in a semi-natural system and for two years invertebrate communities inside and surrounding invasion plots were monitored using pitfalls, sticky traps and suction sampling. In addition, around each invasion plot we placed two native target plants to measure whether changes in invertebrate composition also affected herbivore pressure to nearby native species. The following hypotheses were addressed: (i) given the positive relationship between plant- and invertebrate diversity, the invertebrate richness within invasion plots is expected to be lower compared to the adjacent habitat. Furthermore, (ii) the abundance of invertebrates that are associated with the invader will increase in adjacent vegetation due to spillover. (iii) The incidence of herbivory to co-occurring native plants will increase as a result of apparent competition and spillover effects, especially for plants that are taxonomically related to the invader.

Meta-analysis: effect of invaders on arthropod communities

In chapter 4, we performed a meta-analysis of 56 studies to assess the effect of plant invasions on local arthropod abundance and species richness. In addition, we tested five hypotheses that may be predictive for the direction and magnitude of effect on arthropod communities, namely (i) the time since introduction influences the magnitude and direction of change in native arthropod communities; (ii) arthropod communities are less negatively affected in abundance and richness by an invader when native congeners are already present in the region; (iii) woody invaders cause a stronger change in arthropod communities than herbaceous invaders; (iv) the magnitude of change in arthropod communities will increase with increasing canopy cover of the invasive species, and (v) multiple species invasions cause a bigger change in local arthropod communities than single species invasions.

Exotic plants in the Netherlands

In this final research chapter we used a dataset of more than 10.000 periodically monitored quadrats in the Netherlands to address the question whether exotic plants in the Netherlands have locally reduced native species richness. Subsequently the following hypotheses were addressed: (i) The diversity-resistance hypothesis: under this hypothesis native high diversity habitat is less frequently invaded by exotics than native low diversity habitat. (ii) The resource-enrichment hypothesis: disturbance, measured as the cover of bare ground, is positively correlated with the occurrence of exotic species. (iii) The vacant niche hypothesis: exotic species have different niche characteristics than natives, which may partly explain their success.

In the final chapter we summarized the results and discuss the implications for risk assessment of potential plant invaders. Furthermore, we highlighted several methodological aspects that may be improved in future risk assessment systems. ●

Impact of experimental plant invasions on plant-pollinator and plant-herbivore interactions

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Abstract

Plant invasions can have major impacts on ecosystem functioning and therefore environmental risk assessment (ERA) systems are operational in several countries in order to mitigate their potential risks. However, ERA systems rarely incorporate potential ecological effects of plant invasions. In this study we provide evidence on how plant invasions can indirectly affect native plant-pollinator relations, plant-herbivore interactions and reproductive success of native plants. We used experimental local 'invasions', as well as naturally occurring populations of the invasive model species *Brassica rapa* to study its impact on the recipient ecosystem. Specifically, we tested whether the presence of *Brassica rapa* affected the visitation frequency and reproductive success of a group of five focal native species. In addition, for one native wild relative we measured the incidence of herbivory on fruits and leaves. Depending on the focal native species, we found an increase, decrease or no change in visitation frequency. Yet, these changes did not affect seed output. Furthermore, fruits from the native wild relative growing in the presence of *Brassica rapa* were less often consumed compared to the control. With these results we demonstrate that indirect effects can play an important role in invasion biology and that incorporating these aspects in ERA systems may improve their performance.

Introduction

Plant invasions can affect key ecological processes such as biochemical cycling (e.g. Vitousek et al. 1987), the frequency of disturbances (e.g. Dantonio and Vitousek 1992) and plant-animal interactions (Levine and Rees 2004). Disruption of such processes may threaten native ecosystems and it has been argued that it may eventually lead to loss of plant and animal diversity (Powell et al. 2011, Vilà et al. 2011). In order to mitigate such ecological impact caused by plant invasions, environmental risk assessment (ERA) systems have been developed. An example of a widely implemented and tested system is the Australian weed risk assessment system (Pheloung et al. 1999), which is currently being adopted for application in other parts of the world (Daehler et al. 2004, Gordon et al. 2008, Gassó et al. 2010, Chong et al. 2011). Typically, ERA systems will score potentially invasive species according to a set of criteria related to their biogeography, undesirable attributes (e.g., toxicity of fruits and unpalatability) and ecological properties, such as growth form, reproductive system and dispersal mechanism. Species that fall above a certain threshold will be designated as 'high risk' and will consequently be rejected for introduction as ornamental or crop species.

An increasing number of studies has shown that indirect effects caused by plant invasions can have significant impact on plant community structure and ecosystem

functioning (White et al. 2006, Morales and Traveset 2009, Dangremond et al. 2010, Lau 2012). Nevertheless, the major emphasis of ERA systems is placed on the invasive potential of the species, rather than its potential ecological impact. Incorporating ecological aspects in ERA systems could improve the quality of the risk assessment and can hereby reduce the risk of ecological damage to the recipient system. In particular, two potential indirect effects of plant invasions could be relevant, namely the effect on native plant-pollinator interactions, and on the incidence of herbivory to native co-occurring species.

With respect to the first potential effect it is widely recognized that invasive plant species can disrupt plant-pollinator networks by their integration into native plant-pollinator networks (Memmott and Waser 2002, Bjercknes et al. 2007, Morales and Traveset 2009). As a result, insect visitation frequency to co-flowering plants may be reduced due to increased competition for pollinators with the invasive species (e.g. Chittka and Schurkens 2001, Brown et al. 2002). On the other hand, the visitation frequency to co-flowering species may increase when the invasive plant species attracts and maintains additional populations of pollinators (the 'magnet-species hypothesis', e.g. Johnson et al. 2003, Moeller 2004, Ghazoul 2006, Molina-Montenegro et al. 2008). Pollinator sharing between the invasive and co-flowering native species determines whether, and to what extent, they will interact. In particular taxonomically related species with analogous flower morphology may share pollinators with the invasive species and will therefore interact more strongly (Morales and Traveset 2009). Finally, co-flowering plant species with highly specialized pollination systems (i.e. plants pollinated by one or few species of pollinators) are expected to be more susceptible to disruptions in the plant-pollinator network, because they are less likely to attract alternative pollinator species (Bjercknes et al. 2007).

For outcrossing plant species, successful fertilization of the plants' ovules depends on the pollinator visitation frequency and the availability of resources, but also on the quality (or effectiveness) of the pollinator (Herrera 1987, Aigner 2004). Pollinator quality not only refers to the number of conspecific pollen grains that are successfully transported from flower to flower, but also refers to the composition of the pollen grain mixture that is deposited on the flowers' stigmas. Heterospecific pollen deposition may reduce seed set through a number of processes (Da Silva and Sargent 2011), including stigma clogging and pollen allelopathy (Morales and Traveset 2009, Arceo-Gómez and Ashman 2011). Another factor that may reduce seed set is pollen- and flower consumption (Irwin et al. 2001). For instance, some species of bees, beetles (Hargreaves et al. 2009) and hoverflies (Holloway 1976) feed on pollen and other floral parts.

A second potential indirect effect of plant invasions we address in this study is the effect on the incidence of herbivory in native plants. Apparent competition between native and invasive plants is an important driver that can disrupt native plant-herbivore interactions (Holt and Lawton 1994, Noonburg and Byers 2005, Meiners 2007, Dangremond et al. 2010). Apparent competition is an indirect interaction by which one species increases the abundance or distribution of a shared consumer and thereby alters the consumption rate and population dynamics of another species. Invasive species may increase the abundance and distribution of consumers by providing them with a refuge, or by providing them additional food (Orrock et al. 2010, Dutra et al. 2011). For example, Orrock and Witter (2010) demonstrated that the exotic forb *Brassica nigra* increased the pressure of native consumers on a native bunchgrass species, and by doing so, inhibited its establishment. Similarly, Rand and Louda (2004) showed that spillover of herbivores from an invasive thistle to neighboring habitat increased the incidence of herbivory to a co-occurring native relative.

In this study we provide two examples of how plant invasions can disrupt native plant-animal interactions. For this purpose we used experimental invasions as well as naturally occurring populations of the invasive model species *Brassica rapa* or field mustard - a close relative of the widely cultivated oilseed rape (*Brassica napus*). The results from this study are not only applicable to ERA, but are also relevant for risk assessment of novel GM crops (Craig et al. 2008), since increased weediness of crop-wild hybrids or of feral crop plants is considered to be one of the potential risks of the introduction of GM crops (Warwick et al. 2009).

The specific hypotheses that we test in this study are: (1) competition for pollinators with the invasive species will decrease the flower visitation frequency and seed set of co-flowering native species, provided that there is overlap in pollinator communities between the invasive- and native species. With regard to the potential effects on herbivory we hypothesize that (2) co-occurring native plant species experience increased leaf and fruit consumption due to apparent competition, provided that the native and invasive species share herbivore species.

Material and Methods

We adopted two different experimental approaches, one in which we experimentally introduced potted *Brassica rapa* in the field (experiment 1) and one in which we used naturally occurring *Brassica rapa* populations (experiment 2). In both experiments we studied the effect of invasive species on the reproductive success of native plants, and in experiment 2 we additionally studied effects on herbivory.

Study species

For both experiment 1 and 2 we used *Brassica rapa* L. (Brassicaceae), commonly known as field mustard, as our model invasive species. *Brassica rapa* is an annual or biennial self-incompatible herbaceous plant. Although the species is considered native to the Netherlands (van der Meijden 2005), it probably originates from the Mediterranean region from where it spread to the rest of Europe as it became domesticated (Weeda 1999). Its massive floral display and high nectar rewards attract great numbers of generalist pollinators, such as (bumble)bees, (hover)flies, butterflies and beetles. The peak flowering period is in April and May, but it may continue to flower until August. *B. rapa* is a common weed throughout Europe. In the Netherlands, the species has drastically expanded since 1975 (Luijten and De Jong 2010).

Species in experiment 1: for this experiment we selected four native focal species that are all obligate outcrossers. Two of them had a generalized pollination system, namely *Leucanthemum vulgare* Lam. (Asteraceae) and *Daucus carota* L. (Apiaceae). In addition, we selected two species with a more specialized pollination system, namely *Trifolium pratense* L. (Fabaceae) and *Linaria vulgaris* Mill. (Plantaginaceae). The focal plants were dug out from semi-natural grassland and were selected according to their (similar) size and number of inflorescences.

Leucanthemum vulgare is a perennial herb that flowers from May to August and is mainly pollinated by generalist beetles, (hover)flies, (bumble)bees and occasionally butterflies. *Daucus carota* is a biennial plant attracting a wide range of generalist insects, especially beetles and (hover)flies. It flowers from June to autumn. *Trifolium pratense* is a biennial or perennial herb that flowers from May till autumn. It is usually pollinated by long-tongued bumblebees and occasionally by butterflies or large bees. The fourth native focal species *Linaria vulgaris* is a perennial herb that, owing to its flower morphology, can only be pollinated by long-tongued bumblebees. However, short-tongued bumblebees occasionally rob the nectar through holes bitten into the spurs of the flowers. The species flowers from June to autumn.

Species in experiment 2: for this experiment we used *Diplotaxis tenuifolia* (L.) DC. (Brassicaceae) as native focal species, a perennial plant that flowers from June to August, and has flower morphology and pollination strategy that is similar to that of *Brassica rapa*. Plants were reared from seeds in the greenhouse, were of similar size and contained a similar number of flowers.

Table 1 Geographical coordinates of the field sites of experiment 1 and 2.

Experiment	Site	Latitude	Longitude
1	1	04°56'58.75'	52°21'27.96'
	2	04°45'33.08'	52°24'07.44'
	3	04°44'23.35'	52°24'57.99'
	4	04°46'07.02'	52°24'31.95'
	5	04°58'07.81'	55°22'11.16'
2	1	04°54'31.92'	52°19'28.86'
	2	04°54'57.42'	52°19'33.37'
	3	05°00'17.89'	52°19'16.80'
	4	04°56'51.33'	52°24'32.98'
	5	04°58'28.77'	52°25'31.07'
	6	04°59'42.37'	52°25'54.52'

Experimental design

Experiment 1: We performed experiments at five locations in and around Amsterdam in semi-natural habitat in road verges, on dikes or vacant lots containing a mix of grasses and herbs (Table 1). At three of the five locations we used *Trifolium pratense* and *Leucanthemum vulgare* as native focal species and at the remaining two locations we used *Linaria vulgaris* and *Daucus carota*. At each location we placed 6 plots containing 48 plants in pots (1.5 l, ϕ 16 cm), of which 16 were from the native species (8 for each species), and 32 from *Brassica rapa*. Three of those plots contained flowering *Brassica rapa* plants (from now on referred to as 'Brassica' plots); while in the remaining three plots ('control' plots) all flowers from *Brassica rapa* plants were manually removed. The pots within the plots were arranged in 6 rows of 8 (Appendix S1 a). The two types of plots were separated by 100 m and placed in alternating order in an approximately linear transect in the landscape (Appendix S1 b). In order to trace back the flowers and fruits of the native focal plants that developed during the experiment, we marked the base of the flowers that were about to open at the start of the experiment with a droplet of green acrylic paint (Talens Decorfin, no. 618) applied with a toothpick.

Experiment 2: In order to deal with spatial variation among different geographical locations, we selected 6 experimental sites along drainage ditches, roads, dikes and bicycle paths in and around Amsterdam (Table 1). At every location we created 6 plots with 6 individuals of flowering *Diplotaxis tenuifolia* in pots (2 l, ϕ 16 cm). Three plots were placed on the edge of dense and extensive *Brassica rapa* populations containing at least one thousand flowering plants. The number of *Brassica rapa* flowers in the direct vicinity of the plots (i.e. an area of 1 m² in which the plot was placed in the center) was approximately 2000-3000. Three control plots were placed in standing vegetation containing grasses and herbs and were separated by at least 100 meters from the nearest *Brassica rapa* population. Plots were spaced 20 to 40 meters from each other. The flowers of *Diplotaxis tenuifolia* were marked as described for experiment 1.

Pollinator observations

Experiment 1 and 2: In order to assess the amount of insect visits to the flowers of the native focal species and to *Brassica rapa* we did paired observations in which multiple observers simultaneously observed one control and one *Brassica* plot. By doing so we accounted for temporal fluctuations in pollinator activity resulting from weather conditions and time of the day. Observations were done on dry, non-windy days. Prior to the observation we counted the number of open flowers of the native focal species and estimated the number of open *Brassica rapa* flowers. Pollinators were sorted to seven functional groups: hoverflies (Diptera-Syrphidae), other flies (Diptera), short- and long-tongued bumblebees (Hymenoptera-Apidae-Bombus), other bees (Hymenoptera-Apidae), beetles (Coleoptera) and 'other insects', which included ants (Hymenoptera-Formicidae), butterflies (Lepidoptera) and wasps (Hymenoptera). In experiment 2 we did not distinguish between short- and long-tongued bumbles.

Experiment 1: In this experiment the observations were done from May to July 2009 from 9 am - to 4 pm. During a period of two weeks the plots were observed for four days. On average we did 29 observations of 30 minutes, with a minimum of 25 at location 5 and a maximum of 33 at location 2. Observations were evenly distributed over the plots. Adding up all simultaneous observations the average total observation time per location was 29 hours. During one observation all inflorescences in a given plot were monitored, including flowers of *Brassica rapa*.

Experiment 2: Observations were carried out during the peak flowering period of *Brassica rapa*, from April 21 till May 11, 2011 from 9 am to 4 pm. Due to extremely

dry weather conditions; the flowering period of *Brassica rapa* was exceptionally short compared to previous years. A considerable amount of *Brassica rapa* plants stopped flowering during the observation period at one of the locations (location 1) and we therefore decided to exclude the visitation and seed set data of this location from the rest of the data. We did use data from this location to assess effects on herbivory. The plots were placed in the field for the duration of two weeks, during which they were observed for four days. During one observation we monitored visitation to all focal plants in the plot, including visitation to *Brassica rapa* flowers in an area of 1 m² (with the focal plants situated in the center of this area). On average we did 37 simultaneous observations of 10 minutes with a minimum of 26 and a maximum of 42 per location. The total time of observation was on average 6.1 hours per location.

Seed set analysis

At the end of the two field experiments all native focal plants were transferred to the greenhouse where they were allowed to develop the fruits that resulted from the marked flowers that were receptive during the field experiment. After approximately three weeks the ripe fruits were collected and analyzed using a stereo microscope. Fruits that were damaged by herbivores were not used to determine seed set.

Experiment 1: For *Leucanthemum vulgare* we collected eight capitula per plot (one per individual plant) for seed set analysis, and for *Trifolium pratense* we collected an average of three inflorescences per plant and 24 per plot. For both species we then counted the number of fertilized and aborted ovules. For *Daucus carota* we collected on average 2 inflorescences per plant. Because it was not possible to make a reliable count of the aborted ovules, we counted the number of fertilized ovules and divided this by the surface area of the inflorescence, which was estimated by taking the squared radius of the inflorescence multiplied by π . Nearly all the fruits of *Linaria vulgaris* were severely damaged by insects, which made it impossible to determine the number of fertilized ovules. Instead, the number of developed versus aborted fruits was taken as a measure for pollination success.

Experiment 2: From five *Diplotaxis tenuifolia* plants per plot we collected five fruits. The number of fertilized versus aborted ovules was determined for one (of the two) randomly selected carpel per fruit.

Heterospecific pollen deposition

On the final day of pollinator observations we sampled the stigmas from open flowers of *Diplotaxis tenuifolia* in experiment 2. We excised two randomly selected stigmas per plant and placed them in Eppendorf tubes after which they were stored

at -20 °C. We sampled 5 plants per subplot, summing up to 60 stigmas per field location. To extract the pollen from the stigmas and visualize them under a light microscope we added a 0.2 ml solution of 50% glycerin and 0.25% basic fuchsin staining agent to the Eppendorf tubes. In order to detach the pollen grains from the stigmatic tissue the tubes were transferred to an ultrasonic bath (Sonicor SC-211-22TH) where they were treated for 10 minutes at 55 kHz. The solution was vortexed after which the whole sample was extracted for microscope analysis. Since some samples contained > 4000 pollen grains we counted a maximum of 300 pollen per sample (=pollen sum). We distinguished between pollen from *Diplotaxis tenuifolia*, *Brassica rapa* and 'pollen from other species'. Although *Diplotaxis tenuifolia* and *Brassica rapa* pollen grains are morphologically similar they could be distinguished on the basis of their size (*Diplotaxis tenuifolia* pollen grains are larger than *Brassica rapa* pollen grains).

Leaf and fruit damage

To quantify the amount of herbivore damage to leaves of *Diplotaxis tenuifolia* in experiment 2, we collected 5 randomly selected leaves (> 5 cm) from five plants per plot. Leaves were taped to a sheet of A3 paper which was subsequently scanned (resolution 9921x7015 pixels, Océ variolink 3622c). Then we imported the pictures to the image processing program ImageJ (version 1.44) to determine the total leaf surface that was affected by herbivores. All fruits that were collected for the seed set analysis were classified as either 'undamaged' or 'damaged' (by weevils and gall midges). *Diplotaxis tenuifolia* plants were raised in the greenhouse and were completely intact prior to the experiment.

Statistical analysis

Flower visitation, fruit- and leaf damage, as well as the seed set data from experiment 1 and 2 were analyzed by fitting generalized linear mixed models (GLMM's) with crossed random effects (Baayen et al. 2011) in the statistical framework R (R Development Core Team 2011) using the function *glmer* from the *lme4* package (Bates et al. 2011). Models were fit following the recommendations by Bolker et al. (2009) and Zuur et al. (2009). In our design the factor 'plot type' (*Brassica* vs. control plot) was treated as a fixed factor, and the factor 'location' and 'plot' were treated as random factors. The relationship between 'plot type' and 'location' was specified as crossed. Initially, we also included the number of open inflorescences as co-variable in the model. However, after model evaluation using the *anova* function, we dropped this co-variable because it did not improve the fit in any of the models. Since flower visitation data is based on counts we used a Poisson error distribution and a log link function, while for the seed set data and the fruit- and leaf damage

data we used a Binomial error distribution with logit link function. P-values were calculated on the bases of Wald tests, which are incorporated in the lme4 package. These P-values were confirmed by fitting a reduced model and comparing it with the full model using the *anova* function. The two methods always yielded similar P-values and therefore only Wald tests are presented.

For *Daucus carota*, the response variable for the model was the number of fertilized ovules per inflorescence surface instead of the ratio between fertilized and aborted ovules. Therefore this data was fitted using a general linear mixed model with a log10 transformed response variable using the *lmer* function from the lme4 package (Bates et al. 2011). P-values for this model were calculated using a Markov Chain Monte Carlo approach with 10.000 simulations using the *pvals.fnc* function from the languageR package (Baayen 2011).

A principal component analysis (PCA) was performed on the flower visiting community of *Brassica rapa*, *Leucanthemum vulgare*, *Trifolium pratense*, *Daucus carota* and *Linaria vulgaris* from experiment 1 using the *prcomp* function from the R-package *vegan* (Oksanen et al. 2012).

Results

Experiment 1

Insect visitation and seed set

Effects of *Brassica rapa* invasions plots differed between the four species studied. One of the four native focal species, *Leucanthemum vulgare*, showed an overall increase ($Z = 2.84$, $P < 0.01$) in visitation frequency of 109% when positioned in the proximity of the invasive model species *Brassica rapa*, compared to the control (Fig. 1a). Overall flower visitation rate for the other three native focal species was not significantly different in plots with and without *Brassica rapa* (Fig. 1 b-d). The visitation frequency of the four native focal species differed between locations. For instance, *Daucus carota* received more visits in control plots at one location, but not in a second (overall effect: $Z = 1.35$, $P = 0.18$). The two other native focal species, *Trifolium pratense* and *Linaria vulgaris*, did not respond to the presence of *Brassica rapa* at any of the locations.

Presence or absence of *Brassica rapa* did not affect the seed set of any of the native focal species (Fig. 1 a-d). The higher visitation rate of *Leucanthemum* in the presence of *Brassica rapa* did not translate to an increase in seed set ($Z=0.21, P=0.83$).

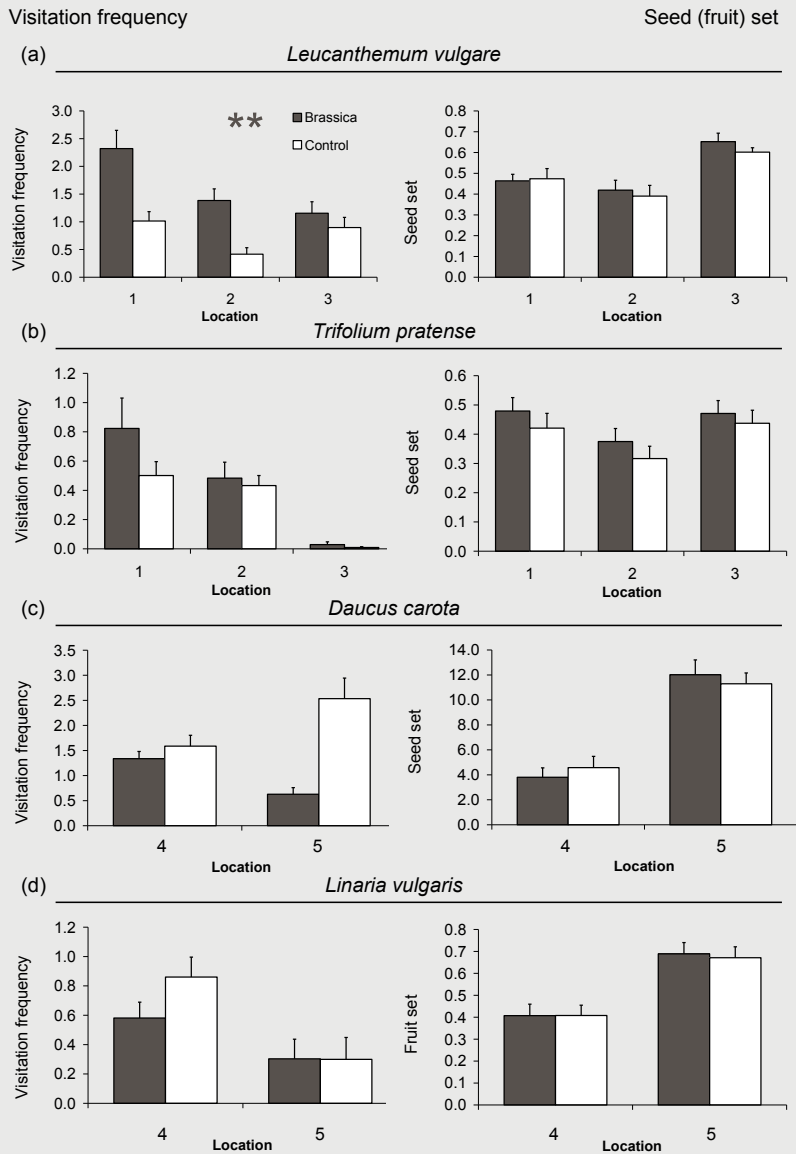


Fig. 1 Visitation frequency and seed (or fruit) set (mean \pm SE) for (a) *Leucanthemum vulgare*, (b) *Trifolium pratense*, (c) *Daucus carota* and (d) *Linaria vulgaris* from Brassica and control plots at 5 locations (Table 1, exp. 1). Visitation is defined as the number of visits per hour per inflorescence (a,b,c), or per flower (d). Seed set is defined as the proportion of fertilized versus aborted ovules (a and b) or the number of fertilized ovules relative to the inflorescence surface (c). For *Linaria vulgaris* (d) the number of developed versus aborted fruits is shown. '**' indicates overall statistical significance at the $P < 0.01$ level.

Shared pollinators

Both *Linaria vulgaris* and *Trifolium pratense* were almost exclusively visited by the long-tongued bumblebee *Bombus pascuorum* Scopoli (84 and 91% of visits, respectively). In contrast, inflorescences of *Leucanthemum vulgare* and *Daucus carota* were visited by a wide variety of insect groups, mainly by flies (32% and 43%, respectively) and hoverflies (42% and 32%, respectively). Also *Brassica rapa* was visited by a mix of insect groups, consisting mostly of hoverflies (42%), short-tongued bumblebees (40%), and bees (14%). The native focal species that shared most insect visitors with the invasive species was *Leucanthemum vulgare*, with an overlap of 49%, followed by *Daucus carota* with 40%. The specialized pollination strategy of *Linaria vulgaris* and *Trifolium pratense* was confirmed by their limited overlap in insect visitor assemblage with *Brassica rapa* (14% and 11%, respectively). The first axis of the principal component analyses of the total visitation community explained 78% of the variation and accounts mainly for the occurrence of long-tongued bumblebees and hoverflies (Fig. 2). *Linaria vulgaris* and *Trifolium pratense* are separated from *Leucanthemum vulgare*, *Daucus carota* and *Brassica rapa* on the basis of the first axis. The second axis explains 19% of the variation and separates *Daucus carota* and *Leucanthemum vulgare* from *Brassica rapa*; mainly on the basis of the occurrence of flies and short-tongued bumblebees.

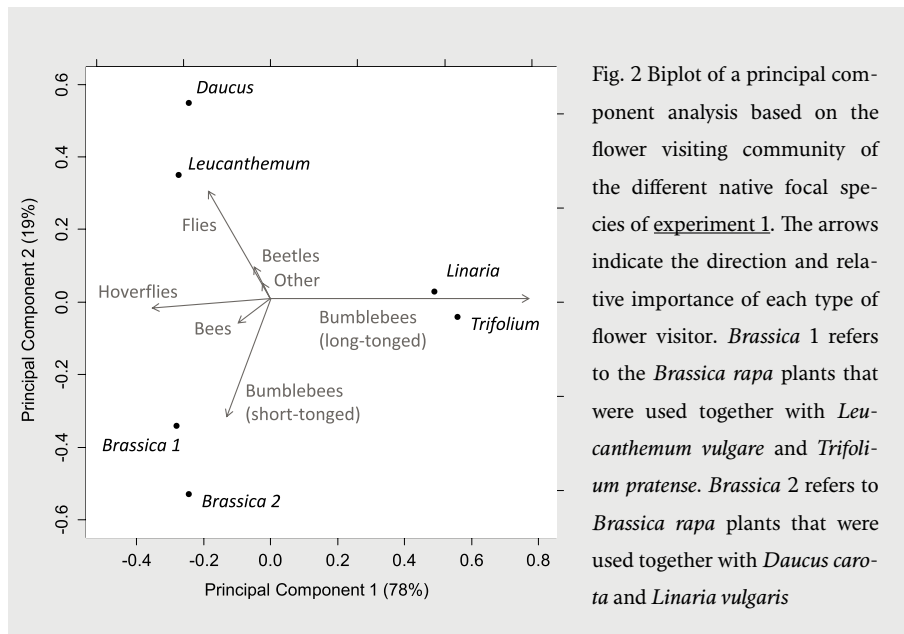


Fig. 2 Biplot of a principal component analysis based on the flower visiting community of the different native focal species of experiment 1. The arrows indicate the direction and relative importance of each type of flower visitor. *Brassica 1* refers to the *Brassica rapa* plants that were used together with *Leucanthemum vulgare* and *Trifolium pratense*. *Brassica 2* refers to *Brassica rapa* plants that were used together with *Daucus carota* and *Linaria vulgaris*

Experiment 2

Insect visitation and seed set

The overall visitation frequency to flowers of *Diplotaxis tenuifolia* near *Brassica* plots was almost three times lower compared to the control ($Z = 3.36$, $P < 0.01$; Fig. 3a). This effect was consistent over all locations. Despite this difference in visitation frequency we found no difference in the resulting seed set between *Brassica* and control plots ($Z = 0.57$, $P = 0.57$; Fig. 3b). The response to *Brassica rapa* on seed set differed markedly between the locations.

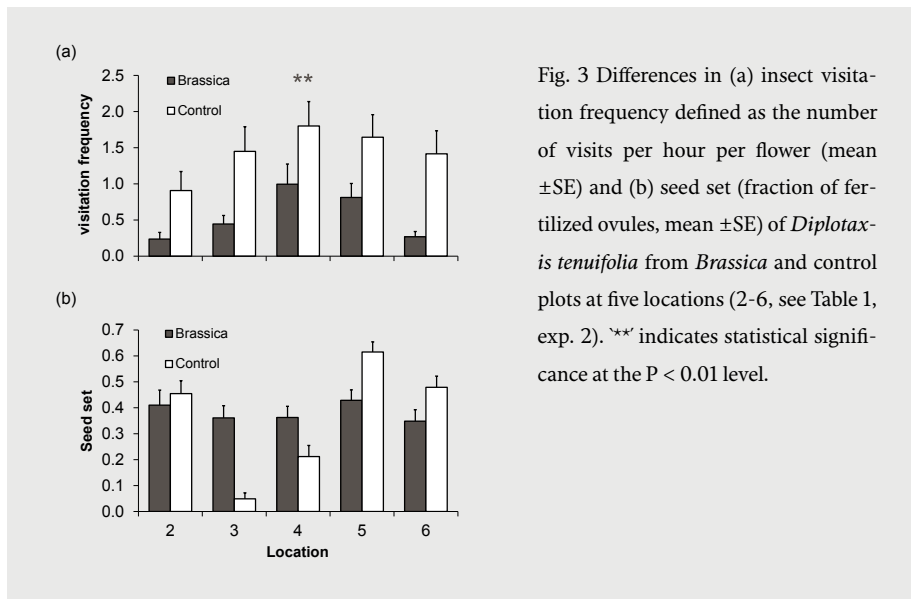


Fig. 3 Differences in (a) insect visitation frequency defined as the number of visits per hour per flower (mean \pm SE) and (b) seed set (fraction of fertilized ovules, mean \pm SE) of *Diplotaxis tenuifolia* from *Brassica* and control plots at five locations (2-6, see Table 1, exp. 2). ** indicates statistical significance at the $P < 0.01$ level.

Pollinator assemblages

The pollinator assemblage of *Diplotaxis tenuifolia* overlapped for 67% with that of *Brassica rapa*, which was mostly visited by Diptera (flies and hoverflies, 34 and 51% respectively), followed by bees (10%), bumblebees (5%) and less than 1% by beetles and other insects. The most important visitor of *Diplotaxis tenuifolia* in terms of the number of visits were flies (54%), followed by hoverflies (28%), beetles (10%), bumblebees (3%), bees (2%) and other insects (2%). The majority of the beetles observed on *Diplotaxis tenuifolia* and *Brassica rapa* were pollen beetles from the genus *Meligethes* (Fabricius) (Coleoptera: Nitidulidae) and *Ceutorhynchus obstructus* (Marsham) or cabbage seedpod weevils (Coleoptera: Curculionidae). Pollinator assemblages varied considerably between locations (Appendix S2).

Heterospecific pollen deposition

We found no overall difference in the percentage of heterospecific pollen deposition on stigmas of *Diplotaxis tenuifolia* between *Brassica* (mean $40.2 \pm \text{SE } 5.3$) and control plots (mean $31.0 \pm \text{SE } 7.8$; $Z = 1.27$, $P = 0.21$; Fig. 4). However, we found proportionally more *Brassica rapa* pollen on the stigmas of *Diplotaxis tenuifolia* that were situated near *Brassica* plots, compared to the control ($Z = 5.00$, $P < 0.001$). The response was similar at all tested locations, except for location 4 where heterospecific pollen deposition was higher in control plots compared to *Brassica* plots (Fig. 4).

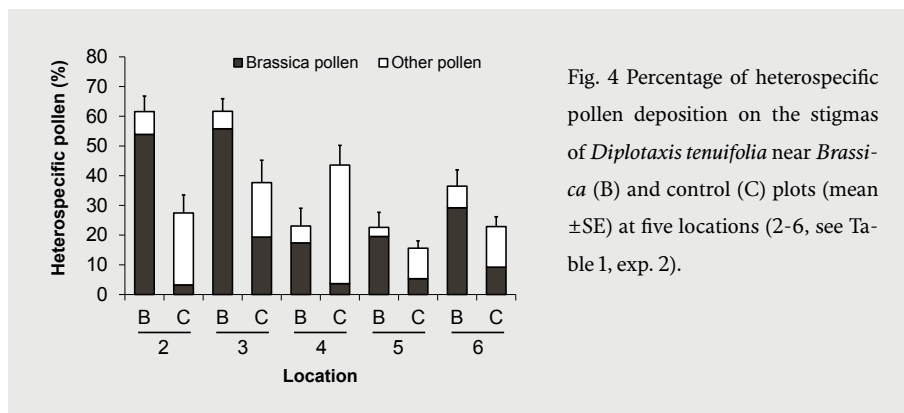


Fig. 4 Percentage of heterospecific pollen deposition on the stigmas of *Diplotaxis tenuifolia* near *Brassica* (B) and control (C) plots (mean \pm SE) at five locations (2-6, see Table 1, exp. 2).

Leaf and fruit damage

Overall, insect damage to the fruits of *Diplotaxis tenuifolia* was significantly higher in control plots compared to *Brassica* plots ($Z = 3.02$, $P < 0.01$; Fig. 5a), and this effect was present in all six locations. In contrast, the incidence of leaf damage did not show any overall difference between the two plot types ($Z = 1.09$, $P = 0.27$; Fig. 5b), and varied considerably among the replicated locations.

Discussion

Our results demonstrate that, in line with our hypothesis, the presence of an 'invasive' model species can disrupt the native plant-pollinator network. The visitation frequency to one native focal species increased in invaded compared to uninvaded plots, while it decreased for a different native focal species. Despite these changes in visitation frequency, the seed set was not affected for any of the native focal species. Furthermore, we did not find evidence for apparent competition between the native species and the invader. Instead, fruits of the native species were less often preyed upon in the invaded- compared to the uninvaded plots.

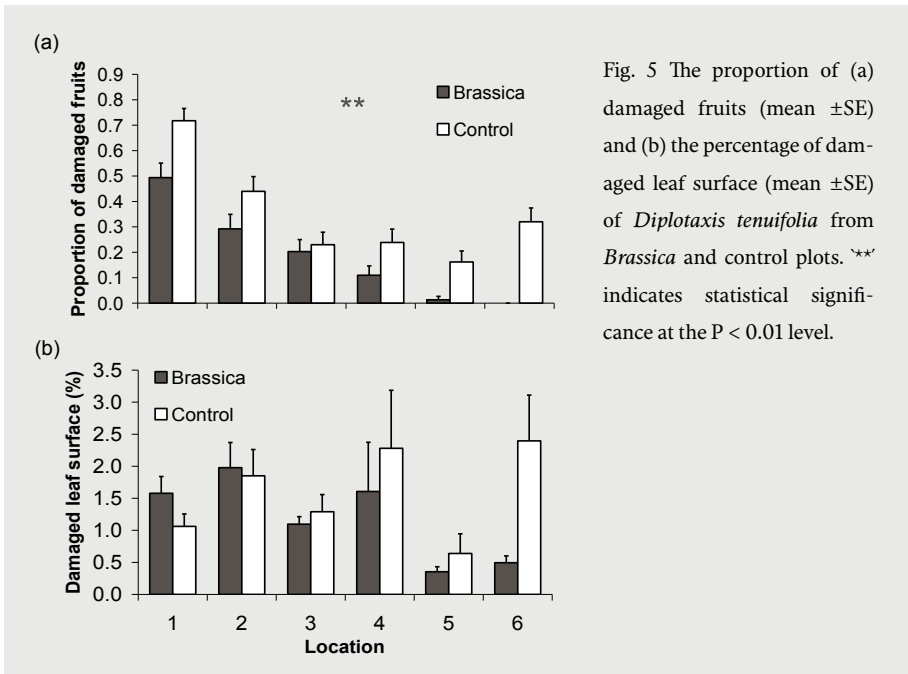


Fig. 5 The proportion of (a) damaged fruits (mean \pm SE) and (b) the percentage of damaged leaf surface (mean \pm SE) of *Diplotaxis tenuifolia* from *Brassica* and control plots. '**' indicates statistical significance at the $P < 0.01$ level.

Plant-pollinator interactions

The native focal species *Leucanthemum vulgare* received more flower visitors in the presence of the invader compared to the control. Similar facilitation effects by invasive species have been observed in other studies (e.g. Johnson et al. 2003, Moeller 2004, Ghazoul 2006), but reports of negative interactions are more common (e.g. Chittka and Schurkens 2001, Brown et al. 2002, Flanagan et al. 2010). In our study, the facilitation effect observed for *Leucanthemum vulgare* may be explained by the 'magnet effect' of the mass flowering *Brassica rapa* populations, drawing additional pollinators to invaded sites (Johnson et al. 2003, Molina-Montenegro et al. 2008). Also, the pollinator community of *Leucanthemum vulgare* overlapped considerably with that of *Brassica rapa* (49% overlap), enabling the species to profit from the increase in pollinator densities. Like *Leucanthemum vulgare*, the native focal species *Daucus carota* shared a substantial part (40%) of its pollinator community with *Brassica rapa*, but the overall visitation frequency was not affected by the presence of the invader. A possible explanation is that pollinators use flower color as a signal for locating flowers (Briscoe and Chittka 2001). Since *Brassica rapa* has yellow flowers, the yellow disc flowers of *Leucanthemum vulgare* may be visited more often than the white *Daucus carota* flowers.

The two remaining native focal species in this experiment, *Trifolium pratense* and *Linaria vulgaris*, both shared less than 15% of their flower visiting community

with *Brassica rapa*. The specialized flower morphology of both *Trifolium pratense* and *Linaria vulgaris* is likely to be responsible for this limited overlap. Their tubular flowers allow access only to those that have a sufficiently long proboscis for harvesting the nectar, which may explain the lack of interaction with *Brassica rapa*.

For *Diplotaxis tenuifolia* an effect on visitation frequency was expected, given the similarities between the native and invader species in flower color and morphology as well as their taxonomic relatedness (Morales and Traveset 2009). Flowers of this species were visited significantly less in the presence of the invader. This negative interaction effect between *Diplotaxis tenuifolia* and *Brassica rapa* may have two possible explanations. Some studies suggest that the higher attractiveness of the flowers of the invader, in terms of nectar reward and flower size, is responsible for the decrease in visitation frequency to co-flowering natives (Chittka and Schurkens 2001, Brown et al. 2002). Alternatively, high densities of flowers from the invasive species may dilute the total number of pollinators over all available flowers, reducing the visitation frequency to co-flowering natives (Holzschuh et al. 2011).

In none of the five native study species seed set declined significantly in the presence of the invasive species; contrasting our hypothesis. It is not uncommon that changes in visitation frequency do not translate to changes in seed set (e.g. Aigner 2004, Totland et al. 2006), yet there is no consensus about the underlying mechanisms that act in the process. Several, non-exclusive explanations have been suggested. A first explanation is of course a lack of statistical power to detect differences given the high variation in the data. However, the coefficient of variation in our data was generally lower than 40 % and the standard errors were generally low. A second explanation is that visitation frequency was not a limiting factor for seed set of the focal plants in our experiments. Some studies suggested that in some systems the availability of resources other than pollination may be limiting seed set (Goulson et al. 1998, Totland et al. 2006). The plants in our experiment were placed in pots containing a constant soil mixture, so we can rule out any differences in seed set caused by the availability of resources in the soil. A third explanation is that the effectiveness of the pollinators caused some of the observed differences (Herrera 1987). For instance, at the control plots of location 3 the low seed set (Fig. 3b) coincided with a high visitation frequency by beetles (Appendix S2). Some flower visitors, such as pollen-consuming beetles from the genus *Melighetes*, may have a net negative effect on pollination success of plants (Kirk and Gray 1992). Additional studies are needed that are specifically designed to examine the effectiveness and potential detrimental effects of flower visitors.

Lastly, a fourth aspect that may decrease reproductive success of plants is a high heterospecific pollen deposition reducing the pollination rate of ovules (Aizen and Harder 2007, Morales and Traveset 2009). The overall proportion of heterospecific pollen on stigmas of *Diplotaxis tenuifolia* was similar between *Brassica* plots and control plots. However, the composition of the heterospecific pollen differed considerably between the two plot types as stigmas from *Brassica* plots contained a significantly higher proportion of *Brassica rapa* pollen. Pollen from different plant species may cause different effects on the reproductive success of the receiving plant. For instance, pollen from some species have allelopathic properties and will therefore have a higher impact on the seed set of the recipient plant than pollen from other (non-allelopathic) species (Arceo-Gomez and Ashman 2011).

Plant-herbivore interactions

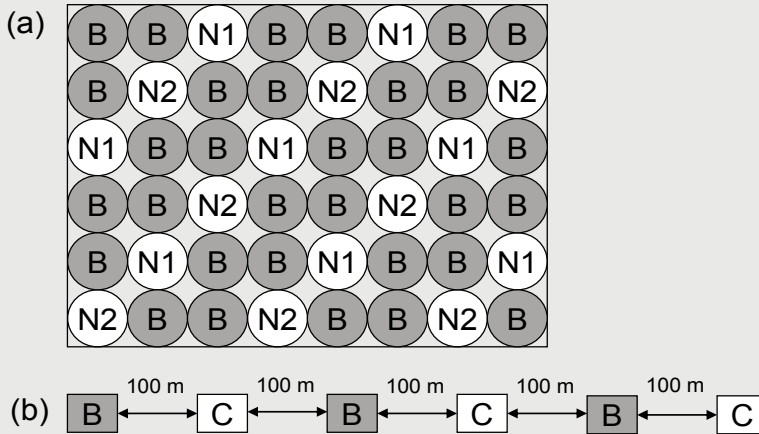
We found a lower incidence of fruit damage for *Diplotaxis tenuifolia* in the presence of the invader, whereas the incidence of leaf herbivory was not affected. This finding contradicts previous studies and our initial hypothesis that apparent competition (Meiners 2007, Dangremond et al. 2010) and spillover of herbivores (Rand and Louda 2004) from invasive populations to neighboring habitat increases the incidence of herbivory on co-occurring native plants. Many direct and indirect interactions may affect the herbivore pressure of neighboring interacting species (Barbosa et al 2009). For instance, *Brassica rapa* may have visually camouflaged *Diplotaxis tenuifolia* by which it could have disrupted host finding by herbivores (cf. Finch et al. 2003). Furthermore, production of volatiles by *Brassica rapa* may have triggered defense responses in *Diplotaxis tenuifolia*, or may have reduced its detection by herbivores (cf. Barbosa et al 2009). Another possibility is that herbivores preferred *Brassica rapa* over *Diplotaxis tenuifolia* as a host plant, causing *Brassica rapa* to act as a sink for herbivores (cf. Tillman 2006). Some studies have observed that preference of herbivorous insects indeed changed in response to the availability of different host plant species (Kuussaari et al. 2000, Gotthard et al. 2004). Alternatively, herbivores feeding on large *Brassica rapa* populations may be saturated by the large amount of available resources. As a consequence, the per capita herbivore pressure on co-occurring plants would decrease. Such a saturating functional response was demonstrated by Rhainds and English-Loeb (2003), who observed a decreasing proportion of damaged fruits with increasing availability of fruits per patch. Note that population dynamics of the herbivore community was not taken into account in these relatively short-term experiments.

Implications for ERA systems

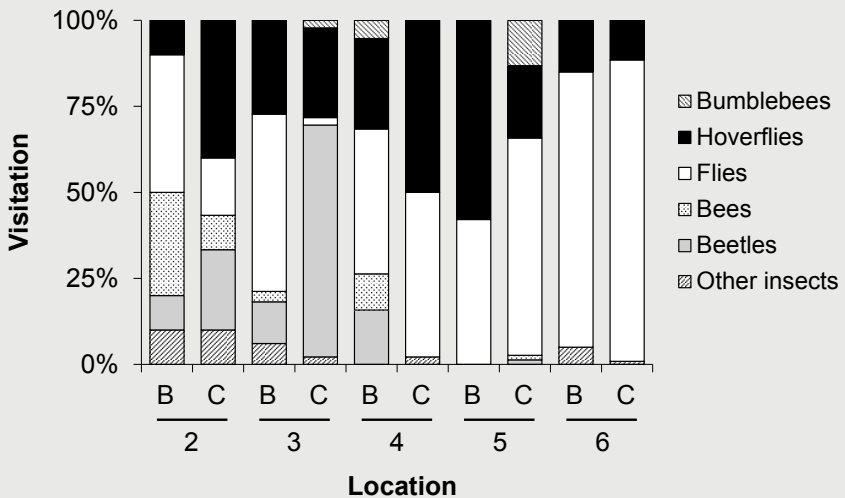
This study demonstrates that invasive plants can have a significant ecological impact on native recipient ecosystems, and that competitive as well as facilitative effects may be expected. Our findings stress the need to incorporate ecological relationships, such as plant-pollinator and plant-herbivore interactions, into ERA systems. Combining ecological indirect effects with the already implemented aspects related to the invasive potential will yield a more comprehensive ERA system than those currently available. We also identified some important challenges to be overcome, of which temporal and spatial variation are the most important ones. We found that the magnitude and direction of effects on visitation frequency, but especially on seed set and leaf herbivory, strongly depends on the location and sampling time. In addition, different densities of the invasive species will likely affect the properties of plant-insect interactions. Another important finding is that plant-insect interactions between native and invasive plants are only to be expected in cases with (at least partial) overlap of pollinator and herbivore communities. Whether effects will be facilitative or detrimental to the native species will depend on factors like the host preference of the shared pollinator or herbivore.

Besides the effect of plant invasions on plant-pollinator and plant-herbivore interactions, changes in invertebrate communities (e.g. Sax 2002, Kappes et al. 2007, Litt and Steidl 2010) and disruption of plant-pathogen interactions (e.g. Malmstrom et al. 2005) are other indirect effects currently not represented in ERA systems. Since they are crucial for ecosystem functioning, including them into ERA systems would further improve their quality and effectiveness. ●

Supplementary material



Appendix S1. Schematic view of the experimental setup of experiment 1: (a) arrangement of the plants within each plot; B: *Brassica* (with or without flowers), N1 and N2: native focal species 1 and 2; (b) spatial arrangement of the plots within each location.



Appendix S2. Pollinator assemblage for flowers of *Brassica* and *Diplotaxis tenuifolia* near *Brassica* (B) plots and control (C) plots (exp. 2).

Contrasting effects of experimental plant invasions on invertebrate communities

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Abstract

Plant invasions often affect local invertebrate communities, which may have cascading effects on ecosystem functions. In order to prevent plant invasions and subsequent ecological impact, risk assessment systems are being implemented to screen (transgenic) crops or ornamental species prior to their introduction in the environment. For the development of these systems, a better understanding of the potential impact of plant invaders is needed. In this study we investigate how plant invasions affect invertebrate communities and how this may affect herbivory on co-occurring native plants. We created experimental 'invasions' of two model species (*Lactuca serriola* and *Brassica napus*) and monitored the invertebrate community within and in the direct surroundings of invasions. At the same time we recorded the incidence of herbivory on two native model species, *Diplotaxis tenuifolia* and *Tragopogon pratensis*, which were placed near the invasion plots.

Within invasion plots the abundance and taxon richness of invertebrates was generally lower than in control plots, while we observed the opposite in the direct surroundings of the invasion plots. We found no effect on the incidence of herbivory of the two selected native species, despite shifts in the invertebrate community. Finally, we show that spatial and temporal variation is considerable in this type of experimental field studies. Based on our findings we propose a number of recommendations that may contribute to the continuing development of risk assessment systems.

Introduction

Plant invasions can have large effects on floral and faunal biodiversity (Davis 2009, Vilà et al. 2011) and ecosystem functioning (Levine et al. 2003, Vitousek 1990). The impact on invertebrate communities is of particular importance, because invertebrates play a key role in ecosystem structure and functioning (Chapman 1998, Matson et al. 2011). Previous studies demonstrated that plant invasions alter invertebrate abundance, diversity and composition (Gerber et al. 2008, Heleno et al. 2009, Kappes et al. 2007, Lindsay and French 2006), as well as trophic interactions (Pearson 2009, Topp et al. 2008). In turn, these changes may indirectly affect different ecosystem functions (White et al. 2006), including interactions of native plants with herbivores (Orrock et al. 2008, Sessions and Kelly 2002) and pollinators (Bjerknes et al. 2007, Morales and Traveset 2009).

Since plant invaders change the local plant community composition (Davis 2009), and plant- and invertebrate communities are strongly interrelated (Haddad et al. 2001, Haddad et al. 2009, Koricheva et al. 2000, Schaffers et al. 2008, Siemann et al. 1998), they are likely to affect local invertebrate communities. Plant invasions

generally have a negative effect on plant species richness (Gordon 1998, Powell et al. 2011), and reduce the associated invertebrate community richness (Haddad et al. 2001, Haddad et al. 2009, Siemann et al. 1998). However, under some conditions, plant invasions locally increase plant species richness and thereby also invertebrate richness and abundance, for example at the edge of the invasive range (Sax and Gaines 2003; Ries and Sisk 2004).

A frequently observed characteristic of plant invaders is that they locally form dense patches and become dominant members of the community (Powell et al. 2011, Sakai et al. 2001). Under such conditions, the 'resource concentration hypothesis' predicts that specialist herbivores become more abundant, because they are more likely to find those dense patches and remain there (Root 1973). Furthermore, the 'enemies hypothesis' predicts a positive correlation between plant species richness (or habitat complexity) and the abundance of predatory insects and parasitoids, presumably because of greater availability of habitats and alternative resources (Russell 1989; Root 1973). This may subsequently increase predation and parasitoid pressure on herbivores.

Another potential consequence of invaded patches is spillover of invertebrates to adjacent habitat, induced by differences in productivity and passive diffusion of invertebrates across habitat edges (Blitzer et al. 2012). This phenomenon has been studied extensively in agricultural settings, in which spillover from crop to non-crop areas has been frequently demonstrated for herbivores (Squires et al. 2009), predators (Rand and Louda 2006) and parasitoids (Gladbach et al. 2011). Spillover can also occur following plant invasions (Didham et al. 2007), but this process has been less well studied. Rand and Louda (2004) present one of the rare examples where an invasive thistle increases the occurrence of a weevil on a native co-occurring species.

Alterations of invertebrate communities can trigger a range of indirect effects (White et al. 2006). For example, spillover of herbivores from agricultural fields to neighboring habitat has led to increased herbivore damage to native species (Mckone et al. 2001, Squires et al. 2009). This indirect interaction effect, called apparent competition (Holt 1977), occurs when prey species indirectly depress each other by increasing the abundance of a shared natural enemy by providing it with additional resources or refuge. Apparent competition has also been observed in response to plant invasions. For instance, Meiners (2007) found significant negative effects on tree regeneration due to increased seed predation in the presence of two exotic shrubs. Similarly, a native *Lotus* species experienced increased herbivory by a weevil, mediated by the presence of an exotic *Medicago* species (Lau and Strauss 2005).

Considerable effort has been put into identifying effects of plant invasions on invertebrate communities (Sax 2002; Magoba and Samways 2010; Heleno et al. 2009). However, most of these studies are observational, i.e. an invaded habitat is compared to an uninvaded habitat (but see Pawson et al. 2010, Simao et al. 2010). A drawback of this approach is that it is difficult to distinguish between ecological effects due to the presence of the invader and local confounding factors, such as soil type, nutrient- and water availability (Belnap et al. 2005, Palmer et al. 2004, Wilkie et al. 2007).

The problem of confounding factors can be resolved by using an experimental approach where invaded and uninvaded plots are situated within the same field (Simao et al. 2010). An additional advantage of an experimental approach is that it allows one to assess potential ecological effects before the species has become invasive. This is important for risk assessment systems, which are designed to prevent invasions and to mitigate potential ecological impact of crops or ornamental plants that are intended for introduction. An example of a widely implemented system is the Australian weed risk assessment system (Pheloung et al. 1999).

In this study we experimentally created controlled invasions of two invasive model species: prickly lettuce (*Lactuca serriola* L., Asteraceae) and oilseed rape (*Brassica napus* L., Brassicaceae). *L. serriola* is a native invader in the Netherlands (Hooftman et al. 2006), whereas *B. napus* is a widely cultivated crop used for the production of food, animal fodder and biofuel. Feral populations of the latter species are found in various countries all over the world, including the Netherlands (Luijten and de Jong 2010).

We test the following hypotheses on how plant invasions affect invertebrate communities and ecosystem function. We hypothesize that, (i) given the positive relationship between plant- and invertebrate diversity, the invertebrate richness within invasion plots is expected to be lower compared to the adjacent habitat. Furthermore, (ii) the abundance of invertebrates that are associated with the invader will increase in adjacent vegetation due to spillover. (iii) The incidence of herbivory to co-occurring native plants will increase as a result of apparent competition and spillover effects, especially for plants that are taxonomically related to the invader. Finally, we discuss the applicability of our results for risk assessment systems.

Material and Methods

Study species

As invasive model species we used *B. napus* (Brassicaceae) and *L. serriola* (Asteraceae). *B. napus* is an annual or biennial crop, which flowers from April to August. This allotetraploid species is a hybrid between *B. rapa* and *B. oleracea* and is cultivated worldwide for the production of animal feed, vegetable oil and biodiesel. *L. serriola* is an annual or biennial species that flowers from July to September. It is closely related to - and completely interfertile with - cultivated lettuce (*L. sativa*). We used *Diplotaxis tenuifolia* (L.) DC. (Brassicaceae) and *Tragopogon pratensis* L. (Asteraceae) as native target species. *D. tenuifolia*, or perennial wall-rocket, flowers from June to autumn. *T. pratensis*, or meadow salsify, is a biennial plant that typically flowers from May to July.

Study area and plots

The experiments were performed from April to September of the years 2010 and 2011. The experimental field was established in a semi-natural grassland area in the harbor area of Amsterdam (52°24'37.4"N, 4°44'11.6"O). Due to the sandy soil, typical dune grassland vegetation has developed at this site. Common species were *Calamagrostis epigejos*, *Carex arenaria*, *Plantago lanceolata*, *Trifolium arvense*, *Achillea millefolium*, *Sedum acre* and *Daucus carota*. The field was mown once a year in September.

Within this field we selected an area of 100 x 100 m in which we created 36 circular plots with a diameter of 2 meters, in a rectangular grid of 6 x 6 plots separated by 20m. Each plot was randomly assigned to *B. napus* (from now on referred to as 'Brassica plots'), *L. serriola* (*Lactuca* plots) and control plots, resulting in 12 plots of each plot type. The original vegetation in *Brassica* and *Lactuca* plots was removed and in order to increase the water holding capacity of the soil, 250 l of unfertilized peat was mixed with the topsoil (\pm 10 cm). In October 2009 and 2010, *Brassica* and *Lactuca* plots were sown at a density of around 100 seeds per m², resulting in a canopy cover of 90-100%. *Brassica* plots were sown with the Maximus® (PR45D03; Pioneer) variety of *B. napus*, while *Lactuca* plots were sown with an inbred line (8th generation of selfing) derived from a single individual from wild *L. serriola* population collected near the village of Eys in the Netherlands. Control plots were left undisturbed and contained local vegetation as described above.

Around each of the 36 plots we dug in 16 pots (ϕ 16 cm, 2 l) at equal distance from each other and at a distance of 80 cm from the edge of the plot. The pots alternately contained adult individuals of two native target species, *D. tenuifolia* and *T. pratensis*.

All plots (including the control) and pots were watered when necessary and slow release fertilizer (Osmocote Exact Standard, NPK: 16:9:12, Code 8840) was added three times a year (30g.m⁻²). *Brassica* and *Lactuca* plots were manually weeded twice a year, during which all species other than *B. napus* and *L. serriola* were removed.

Table 1 Total abundance of sampled invertebrates over all sampling dates and plots.

Order	Abundance
Acarina	11.120
Araneae	9.283
Coleoptera	6.978
Diptera	76.333
Embiodera	1
Ephemeroptera	2
Hemiptera	10.631
Hymenoptera	19.316
Isopoda	214
Lepidoptera	693
Neuroptera	26
Opiliones	422
Opisthoptera	77
Orthoptera	2.201
Siphonaptera	7
Thysanoptera	7.990
Chilopoda (class)	26
Diplopoda (class)	257
Gastropoda (class)	11.530
Total	157.107

Invertebrate sampling and determination

We used three different sampling methods to characterize the invertebrate community in and around plots. The ground dwelling invertebrate community was sampled with pitfall traps filled with a 3 cm layer of 3.7% formaldehyde solution and a drop of detergent to reduce surface tension. Two pitfalls (ø 10 cm, 0.5 l) per plot were positioned opposite to each other at a distance of 30 cm from the edge of the plot. To prevent rain water, mice and frogs or toads from entering the beakers, a hardboard roof was placed over it. To take a sample, pitfalls were opened for a period of 7 days, after which the contents was collected and stored at -20°C. In 2010, samples were taken in April, May, June and August in all three plot types. Presumably due to extremely dry weather conditions in the spring of 2011 the growing season of *B. napus* and *L. serriola* did not coincide, as had been the case in 2010. Therefore we took three pitfall samples in *Brassica* and control plots in April, May and June, whereas *Lactuca* and control plots were sampled in July, August and September.

To sample the airborne invertebrate community we used two yellow two-sided sticky traps (10 x 25 cm) per plot, attached to a bamboo stick and placed next to the pitfalls at a height of 45 cm above the soil surface. Three samples, lasting 7 days each, were taken in 2010 in May, June and August. In 2011, we took two samples in *Brassica* and control plots in May and June, and three samples in *Lactuca* and control plots in July, August and September.

Invertebrates that were present on the specific target plant surrounding the plots (*D. tenuifolia* and *T. pratensis*) were sampled using a suction sampler (STIHL SH55). In order to capture the invertebrates, a fine cloth (mesh size ± 0.1 mm) was placed in front of the air inlet. We took two bulk samples of four plants per plot for each of the two target species. To characterize the invertebrate community within the plots, we suction-sampled two random patches of 800 cm². Samples were transferred to a coffee filter drenched in 96% ethanol and stored at -20°C. In 2010 we sampled all plot types in June and July. In 2011 *Brassica* and control plots were sampled in May, while *Lactuca* and control plots were sampled in August. We always did paired observations between invasion and control plots and the sampling effort per plot type within one year was always kept the same. This allowed us to make direct comparisons between plot types within a sampling year.

Invertebrates from the families Carabidae, Curculionidae, Apionidae and Chrysomelidae from the order Coleoptera were identified to species level, while all other Coleopterans were identified to family level. Invertebrates from the orders Hemiptera, Heteroptera, Orthoptera, Opisthoptera and Neuroptera were identified to family level. Hymenoptera were divided into parasitoid and non-parasitoid individuals, of which the latter group was sorted further to family level. Individuals from the order Diptera were divided in the suborders Nematocera and Brachycera. We also separately identified individuals from the Dipteran families Tephritidae and Cecidomyiidae, because they are potentially important herbivores. All invertebrates were sorted into trophic groups according to literature (Harde and Severa 2006, Koch and Freude 1995, Turin and Museum 2000) and expert knowledge. We distinguished between predators, herbivores, detritivores, parasitoids, fungivores and pollinators. Taxa that contained subtaxa belonging to different trophic groups were labeled as 'miscellaneous'. Formicidae and Collembola were not counted, because their high abundance and patchy distribution obscured differences in the abundance of other invertebrate groups.

In total, we sampled 157.107 invertebrates belonging to 188 operational taxonomic units (OTU = the lowest level taxonomic unit employed in a given numerical taxonomic study) in 2010 and 2011 (Table 1). Average abundances of each individual OTU that was sampled around *Brassica*, control and *Lactuca* plots can be found in the supplementary material (Appendix S1).

Determination of seed set, leaf and silique damage

Herbivore damage to leaves of target species *D. tenuifolia* and *T. pratensis* was assessed by randomly selecting five plants of each target species per plot. From each plant we selected the first 6 leaves > 3 cm in length, counting from the youngest to the older part of the stem. We then checked these leaves for presence or absence (1/0) of damage resulting from chewing, sap-sucking and leaf mining. In 2010 we sampled the target plants from all 36 plots in June and July. In 2011 we sampled the target plants of *Lactuca* and control plots in June and in August we sampled the ones from *Brassica* and control plots.

To assess damage to the siliques of *D. tenuifolia*, we collected five siliques per plant and five plants per plot. We then counted the number of siliques that were damaged by invertebrate herbivores. In 2010 we collected the siliques from *D. tenuifolia* at all 36 plots in June and August. In 2011 the samples were collected in May at *Lactuca* and control plots, while the samples from *Lactuca* and control plots were collected in September.

Data analysis

The data from the pitfalls, sticky traps and suction samples were merged per year. Although the number and timing of the samples was not the same for the 2010 and 2011, we can still compare the relative changes from year to year. All statistical analyses were performed in R (R Development Core Team 2011).

One-way ANOVAs were used to examine the abundance of different invertebrates groups, the number of OTUs and the Shannon diversity around and within the plots, as well as the herbivore abundance on the target plants and the number of damaged siliques on the target plant *D. tenuifolia*. To test for normality of the response variables we conducted Shapiro-Wilk tests, checked whether model residuals were normally distributed and checked for linearity in Q-Q probability plots. Non-normal data were log(10) transformed or, in the case of ratios, arcsin transformed. Plot type (*Brassica*, control and *Lactuca*) was included as fixed factor. We used planned comparisons between *Brassica*-control and *Lactuca*-control plots.

The Shannon index was defined as $H' = -\sum p_i \ln(p_i)$, where p_i is the proportional abundance of species i . For the analysis of the number of *Brassica* specialists within and around the plots the assumptions for ANOVA were not met, and we therefore performed a Kruskal-Wallis rank sum test. Planned comparisons between *Brassica* and control and *Lactuca* and control plots were performed with an ANOVA on the ranked data. The Kruskal-Wallis test is equivalent to performing an ANOVA on data that have been converted to ranks (Conover 1998).

A split-plot ANOVA design was used to examine the invertebrate abundance, the number of OTUs, the herbivore abundance and the abundance of *Brassica* specialists on the two target plant species that surrounded the plots. An interaction term between plot type and target plant was included.

To examine the leaf damage of the two target plant species we performed a General Linear Mixed model. We included plot type and target plant species as fixed factor and included an interaction term between target plant species and plot type. We included plot ($n = 36$) as a random variable to create the appropriate error structure. This analysis was performed using the *lme* function from the R-package *nlme* (Pinheiro et al. 2009).

Nonmetric multidimensional scaling (NMDS), using the *metaMDS* function from the R-package *vegan* (Oksanen et al. 2007), was performed to examine the effect of plot type on the invertebrate community surrounding the plots. NMDS is regarded as a robust unconstrained ordination method in community ecology (Minchin 1987). The NMDS analysis was applied using the default options of *metaMDS* (Bray-Curtis distance measure, two-dimensional solution, maximum of 50 random starts to find the best global solution). We only included OTUs that occurred in >5% of the samples, following recommendations by McCune and Grace (2002).

We applied an analysis of similarity (ANOSIM) on the Bray-Curtis dissimilarity matrix produced by *metaMDS* to identify significant differences in invertebrate communities around the three plot types. For this analysis we used the *anosim* function from the R-package *vegan* (Oksanen et al. 2007) using the program's default values (999 permutations). The program returns an r -value between 1 (two completely separated groups) and 0 (completely random grouping). To test for spatial autocorrelation, we produced Mantel correlograms based on the same distance matrix that was used for the NMDS (Oden and Sokal 1986). Mantel's r statistics were calculated with GenoDive (Meirmans and van Tienderen, 2004). Every distance class was tested for significance using a randomization approach with 999 permutations. Sequential Bonferroni correction was applied to correct significance levels for multiple testing (Hewitt et al. 1997).

Sample-based rarefaction curves were created with *specaccum* from the R-package *vegan* (Oksanen et al. 2007) using the 'exact' method in combination with the programs default values. Sample-based rarefaction curves for 2010 and 2011 reached saturation, which indicates that increasing the number of plot replicates would not have altered the invertebrate diversity patterns observed (appendix S2).

Results

Invertebrate community composition within invasion plots

Given the positive relationship between plant- and invertebrate diversity, we hypothesized that invertebrate richness within invasion plots would be reduced compared to the adjacent habitat. Indeed, in 2010 the total invertebrate abundance within *Brassica* and *Lactuca* plots was much lower than the abundance in control plots, whereas in 2011 we found the same pattern for *Brassica* but not for *Lactuca* (Fig. 1a). Furthermore, in both years invertebrate richness within control plots was higher compared to *Lactuca* plots, but not compared to *Brassica* plots (Fig. 1b). In 2011, *Lactuca* plots had a lower Shannon diversity compared to the control habitat (Fig. 1c). In 2010 Shannon diversity was higher in *Brassica* plots than in control plots. In both years, *Brassica* specialists in *Brassica* plots were considerably more abundant than in control or *Lactuca* plots (Fig. 1d).

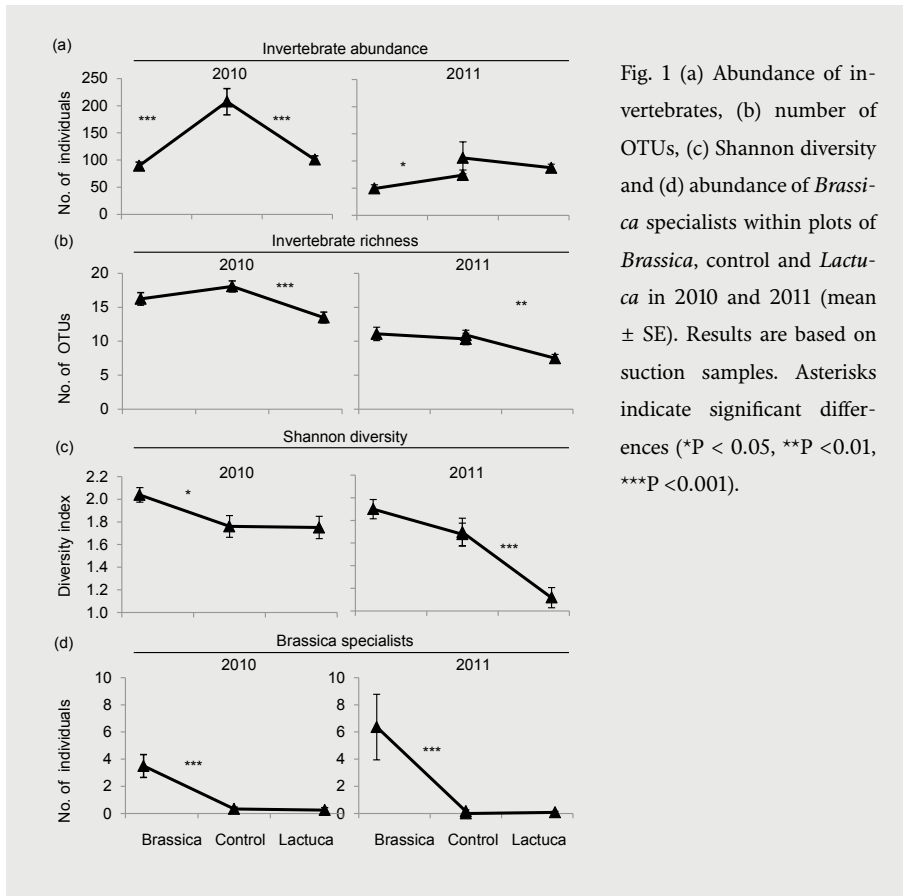


Fig. 1 (a) Abundance of invertebrates, (b) number of OTUs, (c) Shannon diversity and (d) abundance of *Brassica* specialists within plots of *Brassica*, control and *Lactuca* in 2010 and 2011 (mean \pm SE). Results are based on suction samples. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Spillover: invertebrate communities surrounding invasion plots

We hypothesized that the abundance of invertebrates that are associated with the invader will increase in adjacent vegetation due to spillover. In 2011, invertebrate abundance was 21% higher around *Brassica* plots and 25% higher around *Lactuca* plots compared to the surroundings of control plots, while there were no differences between the plot types in 2010 (Fig. 2a). Furthermore, we observed more than

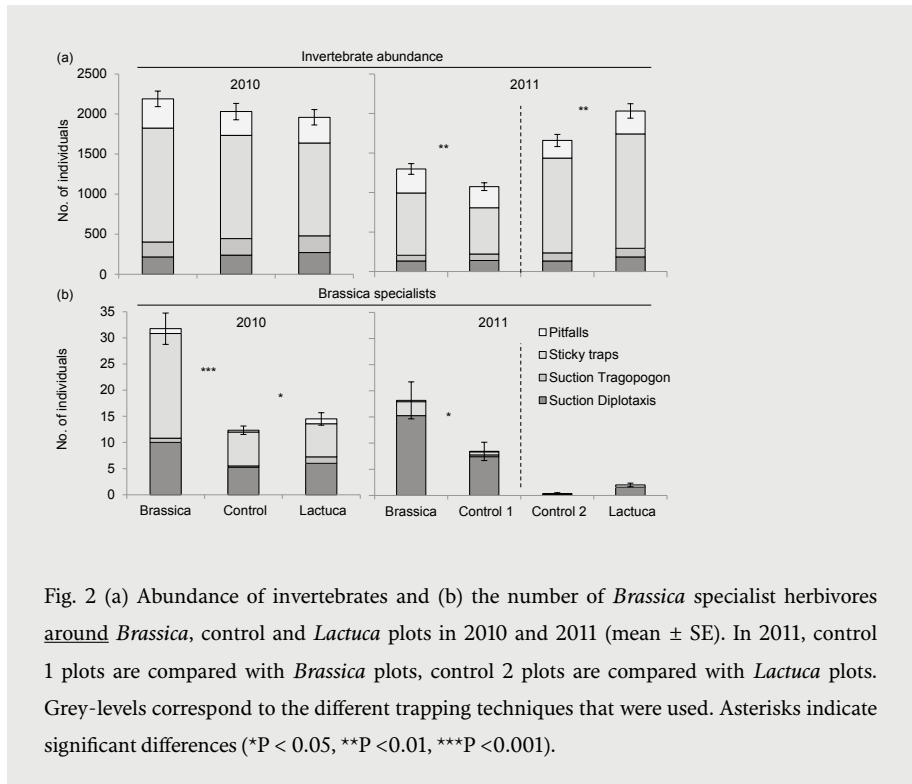


Fig. 2 (a) Abundance of invertebrates and (b) the number of *Brassica* specialist herbivores around *Brassica*, control and *Lactuca* plots in 2010 and 2011 (mean \pm SE). In 2011, control 1 plots are compared with *Brassica* plots, control 2 plots are compared with *Lactuca* plots. Grey-levels correspond to the different trapping techniques that were used. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

twice as many *Brassica* specialist herbivores in the surroundings of *Brassica* plots compared to control and *Lactuca* plots, both in 2010 and 2011 (Fig. 2b). Invertebrate richness in the plot surroundings was higher for *Brassica* plots than for control plots, but only in 2010. The invertebrate richness around *Lactuca* plots was not affected by invasion in neither of the two years (Fig. 3a). We observed a higher Shannon diversity for the invertebrate community around *Lactuca* plots in 2010 compared to the control, but not in 2011. The Shannon diversity around *Brassica* plots never differed from the control (Fig. 3b).

Nearly one third more herbivores occurred around *Lactuca* plots compared to the control plots in 2011, but no such effect was observed in 2010 (Fig. 4a). Para-

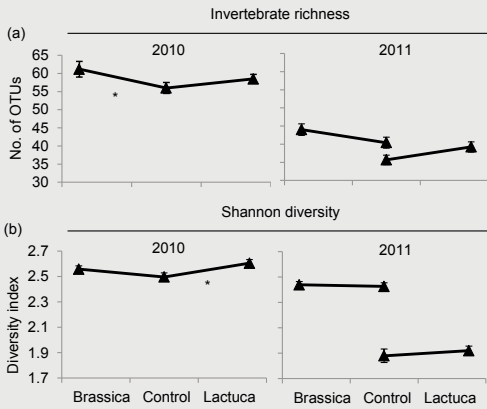


Fig. 3 (a) Invertebrate richness and (b) Shannon diversity around *Brassica*, control and *Lactuca* plots in 2010 and 2011 (mean \pm SE). Asterisks indicate significant differences (* $P < 0.05$).

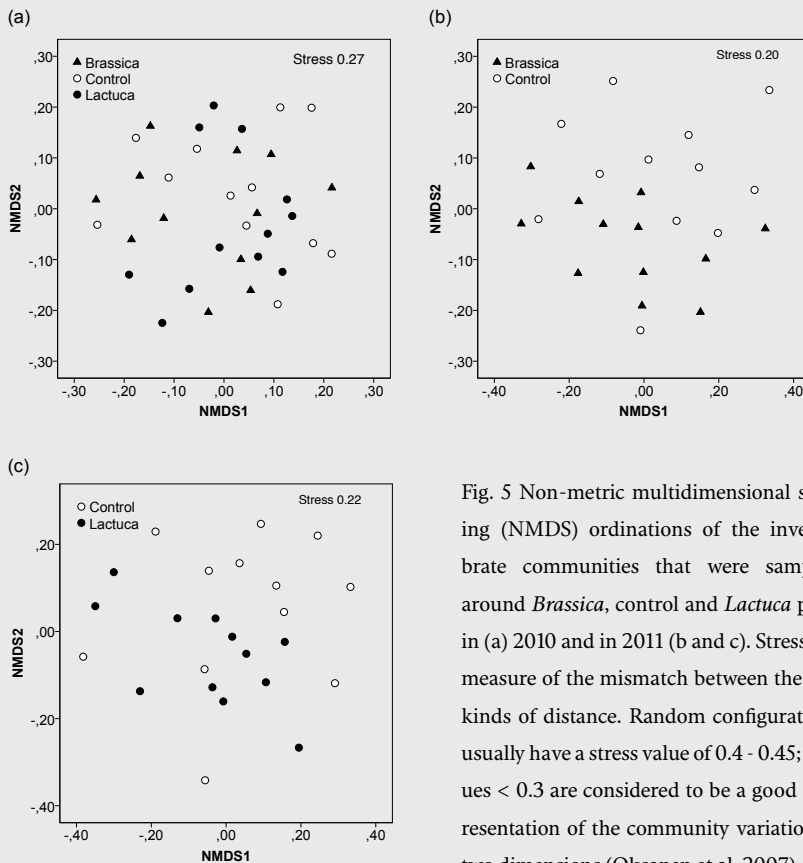


Fig. 5 Non-metric multidimensional scaling (NMDS) ordinations of the invertebrate communities that were sampled around *Brassica*, control and *Lactuca* plots in (a) 2010 and in 2011 (b and c). Stress is a measure of the mismatch between the two kinds of distance. Random configurations usually have a stress value of 0.4 - 0.45; values < 0.3 are considered to be a good representation of the community variation in two dimensions (Oksanen et al. 2007).

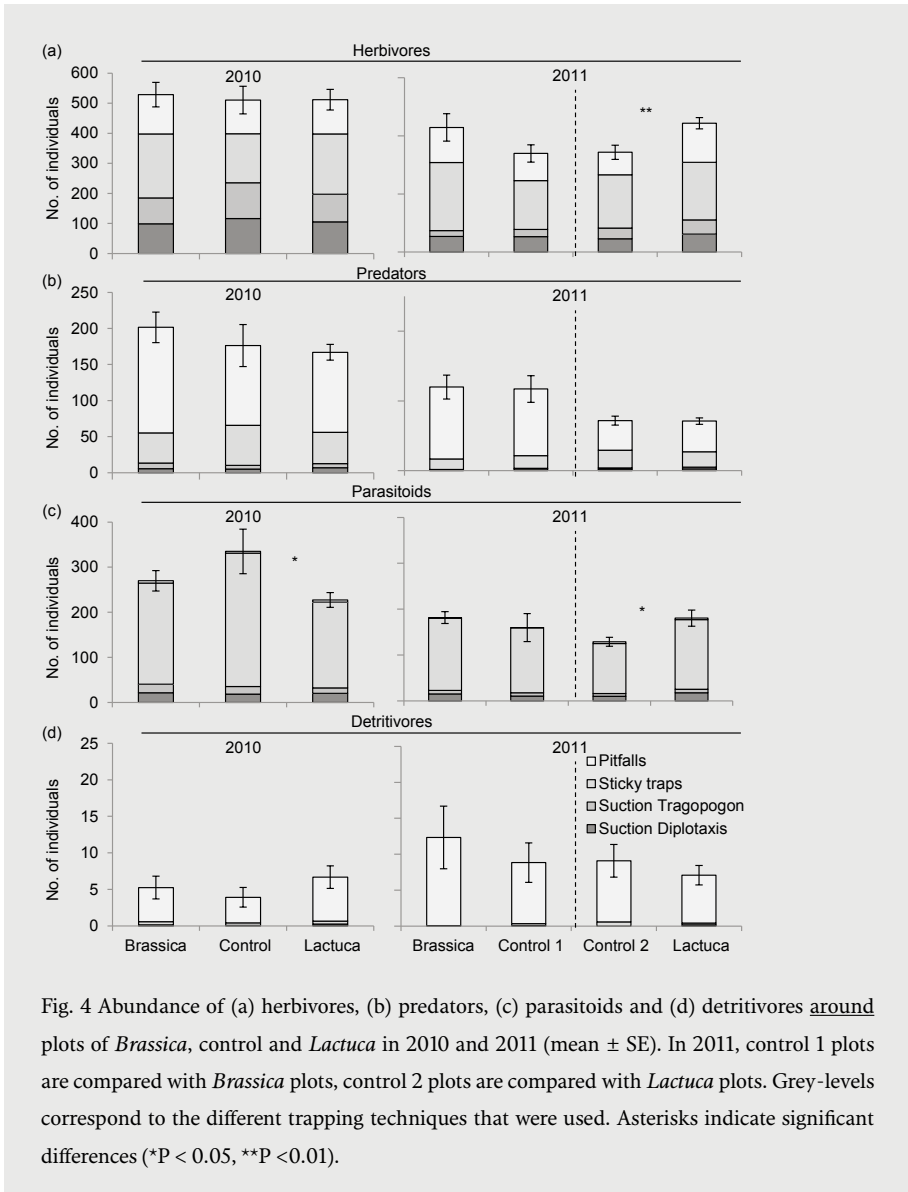
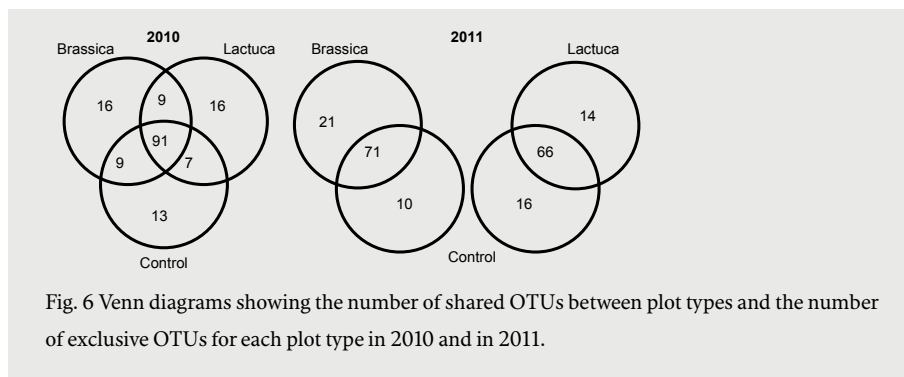


Fig. 4 Abundance of (a) herbivores, (b) predators, (c) parasitoids and (d) detritivores around plots of *Brassica*, control and *Lactuca* in 2010 and 2011 (mean \pm SE). In 2011, control 1 plots are compared with *Brassica* plots, control 2 plots are compared with *Lactuca* plots. Grey-levels correspond to the different trapping techniques that were used. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$).

sitoid abundance in 2010 was one third higher around control plots than around *Lactuca* plots, whereas the opposite, higher parasitoid abundance around *Lactuca* plots, was observed in 2011 (Fig. 4c). Both in 2010 and 2011, the predator and detritivore abundance did not differ among the three plot types (Fig. 4b and d). Omnivores were more abundant around *Brassica* and *Lactuca* plots in both years, while fungivores and pollinators did not show any significant variation among plot types (Appendix S3).

The NMDS that was used to analyze differences in community composition did not yield a clear visual separation between the plot types in 2010, which was confirmed by the statistical analysis (ANOSIM, global $r = 0.032$, $P = 0.216$; Fig. 5a). In contrast, in 2011 the invertebrate community compositions around *Brassica* and control plots were statistically different (ANOSIM, global $r = 0.11$, $P = 0.047$; Fig 5b). The same was true for the difference between the community compositions around *Lactuca* and control plots in 2011 (ANOSIM, global $r = 0.12$, $P = 0.021$; Fig 5c). The Mantel correlograms, which were produced using the same distance matrix as for the NMDS, showed significant spatial autocorrelation in both sampling years, especially for distance classes 0 - 20 and 20 - 40 meters (Appendix S4).

We also checked for the number of OTUs that were exclusively found around one of the plot types (Fig. 6). In 2011, we sampled 21 exclusive OTUs around *Brassica* plots compared to 10 OTUs around control plots, while around *Lactuca* plots this number was similar to that of the control (14 and 16, respectively). In 2010 similar numbers of exclusive OTUs occurred around *Brassica*, control and *Lactuca* plots (16, 13 and 16, respectively).



Herbivory on native target plants

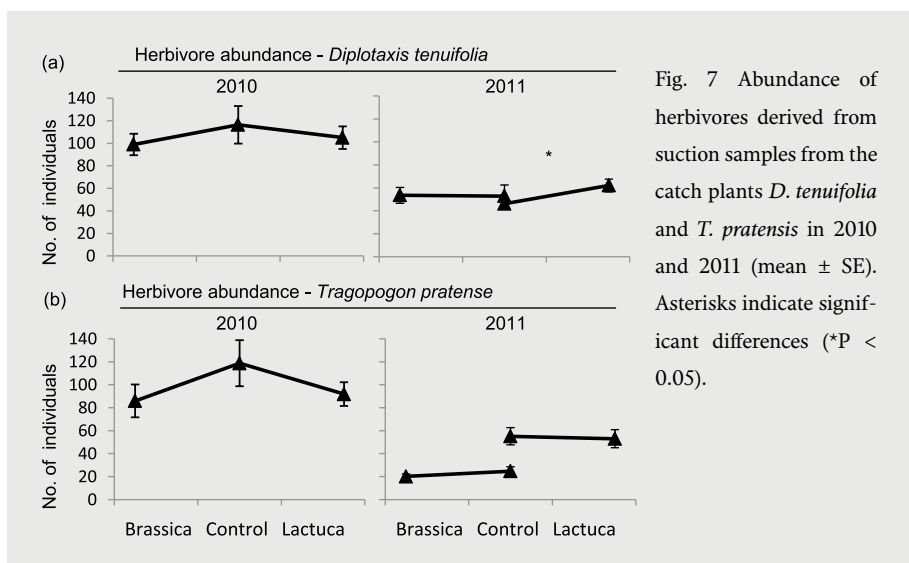
We predicted that the incidence of herbivory to co-occurring native plants would be higher as a result of apparent competition and spillover of herbivores. The two native target plants *D. tenuifolia* (Brassicaceae) and *T. pratensis* (Asteraceae) that were alternately placed around all three plot types always contained a significantly different invertebrate richness and abundance, but between the three plot types no differences in richness or abundance were observed (Table 2). Furthermore, there were no interaction effects between target plant species and plot type (Table 2).

In 2011 about a third more herbivores occurred on target plants of *D. tenuifolia* placed around *Lactuca* plots than on target plants around control plots, but this pattern was not seen in 2010 (Fig. 7; Appendix S5). In both years, similar abundances of herbivores were present on *T. pratensis* around all tree plot types.

Table 2 Results from the split plot ANOVA for the effect of plot type (*Brassica* (B), control (C), *Lactuca* (L)) and catch plant species on the invertebrate and herbivore abundance and number of OTUs on the catch plants (*T. pratensis* and *D. tenuifolia*). Asterisks indicate a significant difference (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$), NS = not significant.

Response	Year	Coefficients	df	F	P
Total abundance	2010 (B,C, L)	Plot type	2, 66	0.75	NS
		Catch plant	1, 66	4.50	*
		Plot type x catch plant	2, 66	0.09	NS
	2011 (B, C)	Plot type	1, 44	0.08	NS
		Catch plant	1, 44	17.63	***
		Plot type x catch plant	1, 44	0.15	NS
	2011 (C, L)	Plot type	1, 39	2.98	NS
		Catch plant	1, 39	17.86	***
		Plot type x catch plant	1, 39	2.26	NS
Invertebrate richness (OTUs)	2010 (B,C, L)	Plot type	2, 66	0.99	NS
		Catch plant	1, 66	12.69	***
		Plot type x catch plant	2, 66	0.39	NS
	2011 (B, C)	Plot type	1, 44	0.73	NS
		Catch plant	1, 44	36.56	***
		Plot type x catch plant	1, 44	1.19	NS
	2011 (C, L)	Plot type	1, 39	5.57	*
		Catch plant	1, 39	8.11	**
		Plot type x catch plant	1, 39	0.88	NS

The number of leaves damaged by herbivores was always significantly different between the target plant species in both years ($P < 0.001$; Appendix S6). However, none of the target plant species showed an effect of plot type. Accordingly, we found no interaction between plot type and target plant species (Appendix S6). Finally, the number of damaged siliques of *D. tenuifolia* showed no plot type effect in 2010 nor in 2011 ($F_{2,33} = 0.76^{NS}$, $F_{1,22} = 0.12^{NS}$, respectively).



Discussion

Experimental invasions of *B. napus* and *L. serriola* significantly altered the local invertebrate communities both within invasion plots as well as in their direct surroundings. Following our expectations, invertebrates within invasion plots were generally less abundant and had lower taxon richness compared to control plots. Similar findings have been repeatedly reported in studies that compared invaded and uninvaded habitats (Gerber et al. 2008, Greenwood et al. 2004, Spyreas et al. 2010, Topp et al. 2008, but see Harris et al. 2004), and may possibly reflect the positive relationship between plant and invertebrate species richness (Haddad et al. 2001, Haddad et al. 2009, Siemann 1998). In addition, the increased invertebrate abundance that was observed within control plots may reflect the fact that diverse plant communities are generally more productive than simple plant communities (Tilman et al. 2001), allowing for an increase in invertebrate abundance.

Spillover from invasion plots to the adjacent habitat was hypothesized to increase the abundance of invertebrates that are associated with the invader. Support for this hypothesis was particularly strong in the case of *Brassica* specialists, especially for the seedpod weevil *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) that was particularly abundant in and around *Brassica* plots. At the same time, this observation provides support for the resource concentration hypotheses that predicts that herbivores, especially specialists, are more abundant in patches with high host plant densities (Root 1973).

In the second sampling year, not only the number of *Brassica* specialists, but also the total number of invertebrates was clearly higher around invasion plots compared to control habitat. Besides spillover effects, such increase could be caused by a positive edge response between the two different habitat types (Ries et al. 2004). Positive edge responses have been demonstrated in several systems (Wirth et al. 2008; Albrecht et al. 2010; Tylianakis et al. 2004) where two bordering habitat types provide complementary resources (e.g. food or refuge) to invertebrates that occur near the habitat edges (Ries et al. 2004; Orrock et al. 2010). In addition, habitat edges may locally alter habitat heterogeneity and microclimatic conditions, which can positively affect invertebrate abundance and richness (Wirth et al. 2008; Dukes and Mooney 2004).

In the second sampling year we found increased herbivore abundance around *Lactuca* plots, which coincided with an increase in parasitoid abundance, but it is unclear whether this represents a causal relationship. Alternatively, the increased parasitoid abundance could be explained by the enemies hypothesis (Root 1973), which predicts a higher predator and parasitoid abundance in more diverse vegetation; such was the case at the edges of invasion plots.

We rejected our hypothesis on the incidence of herbivory: there was no evidence for apparent competition as we observed no significant effect of plant invasions

on the incidence of herbivory of target plants *Diplo-taxis* and *Tragopogon*. At the same time, in the second sampling year, more herbivores were observed on *Diplo-taxis* target plants around *Lactuca* plots compared to target plants around control plots. Moreover, significantly more *Brassica* specialists occurred on target plants surrounding *Brassica* plots compared to the control (data not shown). Although our findings were unexpected, they are in accordance with the results from a meta-analysis focusing on direct and indirect interactions between plants in close proximity. This study concluded that plant taxonomic relatedness affected herbivore abundance, but not plant damage (Barbosa et al. 2008).

We propose two possible explanations for the absence of differences in herbivory activity between the three plot types. First, the additional herbivores that are attracted by the presence of the invader may not (be able to) consume the specific target plant species we selected for this experiment. Second, consumers of *Diplo-taxis* may eat other plant parts than the leaves monitored in this study. For example, adult individuals of the seedpod weevil *C. obstrictus* and the pollen beetle *Meligethes aeneus* (Coleoptera: Nitidulidae), the most commonly observed *Brassica* specialists on *Diplo-taxis*, are known to feed especially on seeds and pollen grains, respectively (Cook et al. 2004, Kirk and Gray 1992). Additional studies need to monitor not only leaf herbivory, but also other damage to flowers, fruits and stems.

Implications for risk assessment

In order to develop risk assessment systems for crop and ornamental species, extensive knowledge on their potential ecological impact on recipient ecosystems is required (Pheloung et al. 1999; Hulme et al. 2009). This study demonstrates that invertebrate communities surrounding invasion patches respond very differently from the communities inside such patches. As a result, depending on whether one samples within or on the edge of invasion patches, negative or positive effects on invertebrate richness and abundance are expected, respectively.

Several methodological issues have emerged that are relevant for the development of risk assessment systems. First, we found considerable differences in invertebrate communities between different sampling times during the growing season, but also between the two sampling years. In the first sampling year, invertebrate communities of the different plot types were not significantly different, while in the second year this was clearly the case. One explanation is that populations of invertebrates may build-up over time (Gladbach et al. 2011; Strayer et al. 2006). Conversely, some initial effects of plant invasions may disappear again in a later stage (Mgobozi et al. 2008). Finally, temporal availability of plant resources (e.g. flowers or fruits) may lead to temporal peaks in the abundance of associated herbivores (Diekotter et al. 2010). To ensure the detection of such temporal peaks it is crucial to sample periodically during the entire growing season, and in several years.

Second, we found significant spatial autocorrelation between the plots within our experimental setup (according to Mantel correlograms; Appendix S4). This spatial effect may be caused by subtle microclimatic gradients in the field or colonization patterns of invertebrates moving from one side of the field towards the other. Independent of the cause of this spatial autocorrelation one can account for this effect by means of a randomized experimental design as was adopted in the current study. Nevertheless, spatial autocorrelation can cause high variation between subplots and may therefore prevent the detection of ecological effects (Lindsay and French 2006).

Third, the three different sampling techniques that we used in this study all sample a specific fraction of the total invertebrate community (Sutherland 2006). For instance, in the current study herbivores were effectively sampled with all three sampling techniques, while predators were mostly sampled with the pitfall traps. In contrast, parasitoids, were almost exclusively sampled with the sticky traps, and detritivores with pitfall traps. This illustrates that the choice of sampling technique will have a major influence on the outcome of a study, and that a combination of different methods will give the best overview of community-wide changes.

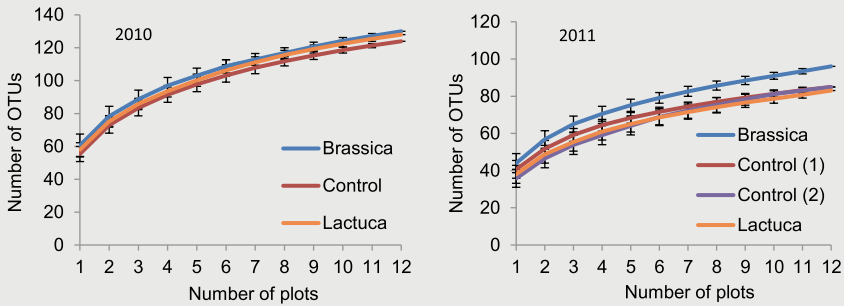
Finally, the size of invasion plots and invader plant density are other factors that are likely to affect study outcomes, given the fact that patch size and invader density are important determinants of insect population densities (Andersson and Hambäck 2011, Bach 1988, Bender et al. 1998, Capman et al. 1990, Grez and González 1995, Pawson et al. 2010). In the current study, it was not feasible to use more than one plot size and invader density for logistic reasons. Yet, for the development of risk assessment systems, we recommend additional studies looking into the effects of patch size and invader density. ●

Appendix S1 Average abundances (± SD) and functional group assignment of all OTUs that were sampled around *Brassica*, control and *Lactuca* plots in 2010 and 2011.

Taxa are ordered alphabetically. p = predator, h = herbivore, d = detritivore, o = omnivore, pl = pollinator, ps = parasitic, f = fungivores and * = miscellaneous.

Order	Family	Operational taxonomic unit (OTU)	Functional group	2010			2011 (period 1)		2011 (period 2)	
				<i>Brassica</i>	Control	<i>Lactuca</i>	<i>Brassica</i>	Control	Control	<i>Lactuca</i>
Acarina	Acarina	Acarina	*	113.5 ± 60.9	124.2 ± 68.7	200.8 ± 190	76.9 ± 66.8	106.4 ± 66.7	36.4 ± 34.6	52.9 ± 55.7
Araneae	Araneae	Araneae	p	169.9 ± 60.7	142.2 ± 73.3	138.3 ± 34.7	105.3 ± 52.9	104.3 ± 53.7	51.3 ± 17.6	47.9 ± 13.4
Coleoptera	Anobiidae	Anobiidae	o	0.1 ± 0.3	-	-	-	-	-	-
	Anthridae	Anthridae	h	5.3 ± 5.7	2.3 ± 2.9	3.9 ± 3.9	2 ± 3	0.3 ± 0.7	0.5 ± 0.7	1.7 ± 2.8
	Apionidae	Apion cruentatum	h	-	0.1 ± 0.3	-	-	0.2 ± 0.6	-	-
		Catapion cf seniculus	h	-	-	0.1 ± 0.3	-	-	-	-
		Catapion pubescens	h	-	-	0.2 ± 0.4	0.1 ± 0.3	-	-	-
		Ceratopion gibbostre	h	-	-	0.1 ± 0.3	-	-	-	-
		Holotrichapion pisi	h	0.1 ± 0.3	-	-	-	-	-	-
		Ischnopterapion virens	h	0.1 ± 0.3	-	0.1 ± 0.3	-	-	-	-
		Oxystoma cracca	h	-	0.1 ± 0.3	-	-	-	-	-
		Oxystoma pomonae	h	0.7 ± 0.7	1.3 ± 2	1.3 ± 1.5	0.1 ± 0.3	-	-	0.1 ± 0.3
		Protapion assimile	o	0.2 ± 0.4	0.1 ± 0.3	-	0.1 ± 0.3	-	-	-
		Protapion dissimile	h	-	-	0.2 ± 0.4	-	-	-	-
		Protapion nigritarse	o	0.1 ± 0.3	-	-	-	0.1 ± 0.3	-	0.1 ± 0.3
		Pseudostenopion simum	h	0.6 ± 0.7	0.6 ± 0.8	2.3 ± 2	-	-	-	-
		Stenopterapion meliloti	h	-	-	0.1 ± 0.3	-	-	-	-
	Byrrhidae	Byrrhidae	h	0.3 ± 0.6	0.1 ± 0.3	-	-	-	-	-
	Cantharidae	Cantharidae	p	3.1 ± 3.8	1.8 ± 1.6	2.8 ± 2.1	1.3 ± 1.4	1.3 ± 2.6	1.6 ± 1.4	1.2 ± 2
		Amara aenea	h	0.9 ± 0.8	1.9 ± 1.4	0.7 ± 0.8	0.3 ± 0.5	0.3 ± 0.5	0.1 ± 0.3	-
		Amara communis	h	-	0.2 ± 0.6	-	-	-	-	-
		Amara consularis	h	12 ± 13.4	6 ± 4.9	9.9 ± 9.5	14.8 ± 7.6	6.7 ± 4.4	0.8 ± 1.6	1.3 ± 2.3
		Amara familiaris	h	4.1 ± 4.1	2.8 ± 4.2	0.7 ± 0.9	8.1 ± 9.3	2.9 ± 3.4	0.2 ± 0.4	0.4 ± 0.7
		Amara fulva	h	-	-	0.7 ± 1.8	0.1 ± 0.3	0.1 ± 0.3	0.3 ± 0.8	0.8 ± 0.9
		Amara lucida	h	-	-	-	0.1 ± 0.3	-	0.3 ± 0.5	1.3 ± 2.3
		Amara lunicollis	h	-	-	-	-	0.3 ± 0.5	0.1 ± 0.3	0.8 ± 2
		Amara ovata	h	0.8 ± 1.4	-	0.1 ± 0.3	1.6 ± 2.3	0.5 ± 0.9	0.1 ± 0.3	-
		Amara spreta	h	-	-	0.2 ± 0.4	-	-	-	-
		Amara tibialis	h	-	-	0.1 ± 0.3	-	-	-	-
		Anchomenus dorsalis	p	0.1 ± 0.3	-	-	-	-	-	-
		Anisodactylus binotatus	o	0.3 ± 0.6	0.3 ± 0.7	0.3 ± 0.5	0.3 ± 0.5	-	-	0.1 ± 0.3
		Badister bullatus	p	0.3 ± 0.5	0.2 ± 0.6	0.2 ± 0.4	0.1 ± 0.3	-	-	0.1 ± 0.3
		Bembidion lampros	p	-	0.1 ± 0.3	-	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.4	-
		Bembidion tetracolum	p	-	0.2 ± 0.4	0.3 ± 0.9	-	-	-	-
		Calathus erratus	p	-	-	-	-	-	0.2 ± 0.4	0.2 ± 0.4
		Calathus melanocephalus	p	0.8 ± 1	0.8 ± 0.8	2.3 ± 3.2	1.4 ± 1.7	0.3 ± 0.6	3.1 ± 4	3.8 ± 3.7
		Clivina fossor	p	0.1 ± 0.3	-	-	-	-	-	-
		Dyschirius politus	p	0.2 ± 0.4	0.2 ± 0.4	-	0.1 ± 0.3	-	-	-
		Elaphropus quadrisignatus	o	0.1 ± 0.3	-	-	-	-	-	-
		Harpalus affinis	h	1.9 ± 2.3	1.7 ± 2.1	1.3 ± 1	0.5 ± 0.7	0.8 ± 0.8	0.2 ± 0.4	0.1 ± 0.3
		Harpalus latus	h	-	-	-	-	-	0.1 ± 0.3	0.1 ± 0.3
		Harpalus ovata	h	-	0.1 ± 0.3	-	-	-	-	-
		Harpalus rubripes	h	0.1 ± 0.3	-	0.2 ± 0.4	-	-	-	0.3 ± 0.9
		Harpalus rufipes	h	6.4 ± 5.2	5.1 ± 5.4	4.7 ± 2.5	6.3 ± 4.3	2.8 ± 2.6	0.5 ± 0.7	0.6 ± 0.7
		Harpalus servus	h	0.1 ± 0.3	0.3 ± 0.6	0.3 ± 0.6	-	0.1 ± 0.3	0.1 ± 0.3	-
		Harpalus tardus	h	0.1 ± 0.3	-	-	-	0.2 ± 0.4	-	-
		Notiophilus biguttatus	p	0.7 ± 0.9	-	0.4 ± 0.9	-	0.1 ± 0.3	-	-
		Notiophilus substriatus	p	-	-	-	0.1 ± 0.3	-	-	-
		Ophonus rufibarbis	h	-	0.2 ± 0.4	0.4 ± 1.4	-	0.1 ± 0.3	-	0.3 ± 0.9
		Poecilus cupreus	o	1.1 ± 3.4	-	0.3 ± 0.6	1 ± 1.3	1.6 ± 3.3	-	-
		Poecilus versicolor	p	6.9 ± 8.9	5.3 ± 10.8	2.5 ± 4.9	6.4 ± 7.9	4.9 ± 11	1.1 ± 1.2	2.7 ± 7.7
		Pterostichus niger	p	0.3 ± 0.5	-	-	0.3 ± 0.6	0.2 ± 0.4	-	-
		Pterostichus nigrita	p	-	-	-	0.1 ± 0.3	-	-	-
		Pterostichus strenuus	o	0.4 ± 0.9	0.1 ± 0.3	0.5 ± 1	0.2 ± 0.4	0.2 ± 0.4	0.1 ± 0.3	0.1 ± 0.3
		Pterostichus vernalis	o	0.2 ± 0.4	0.1 ± 0.3	-	0.1 ± 0.3	0.1 ± 0.3	-	-
		Syntomus foveatus	p	0.9 ± 1.4	1.3 ± 1.8	2.7 ± 2.1	0.7 ± 1.1	0.5 ± 0.8	0.3 ± 0.7	-
	Chrysomelidae	Alicia oleracea	h	-	-	-	-	0.1 ± 0.3	-	-
		Batophila rubi	h	0.1 ± 0.3	0.2 ± 0.4	0.1 ± 0.3	-	-	-	-
		Cassida margaritacea	h	0.1 ± 0.3	0.3 ± 0.5	0.4 ± 0.7	-	-	-	-
		Cassida rubiginosa	h	-	-	-	-	-	-	0.1 ± 0.3
		Cassida sanguinolenta	h	-	-	-	0.2 ± 0.4	0.1 ± 0.3	-	-
		Chaetocnema hortensis	h	0.1 ± 0.3	0.1 ± 0.3	-	-	-	-	-
		Chaetocnema laevicollis	h	-	0.1 ± 0.3	0.1 ± 0.3	-	-	-	-
		Chaetocnema picipes	h	-	-	-	-	-	0.2 ± 0.4	-
		Crepidodera aurata	h	0.3 ± 0.5	0.2 ± 0.4	0.1 ± 0.3	-	-	-	-
		Crepidodera fulvicornis	h	-	-	-	-	-	0.3 ± 0.6	-
		Cryptoccephalus fulvipes	h	5.7 ± 6.2	2.9 ± 3.2	7 ± 6.9	-	-	0.5 ± 0.8	0.8 ± 2.1
		Cryptoccephalus ocellatus	h	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.6	-	-	-	-
		Cryptoccephalus populi	h	0.1 ± 0.3	-	0.3 ± 0.9	0.1 ± 0.3	0.1 ± 0.3	0.3 ± 0.7	0.6 ± 1.2
		Cryptoccephalus rufipes	h	-	-	0.1 ± 0.3	-	-	-	-
		Epitrix pubescens	h	-	-	0.1 ± 0.3	-	-	-	0.1 ± 0.3
		Longitarsus anchusae	h	-	-	-	-	-	0.1 ± 0.3	-
		Longitarsus luridus	h	0.1 ± 0.3	0.2 ± 0.4	0.1 ± 0.3	-	-	0.8 ± 1.3	0.5 ± 0.9
		Longitarsus pellucidus	h	-	0.2 ± 0.6	0.4 ± 1.2	-	-	0.3 ± 1.2	0.1 ± 0.3
		Longitarsus pratensis	h	-	0.1 ± 0.3	-	0.2 ± 0.6	-	0.2 ± 0.4	0.2 ± 0.4
		Longitarsus succineus	h	-	-	-	0.1 ± 0.3	-	-	-
		Neocrepidodera ferruginea	h	0.5 ± 1	0.4 ± 0.7	0.3 ± 0.9	0.3 ± 0.5	-	-	-
		Phyllotreta atra	h	-	-	0.2 ± 0.6	-	0.2 ± 0.4	-	-
		Phyllotreta cruciferae	h	-	-	-	0.1 ± 0.3	0.1 ± 0.3	-	-
		Phyllotreta undulata	h	0.8 ± 1	0.3 ± 0.6	0.6 ± 0.7	0.1 ± 0.3	-	0.1 ± 0.3	0.3 ± 0.7
		Chrysomelidae spec.	h	0.3 ± 0.5	0.4 ± 0.5	0.2 ± 0.4	-	-	0.1 ± 0.3	-
	Coccinellidae	Coccinellidae	p	0.6 ± 0.8	0.6 ± 0.8	0.3 ± 0.5	0.3 ± 1.2	-	0.2 ± 0.4	1.2 ± 0.8
	Cryptophagidae	Cryptophagidae	f	-	-	-	-	-	0.2 ± 0.6	-
	Curculionidae	Anthonomus rubi	h	-	-	-	-	-	0.2 ± 0.6	-
		Ceutorhynchus atomus	h	0.1 ± 0.3	-	-	-	-	-	-
		Ceutorhynchus contractus	h	-	-	-	0.9 ± 1.2	1.2 ± 2.5	-	-
		Ceutorhynchus hirtulus	h	2.5 ± 2.5	0.8 ± 1	2.1 ± 2.5	0.4 ± 0.8	1.3 ± 2.3	-	-
		Ceutorhynchus obstrictus	h	22 ± 10.9	6.8 ± 2	7.7 ± 3.3	5.1 ± 3.7	1.8 ± 2.3	-	0.3 ± 0.6
		Ceutorhynchus pallidactylus	h	-	-	-	0.1 ± 0.3	-	-	-
		Ceutorhynchus quadridens	h	-	-	-	0.2 ± 0.6	-	-	-
		Gloeianus distinctus	h	0.1 ± 0.3	-	-	-	0.1 ± 0.3	-	-
		Grypus equiseti	h	8.6 ± 12	9.4 ± 19	3.4 ± 5.1	2.3 ± 3.6	1.5 ± 2.9	0.3 ± 0.8	0.7 ± 1.1
		Gymnetron pascurum	h	0.1 ± 0.3	0.2 ± 0.4	-	-	-	-	-
		Hadrophlonthus litura	h	-	0.1 ± 0.3	-	-	-	-	-
		Hypera arator	h	-	-	0.3 ± 0.9	-	-	-	-
		Hypera nigrirostris	h	0.1 ± 0.3	-	0.1 ± 0.3	-	-	-	-
		Hypera postica	h	0.8 ± 1.5	0.2 ± 0.4	0.3 ± 0.6	0.2 ± 0.6	0.1 ± 0.3	-	-
		Limobius mixtus	h	-	-	-	-	-	-	0.1 ± 0.3
		Mecinus pyraeaster	h	-	-	0.1 ± 0.3	-	-	-	-
		Nedus quadrimaculatus	h	-	-	-	0.1 ± 0.3	0.1 ± 0.3	-	-
		Otiorynchus ovatus	h	0.1 ± 0.3	-	-	-	-	-	-
		Philopodop plagiat	h	2.7 ± 3.1	2.1 ± 1.8	3.8 ± 3.3	6.2 ± 6	7.3 ± 5.8	0.1 ± 0.3	0.2 ± 0.4
		Rhamphus pulicarius	h	-	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	-	-	-
		Sibinia phalerata	h	-	-	0.2 ± 0.6	-	-	-	-
		Sibinia primita	h	-	-	0.1 ± 0.3	-	-	-	-
		Sitona cylindricollis	h	1.3 ± 1.5	0.2 ± 0.4	0.8 ± 1	-	-	-	0.1 ± 0.3
		Sitona hispidulus	h	1.5 ± 1.6	0.5 ± 0.7	1.3 ± 1.4	0.2 ± 0.6	0.1 ± 0.3	-	-
		Sitona humeralis	h	1.9 ± 4	0.3 ± 0.8	1.7 ± 3.9	0.3 ± 0.6	0.3 ± 0.6	0.3 ± 0.6	0.1 ± 0.3
		Sitona lepidus	h	0.1 ± 0.3	-	-	-	0.2 ± 0.4	-	-
		Sitona puncticollis	h	-	0.1 ± 0.3	-	-	0.1 ± 0.3	-	0.2 ± 0.4
		Stenopelmus rufinatus	h	-	-	-	-	0.1 ± 0.3	-	0.1 ± 0.3
		Trichosirocalus troglodytes	h	5.4 ± 7.3	2.8 ± 4.4	5.1 ± 6.1	2 ± 4.5	1.3 ± 2	0.8 ± 2	0.8 ± 1.2
		Tychius aureolus	h	-	0.2 ± 0.4	-	-	-	-	-
		Tychius meliloti	h	0.3 ± 0.5	0.3 ± 0.6	0.1 ± 0.3	-	-	-	-
		Tychius picirostris	h	0.8 ± 1.5	0.4 ± 0.5	0.6 ± 0.8	0.5 ± 0.7	0.1 ± 0.3	-	-
		Tychius pusillus	h	-	0.1 ± 0.3	-	-	-	0.1 ± 0.3	-
	Elatridae	E								

Appendix S2 Sample-based rarefaction curves for *Brassica*, control and *Lactuca* plots in 2010 and 2011 showing the relationship between the number of plots and invertebrate richness expressed as the number of OTUs. In 2011, control 1 plots are compared with *Brassica* plots, control 2 plots are compared with *Lactuca* plots. Error bars indicate standard deviation.

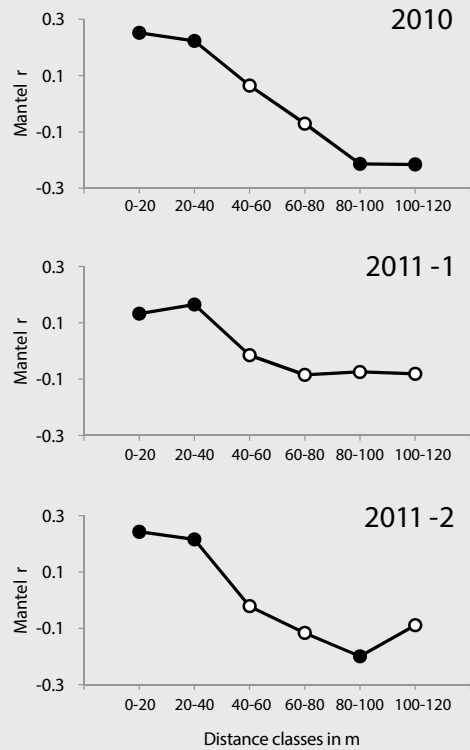


Appendix S3 Mean values (\pm SE) and results from the One-way ANOVAs for the effect of plot type (*Brassica* (B), control (C), *Lactuca* (L)) on the abundance of omnivores, fungivores, pollinators and miscellaneous trophic groups around plots in 2010 and 2011 ($df = 1$). Asterisks indicate a significant difference with the control (* $P < 0.05$, ** $P < 0.01$).

Functional group	Year	Control	<i>Brassica</i>	<i>Lactuca</i>
Omnivores	2010 (B, C, L)	5.6 \pm 0.9	10.7 \pm 1.9*	10.5 \pm 1.7*
	2011 (B, C)	2.8 \pm 0.9	5.8 \pm 0.9**	n/a
	2011 (C, L)	4 \pm 1.2	n/a	6.7 \pm 1.1*
Fungivores	2010 (B, C, L)	0 \pm 0	0 \pm 0	0 \pm 0
	2011 (B, C)	0 \pm 0	0 \pm 0	n/a
	2011 (C, L)	0.2 \pm 0.2	n/a	0 \pm 0
Pollinators	2010 (B, C, L)	5.8 \pm 1.2	6.9 \pm 1.1	5.7 \pm 0.9
	2011 (B, C)	2.5 \pm 0.8	4.2 \pm 1	n/a
	2011 (C, L)	4.8 \pm 0.6	n/a	6.1 \pm 0.9
Miscellaneous ¹	2010 (B, C, L)	985.8 \pm 42.1	1157.3 \pm 52.7*	1021.7 \pm 59.7
	2011 (B, C)	440.5 \pm 22.1	540.5 \pm 20.1**	n/a
	2011 (C, L)	1059 \pm 75.8	n/a	1304.6 \pm 94.3*

¹This category includes taxa with subtaxa belonging to two or more functional groups.

Appendix S4 Mantel correlograms for invertebrate composition data in 2010 and 2011. Filled symbols indicates significant spatial correlation ($P < 0.05$, after sequential Bonferroni correction for multiple comparisons). 2011-1 comprises *Brassica* and control plots, while 2011-2 comprises *Lactuca* and control plots. In 2010, all three plot types are represented.



Appendix S5 Mean values (\pm SE) and results from the One-way ANOVAs for the effect of plot type on the herbivore abundance on the catch plants (*D. tenuifolia* and *T. pratensis*) surrounding the plots in 2010 and 2011. Asterisks indicate a significant difference (* $P < 0.05$), NS = not significant.

Response	Year	Control	<i>Brassica</i>	<i>Lactuca</i>
Herbivore abundance on <i>D. tenuifolia</i>	2010 (B,C, L)	116.4 \pm 16.7	99.0 \pm 9.5	105.0 \pm 10.0
	2011 (B, C)	52.8 \pm 9.7	53.5 \pm 6.9	n/a
	2011 (C, L)	46.1 \pm 4.6	n/a	62.1 \pm 5.5*
Herbivore abundance on <i>T. pratensis</i>	2010 (B,C, L)	118.7 \pm 20.1	85.9 \pm 14.3	91.8 \pm 10.4
	2011 (B, C)	24.8 \pm 3.9	20.2 \pm 2.2	n/a
	2011 (C, L)	55.1 \pm 7.5	n/a	53.0 \pm 7.9

Appendix S6 Results from the General Linear Mixed Model for the effect of plot type (*Brassica*, control, *Lactuca*) and catch plant species on the incidence of leaf damage of the catch plants *T. pratensis* and *D. tenuifolia*. Asterisks indicate a significant difference (*** $P < 0.001$), NS = not significant.

Coefficients	June			July		
	df	F	P	df	F	P
2010	2,33	2.16	NS	2,33	0.99	NS
Catch plant	1,33	13.54	***	1,33	26.12	***
Plot type x catch plant	2,33	2.76	NS	2,33	0.54	NS

Coefficients	June			August		
	df	F	P	df	F	P
2011	1,22	0.25	NS	1,22	0.15	NS
Plot type	1,19	17.01	***	1,18	224.30	***
Catch plant	1,19	4.05	NS	1,18	0.66	NS

Impact of plant invasions on local arthropod communities: a meta-analysis

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Abstract

Invasive plants can have major impacts on local plant and animal communities. However, effects of plant invasions on arthropod communities and potential explanatory mechanisms have rarely been studied. We present a meta-analysis on the impact of plant invasions on abundance and richness of local arthropod communities. Moreover, we study the role of five invader and habitat attributes to assess their influence on the direction and magnitude of effect on arthropod communities. For management prioritisation and development of risk assessment systems, identifying such attributes is essential.

Across the 56 studies included in the meta-analysis, plant invasions reduced both arthropod abundance and taxonomic richness. Moreover, we found that woody invaders had a significantly stronger negative impact on arthropod communities than herbaceous invaders.

Our study demonstrates that arthropod communities are negatively affected by plant invasions, which may in turn have indirect effects on other ecosystem features, for instance on pollination, food web dynamics and decomposition. Management measures aiming at preserving arthropod communities should consider elevating the priority for control of woody invaders.

Introduction

Plant invasions can severely affect the size and composition of native plant and animal communities through disruption of biotic interactions or changes in abiotic ecosystem characteristics (Levine et al. 2003, Belnap et al. 2005, Davis 2009). Although many studies looked into the effects of invaders on plant communities, including several meta-analyses (Lonsdale 1999, Mason et al. 2009, Vilà et al. 2011), little is known about responses of arthropod communities to plant invasions. This is surprising, since arthropods are regarded as important organisms for ecosystem functioning. In many systems arthropods are the main primary consumers, and are crucial for the pollination and dispersal of many plant species (Chapman 1998). Several case studies reported significant changes in arthropod abundance following plant invasions (French and Major 2001, Pearson 2009), as well as changes in species richness (Belnap and Phillips 2001, Greenwood et al. 2004, Harris et al. 2004). Although explanatory mechanisms have been suggested (Palmer et al. 2004, Belnap et al. 2005, Wilkie et al. 2007), so far no comprehensive statistical analysis has been conducted to synthesise and generalise across studies.

A synthesis of the ecological impacts of invasive species is also crucial for the development of better risk assessment systems and guidelines for management prioritisation. Guidelines could be improved by identifying and including habitat

and plant attributes that are good predictors of ecological impact (Pheloung et al. 1999). Only few studies have attempted to identify such factors. For example, Vilà et al. (2011) showed that N-fixing plant invaders did not affect local animal and plant communities more strongly than non N-fixing plants. Mason et al. (2009) did not observe any difference between woody and graminoid invaders in their impact on local plant communities. To our knowledge, no attempts have been made to identify habitat or plant attributes in relation to arthropod responses to plant invasions.

In this study, we report on a meta-analysis of peer-reviewed studies exploring the overall effect of plant invasions on arthropod communities. Moreover, we analyse the role of five often discussed habitat and invader attributes on the direction and magnitude of change in aboveground arthropod communities (Keane and Crawley 2002, Daehler 2005, Davis 2009). First, we examine the 'time since introduction' of the invasive species, an aspect that has been largely neglected in invasion biology (Barney and Whitlow 2008). The magnitude and direction of impact may change over time as a result of evolutionary processes, shifts in plant species composition, accumulation of organic matter, and interaction with abiotic factors (Belnap et al. 2005, Strayer et al. 2006). The second factor tested is the impact of invasion into regions where (native) congeners are already present, as compared to regions without congeners. If specialist herbivores switch from native to invasive species (Keane and Crawley 2002), local herbivore communities (and higher trophic levels) could remain stable: however, evidence for such herbivore switches remains scarce (Tallamy 2004).

Invader woodiness is the third factor that may influence magnitude and direction of ecosystem effects. Daehler (2005) classified 73% of the non-native woody species on Hawaii as disruptive invaders of native communities, whereas only 9% of the non-native herbaceous species were classified as such. The underlying causes might be that woody plants create new niches for many species. Compared to herbaceous species, woody species usually grow taller, root deeper, live longer, and produce more biomass and litter (Knoop and Walker 1985, Hughes et al. 2006), with large effects on light and water availability (Zavaleta 2000, Wearne and Morgan 2004, Blank and Carmel 2012). A shift in plant species composition following the invasion of a woody species could have more pronounced effects on arthropod communities.

Fourth, we examine the impact of invasive canopy cover on arthropod communities, which can be important for management prioritisation (Pawson et al. 2010). Thus far, most studies reported negative relationships between invasive canopy cover and arthropod abundance and richness (Litt and Steidl 2010, Pawson et al.

2010, Spyreas et al. 2010, but see Parr et al. 2010). The final factor we investigate is the impact of multiple species as opposed to single species invasions. Invasive species frequently interact with one another and it has been suggested that this may accelerate impacts on native ecosystems (Simberloff and Von Holle 1999), although the underlying mechanism is not well understood.

Our meta-analysis addresses the following hypotheses, focusing on total arthropod abundance and community richness: (1) the time since introduction influences the magnitude and direction of change in native arthropod communities; (2) arthropod communities are less negatively affected by invaders when native congeners are already present in the region; (3) woody invaders cause a stronger effect on arthropod communities than herbaceous invaders; (4) the magnitude of change in arthropod communities will increase with increasing invasive canopy cover, and (5) multiple species invasions result in a bigger change in local arthropod communities than single species invasions.

Material and Methods

Literature search

Our meta-analysis included those peer-reviewed studies that compared arthropod abundance and richness in invaded compared to uninvaded habitat. Studies were assembled using Google Scholar database searches and inspecting lists of cited references in the selected papers. From each study we extracted mean, standard deviation (SD) and sample size (N) for total arthropod richness and abundance in invaded and uninvaded habitat. These measures were subsequently translated into effect sizes. Across studies, various taxonomic levels were allowed to assess arthropod richness, *viz.*, species, morphospecies, recognizable taxonomic units (RTUs), operational taxonomic units (OTUs), family, genus or order. Means and SD were extracted from tables, figures or from original data sets provided by the authors. Data from figures were extracted using the image processing software ImageJ (Schneider et al. 2012). We included studies that focused on the impact of one invasive species as well as studies on effects of multiple invaders. Both observational and experimental (removal or introduction of the invader) studies were included.

In the meta-analysis we included studies that examined aboveground effects (including the litter layer) on arthropods. When the response variable was measured at different locations we considered splitting the data set. This depended on whether different locations represented clearly distinct regions in terms of ecosystem and

environmental conditions (following Vilà et al. 2011), which was the case for three data sets. For response variables that were measured at different time points (e.g. seasons, years), the pooled mean and SD across all time periods was used to capture natural variability in effects. Furthermore, in four cases where the response variable was measured as a function of degree of invasion (i.e. as percentage cover of the invasive species), we examined the differences between sites with 0-20 % invasive cover (control) and sites with 80-100 % invasive cover.

Study characteristics

The literature search yielded a total of 56 suitable studies comprising 59 independent case studies (sampling locations; Appendix S1). Most studies were done in North America (26), followed by Europe (11), Australia (7), South Africa (6), New Zealand (4) and China (1) and South America (1). In total, the effect of 61 invasive plant species was considered, with a canopy cover that varied between 13 - 95%, with an average of around 60%. For 22 studies no specific data on canopy cover was reported. In 16 studies the invader was woody, but in the majority of the studies (35) the invader was herbaceous. Furthermore, 44 studies studied the effect of a single invader, whereas 11 studies studied the cumulative effect of multiple invading species. For 48 studies we could find information on the year in which the invasive species was introduced, which was between 1650 and 1995, with the majority (31) between 1850 and 1950. Most studies were observational (49) and only 7 were experimental. We also checked whether the invader represented a new genus to the invaded region. For this purpose we performed queries in the global biodiversity information facility (GBIF) using the function `gbif` in the R package *dismo* (Hijmans et al. 2012; version 0.7-17). A complete overview of characteristics of all individual studies is provided in Appendix S2.

Data analysis

We calculated the Hedges' *d* effect size for each data set to estimate the difference in arthropod richness and abundance between invaded and uninvaded habitat as control (Hedges and Olkin 1985). The effect size *d* was expressed as:

$$d = \frac{\bar{X}_1 - \bar{X}_2}{s_{pooled}} J$$

in which \bar{X}_1 and s_{pooled} are the sample means of the two groups (invaded and uninvaded) and s_{pooled} their pooled standard deviation, expressed as:

$$s_{pooled} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}}$$

Here, n_1 and n_2 are the sample sizes and s_1 and s_2 are the standard deviations of the two groups. The weighting factor J was calculated as:

$$J = 1 - \frac{3}{4(n_1 + n_2 - 2) - 1}$$

A positive effect size in our study indicates that the arthropod abundance or richness is higher in the invaded habitat compared to the uninvaded habitat, while a negative effect size implies the opposite.

We used a random effects model to calculate overall effect sizes for arthropod richness and abundance (Borenstein et al. 2009). To define the variation in effect sizes that can be ascribed to differences between the groups that were made, we employed mixed effects models, using the Q-statistic. This model treats the effect of groupings as fixed and adds a random effects variance component that represents the variability across the population effects. We used weighted generalised least squares regression models to test the relationships between 'time since introduction' and 'invasive species canopy cover' and the effect sizes for arthropod abundance and richness.

Calculations were performed in IBM SPSS Statistics (SPSS Inc.; release 20.0.0) using a set of macros developed by Wilson (2005, <http://mason.gmu.edu/dwilsonb/ma.html>). The macros also calculated 95% confidence intervals and Q values for all effects. Variance parameters were estimated using restricted maximum likelihood (REML).

Normality of the distribution of effect sizes was verified by exploring normal quantile plots. The set of effect sizes for arthropod abundance contained one outlier (Ostojca et al. 2009), which caused the data to deviate from a normal distribution (Appendix S3). Conservatively, we decided to include this case study in the meta-analysis, because we could not identify any potential experimental flaws. The effect sizes for arthropod richness were normally distributed (Appendix S3). To assess whether this meta-analysis suffers from potential publication bias we constructed funnel plots describing the relationships between the sample size and effect size. A symmetric funnel shape indicates that a publication bias is unlikely (Egger et al. 1997). The funnel plots based on the data for arthropod richness and abundance showed a symmetrical shape (Appendix S4 a-b). In addition, we found no correlation between sample size and the standardised effect sizes (richness: Spearman $r = 0.111$, $P = 0.44$; abundance: Spearman $r = -0.023$, $P = 0.87$), adding additional support to the statistically unbiased nature of the data.

Table 1 Homogeneity tests among studies for differences among mean effect sizes of each response variable for arthropod abundance and richness. n = number of case studies within each test group; ES = effect size (d); SE = standard error, $df = 1$. A significant Q -score *rejects* the *null hypothesis* of *homogeneity amongst* the average effect sizes of the two groups.

Arthropod abundance						
		N	Mean ES	SE	Q	P
woody invader	yes	15	-1.203	0.324	5.661	0.017
	no	34	-0.288	0.207		
new genus for the region	yes	25	-0.314	0.181	0.703	0.402
	no	19	-0.552	0.218		
multiple invaders	yes	8	-1.239	0.420	3.066	0.080
	no	44	-0.433	0.188		
Arthropod richness						
		N	Mean ES	SE	Q	P
woody invader	yes	16	-1.079	0.190	12.381	<0.001
	no	29	-0.289	0.121		
new genus for the region	yes	24	-0.461	0.169	0.254	0.615
	no	15	-0.602	0.223		
multiple invaders	yes	10	-0.615	0.243	0.115	0.735
	no	40	-0.522	0.134		

Results

Arthropod communities within invaded habitats generally had less individuals (Effect size = -0.57, $n = 52$, $Z = -3.25$, $P = 0.001$) and lower taxonomic richness (Effect size = -0.54, $n = 50$, $Z = -4.60$, $P < 0.001$) than control habitats. In Appendix S5 and S6, we show the individual effect sizes of all studies.

We tested five factors that may explain the negative relationship between invading plant species and arthropods.

- i. There was no relationship between the *time since introduction* of an invasive species and its impact on arthropod abundance ($R^2 = 0.01^{ns}$, $Q = 0.41$; Fig. 1a) or arthropod richness ($R^2 = 0.01^{ns}$, $Q = 0.71$; Fig. 1a; Table 1).
- ii. The *presence of congeners* in the invaded region did not alter arthropod abundance ($Q = 0.70$, $P = 0.40$) or richness ($Q = 0.25$, $P = 0.61$; Fig. 2a; Table 1).
- iii. *Woody invaders* decreased the abundance of arthropods significantly ($Z = -3.71$, $P < 0.001$; Fig. 2b). In contrast, herbaceous invaders had no effect on arthropod abundance ($Z = -1.39$, $P = 0.16$). Both woody and herbaceous invaders significantly lowered arthropod richness ($Z = -5.69$, $P < 0.001$ and $Z = -2.40$, $P = 0.02$, respectively; Fig. 2b), but the effect of woody invaders was about four

times stronger than that of their herbaceous counterparts ($Q = 12.38$, $P < 0.001$; Fig. 2b).

- iv. *Multiple species invasions* resulted in a marginally stronger reduction in arthropod abundance than single species invasions ($Q = 3.10$, $P = 0.08$; Fig. 2c), but we did not detect any difference for arthropod richness ($Q = 0.12$, $P = 0.74$; Fig. 2c).
- v. Contrary to our expectations, there was no correlation between *canopy cover* of the invasive species and arthropod abundance ($R^2 = 0.009^{ns}$, $Q = 0.34$; Fig. 1b) or richness ($R^2 = 0.004^{ns}$, $Q = 0.71$; Fig. 1b).

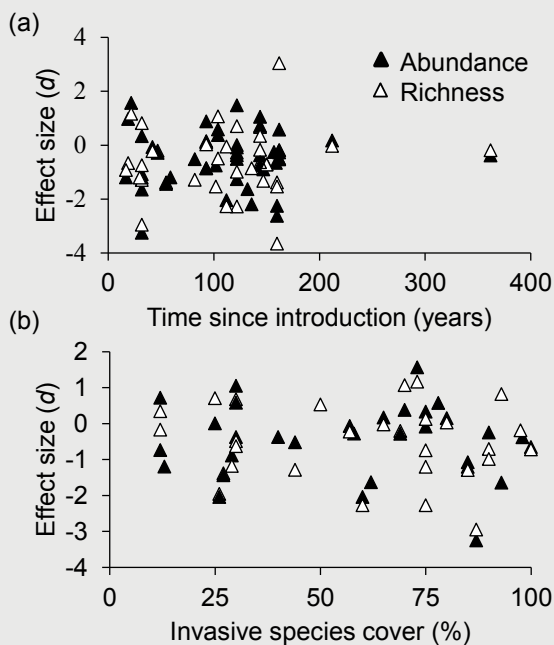


Fig. 1 Relationship between effect size and (a) the time since introduction ($N_{\text{abundance}} = 45$, $N_{\text{richness}} = 40$) and (b) the canopy cover of the invader ($N_{\text{abundance}} = 31$, $N_{\text{richness}} = 30$) for arthropod abundance and richness. No relationships were identified from the weighted generalized least squares regression. One case study with an effect size of 15.2 (time since introduction around 100 years ago; Ostojca et al. 2009) in figure (a) has been removed to improve visual interpretation.

Discussion

Plant invasions significantly reduce local arthropod abundance and taxonomic richness, at least if we can assume that there are no confounding factors that affect both plant invasiveness and arthropods. Moreover, our meta-analysis of 56 studies confirmed that woodiness is an important invader species attribute, increasing the magnitude of change on arthropod abundance and richness following plant invasions fourfold as compared to herbaceous invaders.

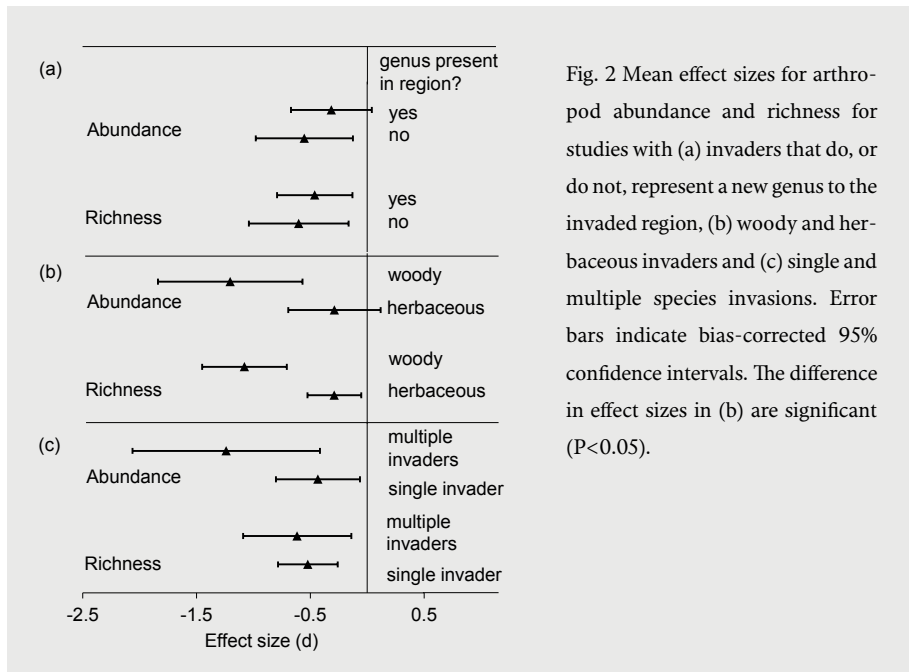


Fig. 2 Mean effect sizes for arthropod abundance and richness for studies with (a) invaders that do, or do not, represent a new genus to the invaded region, (b) woody and herbaceous invaders and (c) single and multiple species invasions. Error bars indicate bias-corrected 95% confidence intervals. The difference in effect sizes in (b) are significant ($P < 0.05$).

In general, plant invasions decrease plant species richness (Mason et al. 2009, Vilà et al. 2011), but increase primary production and plant biomass (Ehrendfeld 2003, Vilà et al. 2011). Since plant species richness is generally strongly positively associated with arthropod diversity (Crisp et al. 1998, Siemann et al. 1998, Haddad et al. 2001), this may explain the observed reduction in arthropod richness. Arthropod abundance is in general positively correlated with primary production (Siemann et al. 1998, Haddad et al. 2001). Nevertheless, across studies arthropod abundances decreased following plant invasions. This suggests that the identity of the invader is more important than its effect on primary production.

We rejected four out of the five hypotheses that were tested in our meta-analysis. However, we confirmed that the changes in arthropod richness caused by woody invaders were substantially (i.e. fourfold) larger than for herbaceous invaders. We

propose four possible explanations for this result.

First, habitat complexity may be reduced by woody invaders, resulting in a reduction in animal richness (Crooks 2002, Tews et al. 2004). Whether an invader increases or decreases habitat complexity depends on its structural properties in relation to the structure of the habitat it invades (Mason and French 2008). The introduced *Spartina alterniflora* increased habitat complexity by colonizing un-vegetated mudflats in a Tasmanian estuary, thereby locally increasing invertebrate abundance and diversity (Hedge and Kriwoken 2000). In contrast, the replacement of structurally diverse native forest by invasive trees reduced habitat complexity and the associated animal abundance and diversity (Crooks 2002).

A second, rather speculative explanation is based on differences between herbs and woody plants as a food source. Woody and herbaceous invaders differ in the chemical composition and toughness of the leaves, affecting their quality and/or decomposability (Cornelissen and Thompson 1997, Haukioja and Koricheva 2000, Kurokawa et al. 2010). The chemical composition of tree foliage makes it less palatable and digestible than herbaceous leaves (Kimmins 2004). In addition, leaves from some woody species have lower nitrogen- and water content (Mattson 1980, but see Wright et al. 2005). These traits are all negatively correlated with food quality (Schädler et al. 2003) and may consequently affect richness and abundance of herbivores and indirectly also organisms from higher trophic levels.

The third explanation is that woody plants are usually taller than herbaceous plants and have a more elaborate root system, which may affect local light and soil conditions for the community as a whole (Holmgren et al. 1997). As a result, arthropod communities may be directly affected due to changed abiotic conditions. Furthermore, arthropod communities may be indirectly affected via a relative reduction in the cover of herbaceous species caused by increased woody plant presence.

Finally, a higher allelopathic potential of woody species could (partly) explain the observed difference in impact between woody and herbaceous invaders (Hierro and Callaway 2003). Of the case studies in the meta-analysis on effects on arthropod abundance, 60% of the woody species are known to have allelopathic properties, against only 21% of the herbaceous invaders ($P = 0.01$, two-tailed Fisher's exact test). In the data set that we used to assess the effect on arthropod richness we found similar, although not significantly different, proportions (56% for woody invaders against 28% for herbaceous ones). This observation may indicate an indirect effect of allelopathy on arthropod communities. Additional studies are needed to verify the exact role of allelopathy.

Several proposed hypotheses were not statistically supported by the results from the meta-analysis. The time since introduction of the invasive species did not influence the magnitude or direction of effects on the arthropod community. We suggest that this could be due to co-occurring of factors that increase or decrease in arthropod abundance and richness in time. For example, increases have been shown in herbivore abundance associated with an invasive tree species (*Sapium sebiferum*) in North America (Siemann et al. 2006). They attributed the effect to selection against energy resource allocation to defensive compounds in plants *sensu* the EICA theory (Evolution of Increased Competitive Ability, Blossey and Notzold 1995). Alternatively, a reduction of arthropod richness and abundance can be observed when invasive species directly decrease water availability and increase salt concentrations in the soil (Di Tomaso 1998, Zavaleta 2000).

We did not find evidence that invaders of habitats containing congeners caused a smaller change to the arthropod community than invaders of habitats without such congeners (Keane and Crawley 2002, Tallamy 2004). Although there is previous evidence of host switching by specialists from native congeners to introduced species (Creed Jr and Sheldon 1995, Jobin et al. 1996), Tallamy (2004) argued that specialists typically comprise only a small percentage of the total insect fauna associated with an introduced species. This may explain why we did not detect general community-wide effects on arthropod abundance and richness in our meta-analysis across all studies. Alternatively, it could be presence of relatives at the genus level is not a suitable taxonomic level for a meaningful comparison.

Furthermore, we could not confirm that invasive species canopy cover affected the magnitude of change on arthropod abundance or richness. In a study by Hejda et al. (2009), invasive cover and plant height were found to determine the magnitude of change in plant communities, but this apparently is not reflected in the arthropod community.

Finally, an unexpected result was that in comparison to single-species invasions, invasion events involving multiple species only marginally magnified the resulting changes in arthropod abundance and richness. We expected that interactions between multiple invader species could result in a higher impact on the recipient system (Simberloff and Von Holle 1999). Across the included studies such an effect was not found, but this hypothesis would deserve more empirical testing in the future.

Conclusions and future directions

In our meta-analyses we showed that the plant invaders lower local arthropod abundance and species richness. Furthermore, characteristics associated with woodiness seem to be important determinants of the magnitude of change in

arthropod communities. In addition to concluding that our findings deserve more research, three potential methodological issues have emerged during our synthesis that we suggest to incorporate in future studies. Most studies up to now are observational - directly comparing invaded with uninvaded habitat - and only few used experimental designs (e.g. Heleno et al. 2010, Magoba and Samways 2010, Simao et al. 2010). The fundamental problem with observational studies is that it is not possible to identify the true causes of the observed differences between invaded and uninvaded habitat, because other factors, such as local resource availability and soil type, may act independently (Palmer et al. 2004, Belnap et al. 2005, Wilkie et al. 2007). If containment is possible we would recommend an approach in which the invader is experimentally introduced, controlling for as many environmental factors as possible (Simao et al. 2010).

The second concern is the use of 'arthropod richness' as such. For instance, studies might not detect differences in arthropod richness, whereas the functional and species composition may have changed drastically (e.g. Wilkie et al. 2007, Wu et al. 2009). Unfortunately, the current studies of functional and taxonomic composition did not provide sufficient statistical data and power to analyse the effects of species invasions on arthropod community structure, which deserves to become a clear research priority for the future.

Finally, most studies focus on a particular taxonomic group of arthropods. It is not unrealistic to assume that different arthropod (functional) groups respond differently to plant invasions (Simao et al. 2010). By using a combination of multiple insect sampling techniques - e.g. pitfalls, vacuum samplers and flight interception traps - this problem could be avoided (Sutherland 2006).

Irrespective of these experimental recommendations, predictive theories on the impacts of invasive species are crucial for management prioritisation and environmental risk assessment (Pheloung et al. 1999) and are currently extensively sought after for plants (Theoharides and Dukes 2007, Ramula et al. 2008). Our study clearly shows that indirect effects on arthropod communities are an important ecological consequence of invasive plants that should be included in these assessments.

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Supplementary material

Appendix S1. List of studies that were used in the meta-analysis.

- Almeida-Neto M, Prado PI, Lewinsohn TM (2011). Phytophagous insect fauna tracks host plant responses to exotic grass invasion. *Oecologia* 165:1051-1062
- Burghardt KT, Tallamy DW, Gregory Shriver W (2009). Impact of native plants on bird and butterfly biodiversity in suburban landscapes. *Conserv Biol* 23:219-224
- Davalos A, Blossey B (2004). Influence of the invasive herb garlic mustard (*Alliaria petiolata*) on ground beetle (Coleoptera : Carabidae) assemblages. *Environ Entomol* 33:564-576
- De Groot M, Kleijn D, Jogan N (2007). Species groups occupying different trophic levels respond differently to the invasion of semi-natural vegetation by *Solidago canadensis*. *Biol Conserv* 136:612-617
- DeLay L, Finch DM, Brantley S, Fagerlund R, Means MD, Kelley J (1999). Arthropods of native and exotic vegetation and their association with willow flycatchers and Wilson's warblers. USDA Forest Service Proceedings RMRS-P-7
- Derraik JGB, Rufaut CG, Closs GP, Derraik K (2005). Ground invertebrate fauna associated with native shrubs and exotic pasture in a modified rural landscape, Otago, New Zealand. *N Z J Ecol* 29:129-135
- Durst SL, Theimer TC, Paxton EH, Sogge MK (2008). Temporal variation in the arthropod community of desert riparian habitats with varying amounts of Saltcedar (*Tamarix ramosissima*). *J Arid Environ* 72:1644-1653
- Ernst CM, Cappuccino N (2005). The effect of an invasive alien vine, *Vincetoxicum rossicum* (Asclepiadaceae), on arthropod populations in Ontario old fields. *Biol Invasions* 7:417-425
- French K, Major RE (2001). Effect of an exotic acacia (Fabaceae) on ant assemblages in South African fynbos. *Austral Ecol* 26:303-310
- Gerber E, Krebs C, Murrell C, Moretti M, Rocklin R, Schaffner U (2008). Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. *Biol Conserv* 141:646-654
- Gratton C, Denno RF (2005). Restoration of arthropod assemblages in a *Spartina* salt marsh following removal of the invasive plant *Phragmites australis*. *Restor Ecol* 13:358-372
- Greenwood H, O'Dowd DJ, Lake P (2004). Willow (*Salix x rubens*) invasion of the riparian zone in Southeastern Australia: Reduced abundance and altered composition of terrestrial arthropods. *Divers Distrib* 10:485-492
- Hansen AK, Ortega YK, Six DL (2009). Comparison of ground beetle (Coleoptera: Carabidae) assemblages in rocky mountain savannas invaded and uninvaded by an exotic forb, spotted knapweed. *Northwest Science* 83:348-360
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- Harris RJ, Toft RJ, Dugdale JS, Williams PA, Rees JS (2004). Insect assemblages in a native (Kunzea - *Kunzea ericoides*) and an invasive (Gorse - *Ulex europaeus*) shrubland. *N Z J Ecol* 28:35-47
- Harvey KJ, Britton DR, Minchinton TE (2010). Insect diversity and trophic structure differ on native and non-indigenous congeneric rushes in coastal salt marshes. *Austral Ecol* 35:522-534
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- Ivanov K, Keiper J (2011). Potential impacts of the invasive herb garlic mustard (*Alliaria petiolata*) on local ant (Hymenoptera: Formicidae) communities in northern temperate forests. *Jeffersoniana* 26:1-14
- Jonas JL, Whiles MR, Charlton RE (2002). Aboveground invertebrate responses to land management differences in a central Kansas grassland. *Environ Entomol* 31:1142-1152
- Kappes H, Lay R, Topp W (2007). Changes in different trophic levels of litter-dwelling macrofauna associated with giant knotweed invasion. *Ecosystems* 10:734-744
- Lambrinos JG (2000). The impact of the invasive alien grass *Cortaderia jubata* (Lemoine) Stapf on an endangered Mediterranean type shrubland in California. *Divers Distrib* 6:217-231
- Lindsay EA, French K (2006). The impact of the weed *Chrysanthemoides monilifera* ssp *rotundata* on coastal leaf litter invertebrates. *Biol Invasions* 8:177-192
- Litt AR, Steidl RJ (2010). Insect assemblages change along a gradient of invasion by a nonnative grass. *Biol Invasions* 12:3449-3463
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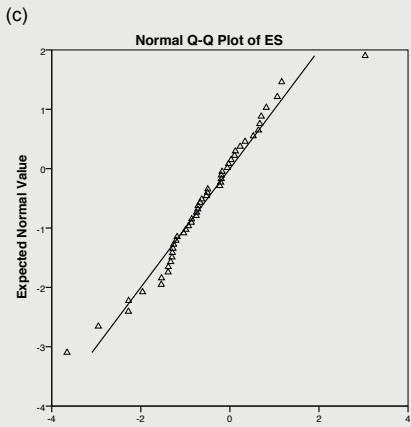
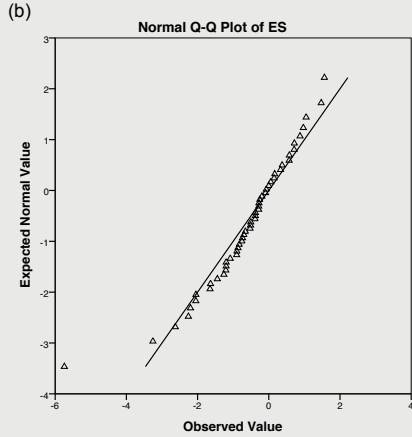
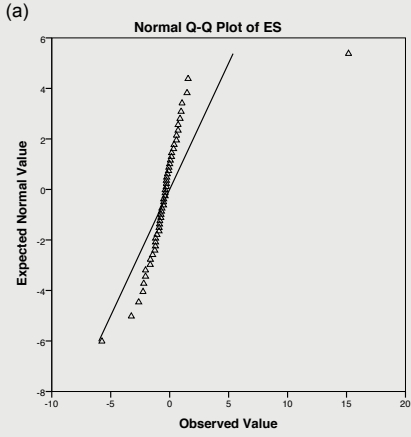
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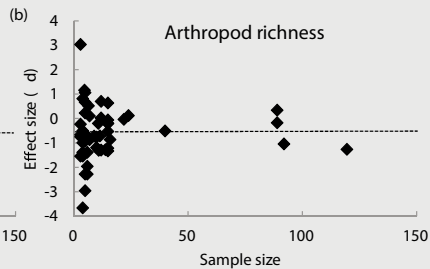
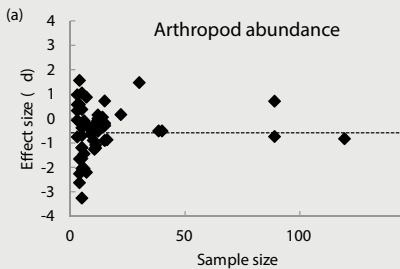
Appendix S2. Individual study characteristics used in meta-analysis. Some characteristics are not used in the statistical analyses and are presented to provide additional background information.

study	type of data ¹	vegetation community	invasive species	plant family	study area	native range	estimated year of introduction	average cover invasive species	new genus for region	stem ²	sampling design	sampling technique	studied taxa	taxo-nomic level ³
Almeida-Neto et al. (2011)	r	tropical savanna	Mostly <i>Andropogon gayanus</i> , <i>Urochloa decumbens</i> , <i>Hyparrhenia rufa</i> , <i>Melinis minutiflora</i> , <i>Urochloa maxima</i>	Poaceae	Brazil	n/a	n/a	density gradient	n/a	h	invasion gradient	emergence from flowerheads	insect herbivores	genus, OTUs
Burghardt et al. (2008)	a, r	suburban mixed vegetation	not specified	n/a	Pennsylvania, USA	n/a	n/a	26%	n/a	n/a	invaded vs uninvaded	visual search	Lepidoptera	(morpho)species
Davalos and Blossey (2004)	a, r	forest	<i>Alliaria petiolata</i>	Brassicaceae	New York, USA	Eurasia	1868	12%	yes	h	invaded vs uninvaded	hand collection, cover boards	Carabidae	species
DeLay et al. (1999)	a	riparian forest	<i>Tamarix ramosissima</i>	Tamaricaceae	New Mexico, USA	Europe, Asia	late 19th century	dominant	yes	w	invaded vs uninvaded	sticky traps	whole community	order, family
Derraik et al. (2005)	a	shrubland	<i>Agrostis capillaris</i> , <i>Anthoxanthum odoratum</i>	Poaceae	New Zealand	Eurasia (both species)	n/a	dominant	n/a	h	invaded vs uninvaded	pitfalls	whole community	(morpho)species
Durst et al. (2008)	r	riparian forest	<i>Tamarix ramosissima</i>	Tamaricaceae	Arizona, USA	Europe, Asia	late 19th century	>90%	yes	w	invaded vs uninvaded	malaise traps	whole community	morphospecies
Ernst and Cappuccino (2005)	a	woodlots and old fields	<i>Vincetoxicum rossicum</i>	Apocynaceae	Ontario, Canada	Russia, Ukraine	1930	?	yes	h	invaded vs uninvaded	pitfalls	whole community	order, family, species
French and Major (2001)	a, r	fynbos	<i>Acacia saligna</i>	Fabaceae	South Africa	Australia	1876-1885	dominant	no	w	invaded vs uninvaded	pitfalls	Formicidae	species
Gerber et al. (2008)	a, r	riparian grassland/shrubland	<i>Reynoutria japonica</i> var. <i>japonica</i> and <i>R. sachalinensis</i>	Polygonaceae	Germany, Switzerland, France	Eastern Asia	19th century	dominant	yes	h	invaded vs uninvaded	pitfall and window traps	whole community	species
Gratton and Denno (2005)	a, r	salt marsh	<i>Phragmites australis</i>	Poaceae	New Jersey, USA	probably Europe	1850s	>90%	no	h	experimental removal	suction sampling	whole community	(morpho)species
Greenwood et al. (2004)	a, r	riparian woodland	<i>Salix x rubens</i>	Salicaceae	Australia	Europe, western and central Asia	around 1900	>60%	yes	w	invaded vs uninvaded	sticky traps, hand collection, pan traps	whole community	morphospecies
Groot et al. (2007)	a, r	managed fields	<i>Solidago canadensis</i>	Asteraceae	Slovenia	North America	17th century	95-100%	no	h	invaded vs uninvaded	pitfalls, visual search	Lepidoptera, Syrphidae, Carabidae	species
Hansen et al. (2009)	a, r	savanna	<i>Centaurea maculosa</i>	Asteraceae	Montana, USA	Europe	late 19th century	>25%	no	h	invaded vs uninvaded	pitfalls	Carabidae	species
Hanula and Horn (2011)	a, r	riparian forest	<i>Ligustrum sinense</i>	Oleraceae	Georgia, USA	Asia	1852	dominant	no	w	experimental removal	pan traps	Apidae	species
Hanula and Horn (2011)	a, r	riparian forest	<i>Ligustrum sinense</i>	Oleraceae	Georgia, USA	Asia	1852	dominant	no	w	experimental removal	pan traps	Lepidoptera	species
Harris et al. (2004)	a, r	shrubland	<i>Ulex europaeus</i>	Fabaceae	New Zealand	Europe	mid-19th century	78%	yes	w	invaded vs uninvaded	malaise traps, pitfalls	Coleoptera, Lepidoptera Diptera	RTU
Harvey et al. (2010)	a, r	salt marsh	<i>Juncus acutus</i>	Juncaceae	Australia	Europe, North Africa (?)	1957	27%	no	h	invaded vs uninvaded	sweeping, suction sampling	whole community	family, morphospecies
Heleneo et al. (2009)	a, r	forest	Mostly <i>Cryptomeria japonica</i> , <i>Pittosporum undulatum</i> , <i>Hedychium gardnerianum</i> , <i>Clethra arborea</i>	n/a	Azores, Portugal	n/a	n/a	density gradient	n/a	n/a	invasion gradient	fruit collection	insect herbivores and parasitoids	(morpho)species
Heleneo et al. (2010)	a, r	forest	Mostly <i>Pittosporum undulatum</i> , <i>Hedychium gardnerianum</i> , <i>Clethra arborea</i> , <i>Acacia melanoxylon</i>	n/a	Azores, Portugal	n/a	n/a	?	n/a	n/a	experimental removal	fruit collection	insect herbivores and parasitoids	(morpho)species
Herrera and Dudley (2003)	a, r	riparian forest	<i>Arundo donax</i>	Poaceae	California, USA	India	mid-19th century	pure stands	yes	h	invaded vs uninvaded	pitfalls, sticky traps	whole community	family
Holmquist et al. (2011)	a, r	desert spring	<i>Phoenix dactylifera</i> , <i>Washingtonia filifera</i>	Arecaceae	California, USA	Western-Asia (Gulf region), SW N-America	1912, n/a	17%	-	w	invaded vs uninvaded	suction sampling	whole community	order, genus, species
Houston and Duivenvoorden (2002)	a, r	wetland (pasture)	<i>Hymenachne amplexicaulis</i>	Poaceae	Australia	Central and South America	1980s	93%	no	h	invaded vs uninvaded	sweeping	whole community	family
Ivanov and Keiper (2011)	a, r	forest	<i>Alliaria petiolata</i>	Brassicaceae	Ohio, USA	Eurasia	1868	>30%	yes	w	invaded vs uninvaded	litter extraction	Formicidae	species
Jonas et al. (2002)	a, r	grassland	<i>Bromus inermis</i>	Poaceae	Kansas, USA	Eurasia	1970	57%	no	h	invaded vs uninvaded	pitfall an drop-traps	whole community	order, (morpho)species
Kappes et al. (2007)	a, r	riparian forest, ruderal riverbank	<i>Reynoutria japonica</i> var. <i>japonica</i> and <i>R. sachalinensis</i>	Polygonaceae	Germany	Eastern Asia	19th century	10.9 stems per m ²	yes	h	invaded vs uninvaded	pitfalls	Opliones, Diplopoda and Isopoda	family, species
Lambrinos (2000)	a	coastal shrubland	<i>Cortaderia jubata</i>	Poaceae	California, USA	North-eastern Andes	around 1880	62%	yes	h	invaded vs uninvaded	sticky traps	whole community	order
Lindsay and French	a, r	coastal shrubland	<i>Chrysanthemoides monilifera</i>	Asteraceae	Australia	South Africa	1908	>70%	yes	h	invaded vs uninvaded	litter extraction	whole community	class, family
Litt and Steidl (2010)	a, r	grasslands	<i>Eragrostis lehmanniana</i>	Poaceae	Arizona, USA	South Africa	1930s	density gradient	no	h	invasion gradient	pitfalls	whole community	family, morphospecies
Magoba and Samways (2011)	a, r	fynbos	mostly <i>Acacia mearnsii</i> , <i>A. longifolia</i> , <i>A. saligna</i> , <i>Hakea sericea</i> , <i>H. drupacea</i> , <i>Pinus pinaster</i> , <i>P. radiata</i> , <i>Eucalyptus lehmannii</i> , <i>E. diversicolor</i> , <i>Populus sp.</i>	Fabaceae, Pinaceae, Myrtaceae, Proteaceae, Salicaceae	South Africa	mostly Australia	n/a	>90%	n/a	w	experimental removal	pitfalls	whole community	(morpho)species
Magoba and Samways (2010)	r	riparian forest	mostly <i>Solanum mauritianum</i> , <i>Acacia mearnsii</i> , <i>Pinus patula</i> , <i>Ceasalpinia decapitala</i> , <i>Eucalyptus gomphocephala</i>	Solanaceae, Fabaceae, Pinaceae, Myrtaceae	South Africa	mostly Australia	n/a	>75%	n/a	n/a	invaded vs uninvaded	visual search	Odonata	species
Marshall and Buckley (2009)	a, r	forest	<i>Microstegium vimineum</i>	Poaceae	Tennessee, USA	Southeast Asia, East Asia, South Asia	1919	80%	yes	h	invaded vs uninvaded	suction sampling	whole community	family
Marshall and Storer (2008)	a	coastal dune	<i>Centaurea maculosa</i>	Asteraceae	Michigan, USA	Europe	late 19th century	58%	no	h	invaded vs uninvaded	pitfalls	whole community	family
McGrath and Binkly (2009)	a, r	forest	<i>Microstegium vimineum</i>	Poaceae	Tennessee, USA	Southeast Asia, East Asia, South Asia	1919	dominant	yes	h	invaded vs uninvaded	litter extraction	whole community	order
Nelson and Anderson (1999)	a, r	riparian forest	<i>Tamarix ramosissima</i>	Tamaricaceae	Arizona and California, USA	Europe, Asia	late 19th century	70-80%	yes	w	invaded vs uninvaded	visual search	Lepidoptera	species
Ostoja et al. (2009)	a	grassland	<i>Bromus tectorum</i>	Poaceae	Utah, USA	Eurasia	1861-1928	dominant	no	h	invaded vs uninvaded	pitfalls	Formicidae	species
Osunkoya et al. (2011)	a, r	forest	<i>Macfadyena unguis-cati</i>	Bignoniaceae	Australia	South and Central America	1865	dominant	yes	w	invaded vs uninvaded	pitfalls	Formicidae	species
Palmer et al. (2004)	r	coastal rocky slope	<i>Carpobrotus acinaciformis</i>	Aizoaceae	Mallorca, Spain	South Africa	early 20th century	?	yes	h	invaded vs uninvaded	pitfalls	Coleoptera, Arachnida, Isopoda, Gastropoda	(morpho)species
Pawson et al. (2010)	a, r	grassland	<i>Pinus nigra</i>	Pinaceae	New Zealand	Southern Europe, North Africa	1993	density gradient	yes	w	invasion gradient	pitfalls, flight traps	whole community	(morpho)species
Pearson (2009)	a	grassland	<i>Centaurea maculosa</i>	Asteraceae	Montana, USA	Europe	late 1800s	?	no	h	invaded vs uninvaded	sticky traps	Araneae	order, family
Petillon et al. (2005)	a, r	salt marsh	<i>Elymus athericus</i>	Poaceae	France	Europe	n/a	73%	no	h	invaded vs uninvaded	pitfall, hand collection	Araneae	species
Robertson et al. (2011)	a	savanna	<i>Opuntia stricta</i>	Cactaceae	South Africa	North and Central America, Northern South America	1953	13%	yes	h	invaded vs uninvaded	pitfalls, litter extraction, visual search	Carabidae, Araneae	species
Samways and Sharratt (2010)	a, r	riparian forest	<i>Acacia mearnsii</i> (and <i>A. longifolia</i> , <i>Eucalyptus camaldulensis</i>)	Fabaceae, Myrtaceae	South Africa	Australia	1853, 1827, 1900	?	n/a	w	experimental removal	visual search	Odonata	species
Samways et al. (1996)	r	grassland	<i>Lantana camara</i> , <i>Solanum mauritianum</i> , <i>Acacia longifolia</i> , <i>Acacia mearnsii</i> , <i>Eucalyptus grandis</i> , <i>Pinus patula</i>	Verbenaceae, Solanaceae, Fabaceae, Myrtaceae, Pinaceae	South Africa	mostly Australia	n/a	dominant	n/a	n/a	invaded vs uninvaded	pitfalls	whole community	family, species
Sax (2002)	a, r	forest	<i>Eucalyptus globulus</i>	Myrtaceae	California, USA	Australia	1910	dominant	yes	w	invaded vs uninvaded	litter extraction	whole community	(morpho)species
Schirmel (2011)	a	coastal dune	<i>Campylopus introflexus</i>	Dicranaceae	Germany	South America, South Africa and Australia	1980s	>85%	no	h	invaded vs uninvaded	pitfalls	Orthoptera	species
Schirmel et al. (2011)	a, r	coastal dune	<i>Campylopus introflexus</i>	Dicranaceae	Germany	South America, South Africa and Australia	1980s	>85%	no	h	invaded vs uninvaded	pitfalls	Carabidae, Araneae	species
Simao et al. (2010)	a, r	grassland	<i>Microstegium vimineum</i>	Poaceae	Indiana, USA	Southeast Asia, East Asia, South Asia	1919	690 seeds per m ²	yes	h	experimental introduction	sweeping	whole community	order, family, genus, RTU, species
Spyreas et al. (2010)	a, r	wetland	<i>Phalaris arundinacea</i>	Poaceae	Illinois, USA	Eurasia	1800	>65%	no	h	invaded vs uninvaded	sweeping	whole community	morphospecies
Standish (2004)	a, r	forest	<i>Tradescantia fluminensis</i>	Commelina-ceae	New Zealand	South America	1980	>75%	yes	h	invaded vs uninvaded	pitfalls	whole community	RTU
Ulyshen et al. (2010)	r	forest	<i>Ligustrum sinense</i>	Oleaceae	Georgia, USA	Asia	1852	dominant	no	w	experimental removal	flight intercept traps	Carabidae	morphospecies
Valtonen et al. (2006)	a, r	road verge	<i>Lupinus polyphyllus</i>	Fabaceae	Finland	western North America	1965	69%	no	h	invaded vs uninvaded	visual search	Lepidoptera	species
Vogels (2005)	a, r	coastal dune	<i>Campylopus introflexus</i>	Dicranaceae	Netherlands	South America, South Africa and Australia	1980s	80-94%	no	h	invaded vs uninvaded	pitfalls, emergence traps	Sciaridae, Empididae, Carabidae, Araneae	species
Webb et al. (2000)	r	coastal dune	<i>Ammophila arenaria</i>	Poaceae	Australia	Europe, North Africa	1982	>75%	yes	h	invaded vs uninvaded	pitfalls	whole community	order, morphospecies
Wilkie et al. (2007)	a, r	coastal heath	<i>Chrysanthemoides monilifera</i>	Asteraceae	Australia	South Africa	1908	30%	yes	h	invaded vs uninvaded	pitfalls, beating	Araneae, Heteroptera Coleoptera, Formicidae	species, RTU
Wolkovich et al. (2009)	a	coastal shrubland	Mostly <i>Brachypodium distachyon</i> , <i>Bromus madriensis</i> spp. <i>Rubens</i> and <i>Avena barbata</i>	Poaceae	California, USA	Europe	n/a	>40%	n/a	h	invaded vs uninvaded	pitfalls	whole community	morphospecies
Wu et al. (2009)	a, r	salt marsh	<i>Spartina alterniflora</i>	Poaceae	China	North America	1995	dominant	yes	h	invaded vs uninvaded	sweeping, hand collection	whole community	order, family, species

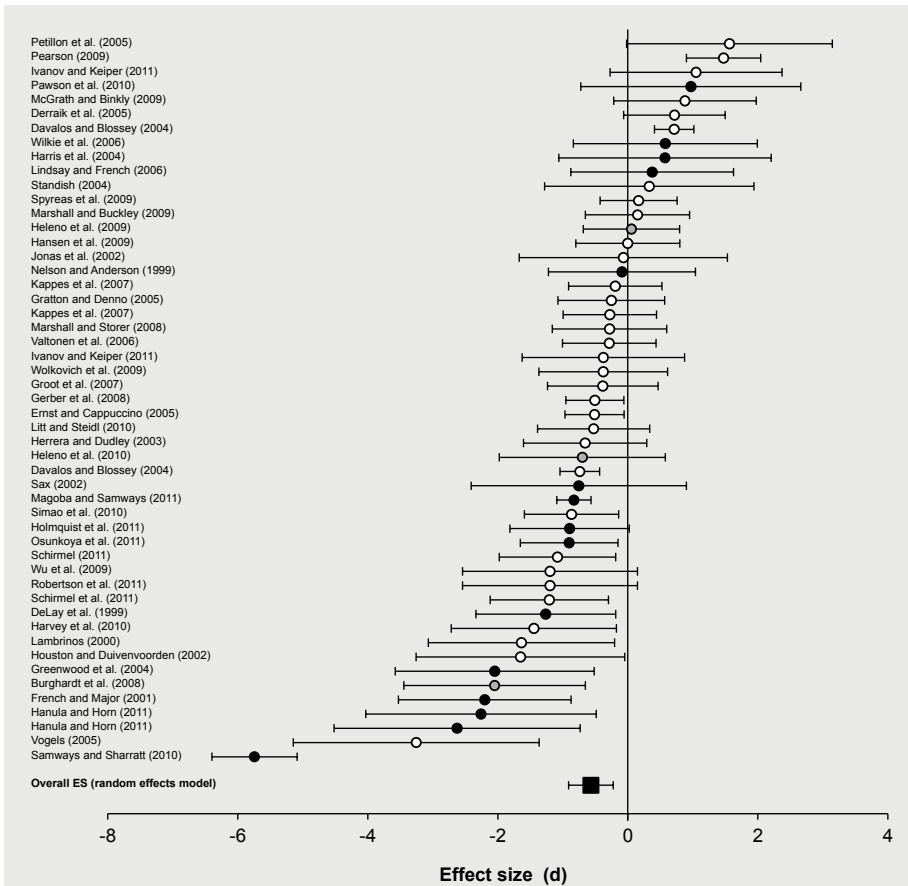
¹ a = abundance data, r = richness data² h = herbaceous, w = woody³ RTU = Recognizable Taxonomic Unit, OTU = Operational Taxonomic Unit



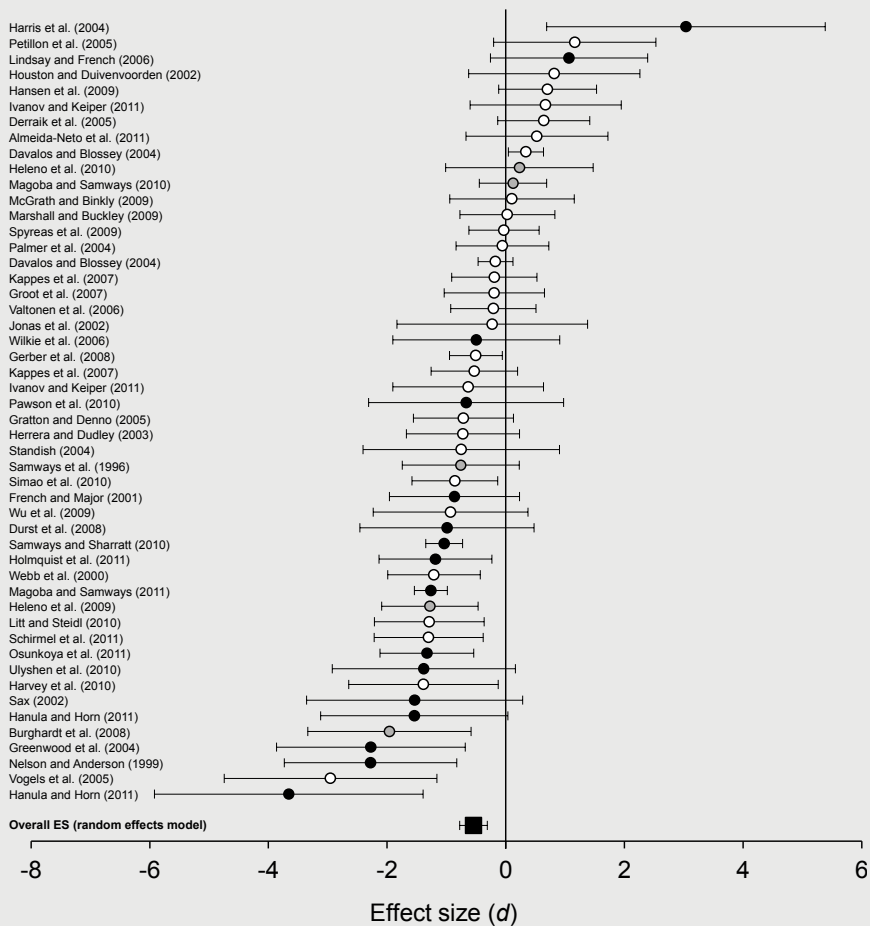
Appendix S3. Normal quantile plots for response variable arthropod (a) abundance, (b) abundance with one outlier excluded, and (c) richness.



Appendix S4. Funnel plots showing the relationship between effect size and sample size for the response variable arthropod (a) abundance and (b) richness. The dashed line indicates the overall effect size. In figure (a) we removed two outliers to facilitate visual interpretation of the data.



Appendix S5. Individual effect sizes (Hedges' d) of differences in arthropod abundance between invaded and uninvaded habitat. The effect size value on the bottom of the graph indicated by a square box denotes the weighted bias-corrected mean effect size based on the random effects model. Black (filled) circles indicate effect sizes of studies with woody invaders, open circles indicate herbaceous invaders, and grey circles indicate mixed or unknown invaders. The bars around the effect sizes denote bias-corrected 95% confidence intervals. One case study, which investigated the effect of a *Bromus tectorum* invasion on ant communities (Ostoja et al. 2008: ES = 15.2, CI = 6.44 - 23.9), was excluded to facilitate visual interpretation of the graph.



Appendix S6. Individual effect sizes (Hedges' d) of differences in arthropod richness between invaded and uninvaded habitat. The effect size value on the bottom of the graph indicated by a square box denotes the weighted bias-corrected mean effect size based on the random effects model. Black (filled) circles indicate effect sizes of studies with woody invaders, open circles indicate herbaceous invaders, and grey circles indicate mixed or unknown invaders. The bars around the effect sizes denote bias-corrected 95% confidence intervals.

Causes and consequences of plant introductions: insights gained from long-term monitoring

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Abstract

Exotic plants are becoming increasingly common across the world and can have drastic effects on native communities and ecosystem functioning. However, much remains unclear about the impact of introduced plants on native plant diversity and the factors determining the success of exotic invaders. Long-term monitoring can serve as a useful tool to fill in some of these knowledge gaps.

In this study we analyse a dataset of more than 10.000 periodically monitored quadrats from the Netherlands to address the following questions:

1. Do exotics occur in less diverse habitats than native species, and if so, how can this be explained?
2. Is the spread of exotic species linked to habitat disturbance?
3. Do exotic species occupy a different niche than native species?

We demonstrate that plots with exotic species have a significantly lower alpha diversity and native species richness compared to plots with only natives. However, this could not be explained by direct competitive effects of exotic species, since a local increase in the number of exotics over time did not decrease native species richness. Exotic species frequently invaded habitats that were low in species richness, providing support for the diversity-resistance hypothesis. We also found that invasive exotics have a broader niche than equally common natives. Finally, we show that exotics are found in habitats that are shadier, warmer, more continental and more nutrient rich compared to those of native species.

Long-term monitoring can provide important insight into the causes and consequences of plant invasions and this will help to guide management decisions.

Introduction

In many parts of the world plant and animal species richness has increased due to the continuous introduction of exotic species that now represent a considerable proportion of many plant and animal communities (Sax and Gaines 2003). When conditions are favourable, exotic species may become invasive and can severely alter communities and ecosystems (Davis 2009, Levine et al. 2003), which may ultimately lead to (local) species extinctions (Pimm and Raven 2000). Although considerable effort has been made to identify the causes and consequences of exotic species invasions, many aspects require further exploration (Mack et al. 2000, Sax and Gaines 2008). Knowledge on the causes and consequences of exotic invasions is essential for effective management and mitigation of ecological impact caused by exotic species.

A first important challenge is to understand why some communities are more susceptible to colonization by exotic species than others (Lonsdale 1999). One of the most prominent hypothesis that attempts to explain the differences in habitat susceptibility to exotic invasions is the diversity-resistance hypothesis (Elton 1958), which is based on the idea that in species-rich habitats limited resources are used more fully, leaving less opportunities for new species to enter the community. On the other hand, species-poor habitat has fewer interspecific interactions and less complete resource use, making it more susceptible to invasions (Stohlgren et al. 1999).

Studies that tested the diversity-resistance hypothesis reported mixed results (e.g. Levine and D'Antonio 1999, Shea and Chesson 2002). In cases where plant communities are experimentally manipulated, negative correlations between native and exotic species richness are usually reported, i.e., support for the diversity-resistance hypothesis (e.g. Kennedy et al. 2002, Naeem et al. 2003). In contrast, studies based on field surveys often report positive correlations (Fridley et al. 2004, e.g. Stohlgren et al. 2003). Part of this difference may be explained by the scale at which the studies are performed; small-scale studies where direct competition for space is expected usually yield negative correlations, while studies performed at larger scales usually yield positive correlations (Brown and Peet 2003, Fridley et al. 2007). The positive relationship at large scales may be driven by spatial heterogeneity in species composition, which in turn is driven by spatial heterogeneity in the environment (Davies et al. 2005).

Although many studies have investigated the role of diversity-resistance, the cause-effect relationships have seldom been confronted with empirical evidence (Levine and D'Antonio 1999). Positive correlations are interpreted as resulting from environmental conditions that favoured both native species and exotic species (Gilbert and Lechowicz 2005, Stohlgren et al. 2003). On the other hand, negative correlations are interpreted as evidence for the diversity-resistance hypothesis, but it is often unclear whether this correlation indeed arose from a lower chance to invade in already diverse habitats, or that colonization by exotics subsequently reduced native species diversity as a result of competition (Richardson and Pyšek 2006). Yet others suggested that the effect of diversity-resistance may be overruled by spatially covarying factors, such as propagule pressure, resource availability and disturbance (Levine 2000, Lonsdale 1999, Meiners et al. 2004, Rejmánek et al. 2005). The latter two factors are part of the resource-enrichment hypothesis (Davis et al. 2000), which states that intermittent resource enrichment (eutrophication) or release (e.g. following disturbance) increases a community's susceptibility to invasions. Long-term monitoring can be used as a tool to test these kind of hypotheses (Blossey 1999).

A second issue that needs further attention are the factors that make exotic species successful outside their native range. Only few exotic species are able to persist outside their native range and an even smaller number will successfully expand their ranges and become invasive (Rejmánek et al. 2005, Sax and Brown 2001). Several hypotheses have been postulated to explain the success of exotic invaders (Hierro et al. 2005, Mack et al. 2000, Sax and Brown 2001), of which the enemy release hypothesis is best supported by empirical evidence (Keane and Crawley 2002; Engelkes et al. 2008). However, for other, related hypotheses, such as the vacant (or empty) niche hypothesis, empirical evidence remains scarce (Herbold and Moyle 1986, Mack et al. 2000). The vacant niche hypothesis predicts that exotics can be successful because they can access resources that are not utilized by native species (Elton 1958, Levine and D'Antonio 1999, Mack et al. 2000). These vacant niches may be present due to the absence of suitable native species, or they may have become available following changes in environmental conditions, for example due to climate change or changes in disturbance frequencies and intensities (Dukes and Mooney 1999).

Here, we will use a 12-year monitoring data set from the Netherlands to assess the consequence of exotic plant invasions for native diversity. Moreover, we will test three hypotheses related to the causes of exotic invasions: (1) The diversity-resistance hypothesis: under this hypothesis high native diversity habitat is less frequently invaded by exotics than low native diversity habitat. (2) The resource-enrichment hypothesis: the cover of bare ground as a proxy for disturbance is positively correlated with the occurrence of exotic species. (3) The vacant niche hypothesis: exotic species have different niche characteristics than natives, which could partly explain their success.

Material and Methods

Data set

To test our hypotheses we used a large data set comprising 30.861 censuses from 10.973 permanent quadrats in the Netherlands spanning a period of 12 years (1999 - 2010). The data set was derived from the Dutch monitoring program 'National Flora Monitoring Scheme for Environmental- and Nature Quality', initiated in 1999. Quadrats were monitored every fourth year following strict protocols. In each quadrat the occurrence of vascular plants and their canopy cover was recorded. Quadrat size was based on the principle of minimum-area (Mueller-Dombois and Ellenberg 1974) and varied between 2 x 2 m for species-rich grasslands to 50 x

50 m for species-poor forests. The distribution of the quadrats followed a stratified design, striving for an optimal representation of all habitat types in the Netherlands. Quadrats were positioned in homogeneous vegetation structure and were located at least 2 km apart to minimize the effect of spatial autocorrelation. The region of Zuid-Limburg was not included in the monitoring network.

Data preparation

Species were classified as either native or exotic using the following definition of an exotic species: any species that is introduced by humans, either by accident or intentional, into an area other than the native range. Species that were introduced before 1500 and have maintained populations ever since, were not considered exotic species (after the definition used by the 'Nederlands Soortenregister', van der Meijden 2005; www.nederlandsesoorten.nl). Using this definition, from the total list of 1213 species present in the dataset, 437 were characterised as exotic. We only included relevés in the analyses that were situated in (semi-)natural habitat, excluding relevés that contained a putative (exotic) crop or plantation species (e.g. *Pinus nigra* and *Zea mays*) with a canopy cover >60%. There were a few cases where a species could not be unambiguously identified and two names were given to a species (e.g. '*Agrostis stolonifera* or *A. gigantea*'). In these cases, we randomly assigned one of the two species, where the probability of a species being assigned was relative to their frequency of occurrence in the whole data set.

Statistical analyses

For each relevé we calculated the following statistics: species richness, native species richness, the cumulative cover of exotic species, the cumulative cover of native species, the percentage of bare ground and the Shannon diversity.

To test for differences in diversity and bare ground cover between habitat of exotic and habitat of native species, we included the most recent relevés ($N = 11.631$). For every species we calculated the mean species richness of all relevés in which the species was found. In the same way, we calculated for each species the mean percentage of bare ground and the mean Shannon diversity. To avoid heteroscedasticity we excluded species that occurred in less than 10 relevés in the most recent census. This threshold of 10 relevés was chosen by testing various thresholds and performing Breusch-Pagan tests for heteroscedasticity *bptest* from the R-package *lmtest* (Zeileis and Hothorn 2002). After application of this threshold, 593 native and 52 exotic species remained. ANCOVAs were performed to test for differences between the habitats of exotic and native species in species richness, alpha diversity, percentage bare ground (log10). The model included the fixed factor 'exotic or native species' (from now on referred to as 'species identity') and the co-variable 'frequency

of occurrence' (i.e. the number of relevés in which the species was found), as well as the interaction between these two factors.

In a second approach we tested for the difference in species richness between plots with (one or more) exotics and plots with only native species. Using only the data from the most recent survey, there were 3914 plots containing both exotic and native species, and 7717 plots with only native species. The difference in species richness between the two habitat types was analysed using two different analyses of variance (ANOVA), both with 'species identity' as fixed factor for the presence/absence of exotics: the first with the number of native species per plot as the response variable, the second using with the total number of species per plot.

The diversity-resistance hypothesis was tested by performing a univariate linear regression between the number of native species that were present at the time of the first vegetation census and the change in exotic species richness between the first and the last vegetation census. To test the relationship between native and exotic species richness, we performed a Pearson correlation including only the most recent relevés ($N = 11.631$). In addition, Pearson correlations were performed between (i) the difference in cumulative cover of native versus exotic species between the first and the last census, and (ii) the change in the number of native (range -42 to +42) versus the change in the number of exotic species (range -4 to +5) between the first and the last census.

In order to test the resource enrichment hypothesis, we used the percentage of bare ground at the time of the first census as a proxy for disturbance and performed a univariate regression to test this factor against the change in the number - and in a second analysis the change in relative cover - of exotic species between the first and the last vegetation census. The relative cover of exotics was calculated by dividing the cumulative cover of exotics by the cumulative cover of all species present in the relevé. The average time between the first and the last census was 7 years.

Niche width for each species was estimated using two methods: beta-diversity and 'the percentage of variance explained' (\log_{10}) by a Canonical Correspondence Analysis (CCA; Ter Braak 1986). Beta diversity was calculated with Shannon's diversity index, using the partitioning method of Jost (2007). The CCA method was calculated by performing the following steps for every species. First, we created a subset of the data by taking the entire dataset and removing the focal species. Second, we constructed a vector that contained for every site in the dataset a zero when the focal species was absent or a one when it was present. We then performed a CCA on the subset of the data, using the presence/absence vector as an explanatory variable. This way, it is possible to calculate for every species the relative impact of

its presence and absence on the variation in the dataset. Therefore, the percentage of explained variance in the CCA can be used as an estimate of niche width. If all sites in which a species occurs are highly similar, the percentage will be relatively low, if the sites in which the species occurs are widely differing, the percentage will be relatively high. The beta diversity and CCAs were calculated in R using the function `h` from the package *vegetarian* (Charney and Record 2012) and the function `cca` from the package *vegan* (Oksanen et al. 2007), respectively. We used these two parameters as response variables in two separate ANCOVAs, in which 'species identity' was included as fixed factor, and the 'frequency of occurrence' (log10 transformed for the ANCOVA with beta-diversity) of a species as co-variable. An interaction term between 'frequency of occurrence' and 'species identity' was included in the model. Finally, for each species we estimated the average habitat characteristics related to light intensity (arcsine), availability of moisture (log10) and nitrogen (arcsine), soil acidity (arcsine), temperature and continentality (arcsine). These habitat values were calculated by averaging the specific Ellenberg indicator values (Ellenberg et al. 1991, Wiertz 1992) of all species, present in relevés in which the species occurred, weighted by their canopy cover. These average Ellenberg variables were then used as response variables in an ANCOVA with fixed factor 'species identity' and co-variable 'frequency of occurrence', as well as their interaction term.

All statistical analyses were performed in the statistical framework R (R Development Core Team 2012). The statistical significance of ANCOVAs and correlations was determined using permutations (100.000 iterations). ANCOVAs were performed using the `avp` function from the R-package *lmPerm* (Wheeler 2010). Correlations were performed using a custom script to do the permutations, using Pearson's correlation coefficient as the test statistic. Univariate linear regressions were performed with the function `lm` (R Development Core Team 2012). Some variables were transformed (if so, the type of transformation is given between brackets) to meet the assumption of a normal error distribution.

Table 1 Analyses of co-variance based on permutation tests (df = 1, 641; number of iterations: 100.000). We tested the difference between habitat of exotic versus native species (i.e. identity) for Alpha diversity based on the Shannon index, native species richness, percentage of bare ground and total canopy cover. The co-variable was the species' frequency of occurrence. P-values were calculated using permutation tests (100.000 iterations).

Response variable		P-value
Alpha diversity (Shannon)	Identity	<0.001
	Frequency	0.0096
	Identity x Frequency	0.086
Native species richness	Identity	<0.001
	Frequency	0.01
	Identity x Frequency	0.08
Bare ground	Identity	0.58
	Frequency	0.28
	Identity x Frequency	0.57

Results

Differences in diversity of habitats of exotic and native species

In terms of plant diversity, exotic species occurred in less diverse habitats than native species: the Shannon alpha diversity of the habitats of exotics was nearly 10% lower than that of native species (based on estimated marginal means; Fig. 1a). Moreover, we found that alpha diversity decreased with increasing 'frequency of occurrence', i.e., more common species occurred in less diverse habitats. This relationship was similar between native and exotic species, as indicated by the absence of a statistically significant interaction (Table 1). Similar results were obtained when plant diversity was measured using native species richness: on average, the habitats of native species contained nearly 5 native species more than the habitats of exotic species (Fig. 1b). Again, more common species occurred in habitats with less native species: the native species richness decreased with an increasing frequency of occurrence. The level of disturbance, estimated by using the percentage of bare ground, was not different between the habitats of exotic species and native species (Fig. 1c).

A similar picture emerged when looking at the plot level: the species richness of the 3914 relevés containing exotics was on average 4% higher than for the 7717 relevés without any exotics (df = 1, 11629, $P < 0.001$). However, when looking only at the number of native species, plots containing exotics had fewer native species than plots without exotics (Fig. 2; df = 1, 11629, $P < 0.001$).

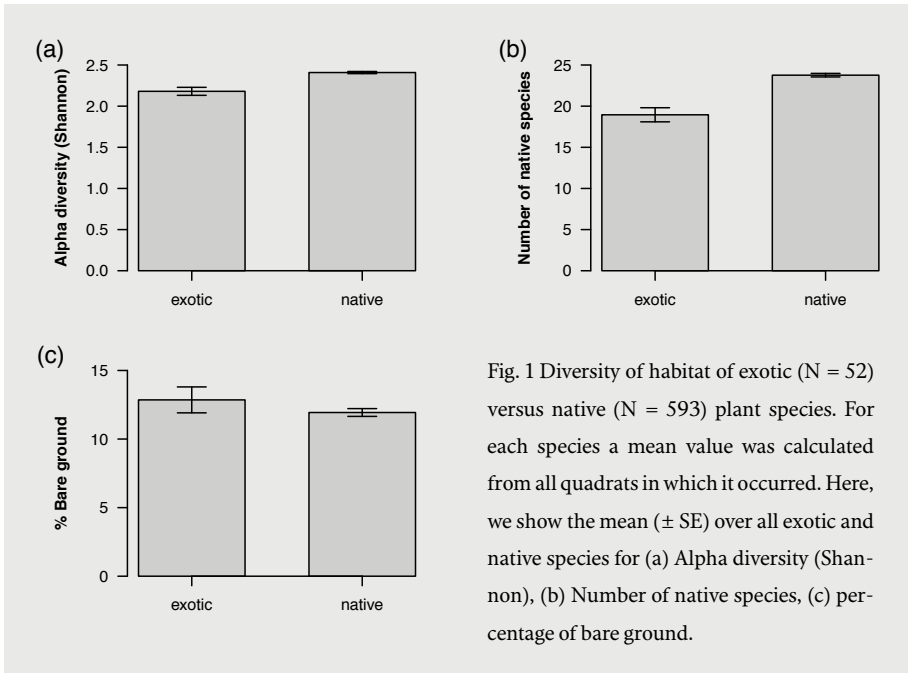


Fig. 1 Diversity of habitat of exotic ($N = 52$) versus native ($N = 593$) plant species. For each species a mean value was calculated from all quadrats in which it occurred. Here, we show the mean (\pm SE) over all exotic and native species for (a) Alpha diversity (Shannon), (b) Number of native species, (c) percentage of bare ground.

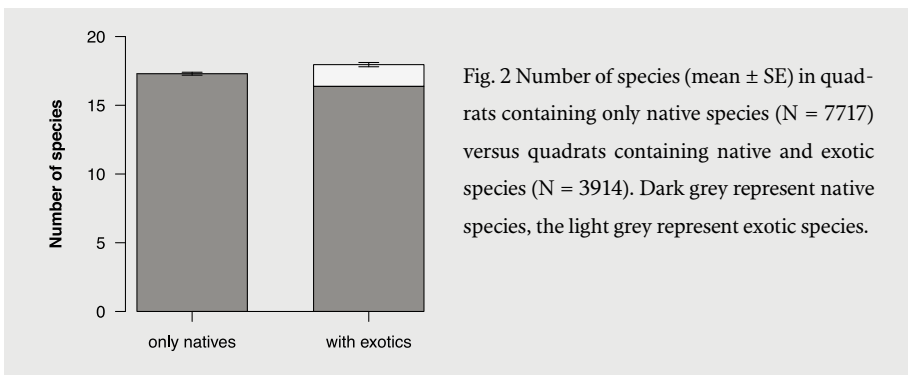
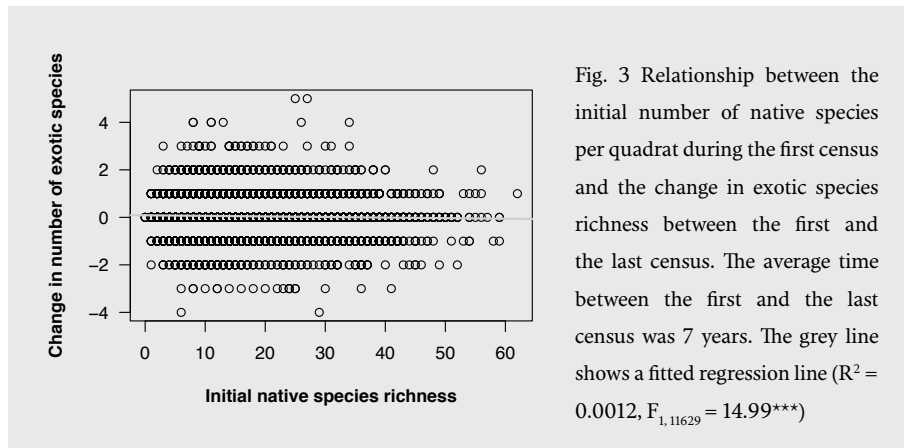


Fig. 2 Number of species (mean \pm SE) in quadrats containing only native species ($N = 7717$) versus quadrats containing native and exotic species ($N = 3914$). Dark grey represent native species, the light grey represent exotic species.

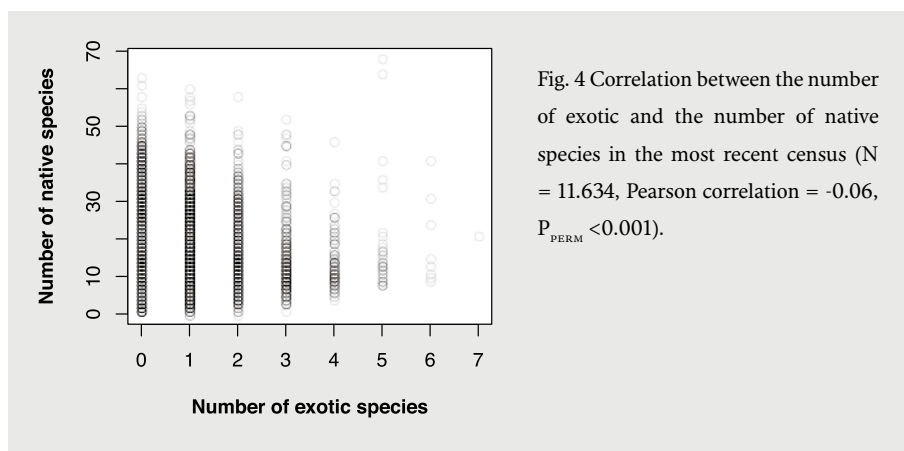
The diversity-resistance and resource enrichment hypothesis

Plots with high native species richness at the start of the monitoring had a lower chance of being invaded by exotics than plots with a low initial species richness (Fig. 3; $F_{1,11629} = 14.99$, $P < 0.001$). However, while this relationship was statistically strongly significant, the effect was small ($R^2 = 0.0012$, slope $b = -0.0024$, intercept $a = 0.1184$). As an example, during an average period of 7 years, plots containing only 5 native species were invaded on average by 0.11 exotic species ($bX + a = -0.0024 \times 5 + 0.1184$), while plots containing 50 native species did not gain (or lose) any exotic species ($-0.0024 \times 50 + 0.1184 = -0.00$). A linear regression on initial total diversity

(exotic + native species) instead of initial native species richness yielded a similar negative relationship ($R^2 = 0.0039$, $F_{1,11629} = 45.49$, $P < 0.001$).



We determined that native species richness was negatively correlated with exotic species richness (Fig. 4; $N = 11631$, Pearson correlation = -0.06 , $P_{\text{PERM}} < 0.001$). At the same time, a change in the number of native species between the first and the last census, was positively correlated with a change in the number of exotic species (Fig. 5a; $N = 4411$, Pearson correlation = 0.215 , $P_{\text{PERM}} < 0.001$), indicating that entry of exotic species was not associated with a reduction in the number of native species. In contrast, a change in cumulative cover of exotics was negatively correlated with a change in cumulative cover of natives (Fig. 5b; $N = 4411$, Pearson correlation = -0.092 , $P_{\text{PERM}} < 0.001$). This indicates that an increase of exotic species' cover reduced native species cover, and vice versa.



Finally, the intensity of disturbance, measured as the percentage of bare ground during the first census, did not predict changes in exotic species richness over time ($F_{1,4409} = 0.02$, $P = 0.90$; Fig. 5c). Also the exotic canopy cover remained unaffected by the percentage of bare ground ($F_{1,4409} = 1.36$, $P = 0.24$; Fig. 5d).

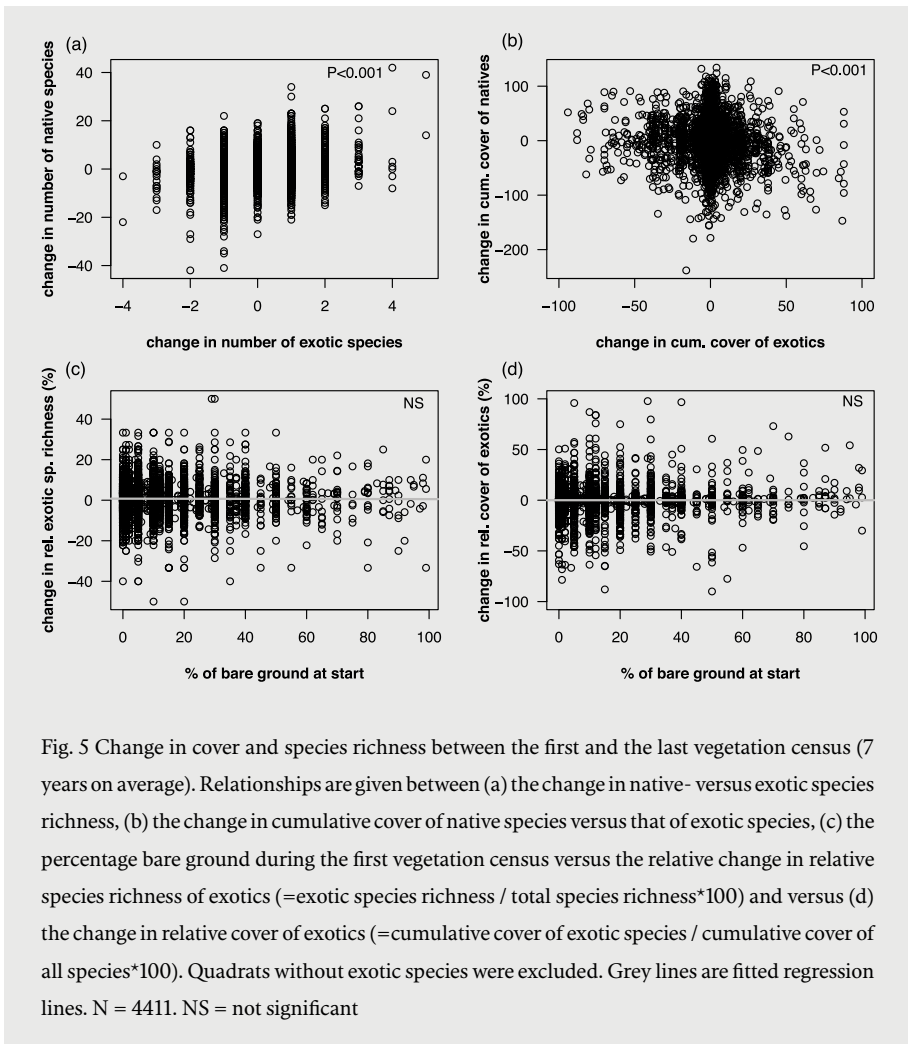


Fig. 5 Change in cover and species richness between the first and the last vegetation census (7 years on average). Relationships are given between (a) the change in native- versus exotic species richness, (b) the change in cumulative cover of native species versus that of exotic species, (c) the percentage bare ground during the first vegetation census versus the relative change in relative species richness of exotics (=exotic species richness / total species richness*100) and versus (d) the change in relative cover of exotics (=cumulative cover of exotic species / cumulative cover of all species*100). Quadrats without exotic species were excluded. Grey lines are fitted regression lines. $N = 4411$. NS = not significant

The vacant niche hypothesis

The niche width, expressed in the percentage of variance explained by the CCA, did not differ between native and exotic species (Fig. 6a; Table 2). Niche width increased as a function of frequency of occurrence both exotic and native species, and this increase was stronger for exotics than natives. Thus, common exotic species have

a broader niche, as inferred from the CCA, than common native species. Similarly, niche width based on beta diversity (Shannon) did not differ between natives and exotics, and increased for both with frequency of occurrence. For this estimator of niche width we did not find that common exotics had a broader niche than common natives (Fig. 6b; Table 2).

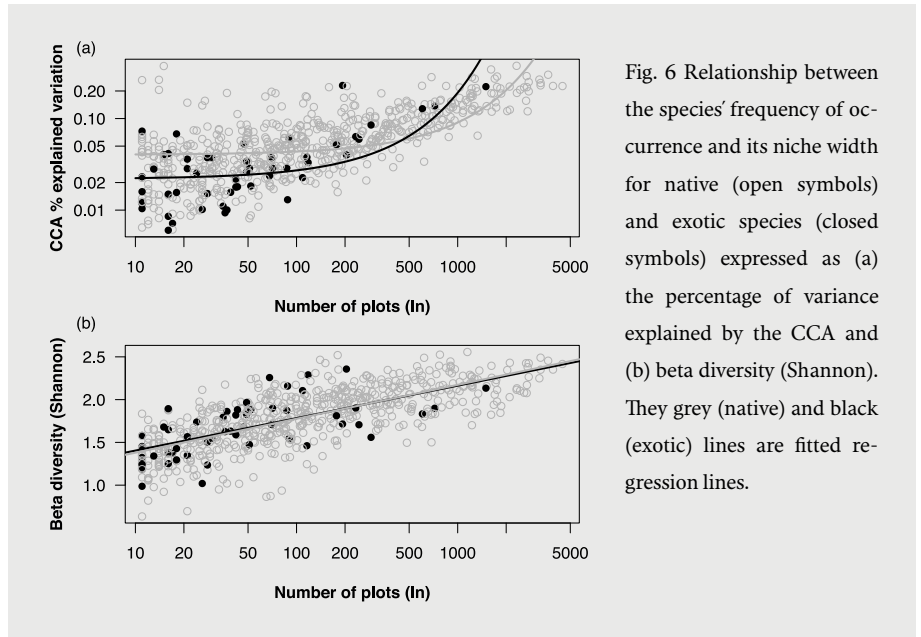


Fig. 6 Relationship between the species' frequency of occurrence and its niche width for native (open symbols) and exotic species (closed symbols) expressed as (a) the percentage of variance explained by the CCA and (b) beta diversity (Shannon). They grey (native) and black (exotic) lines are fitted regression lines.

Table 2 Analyses of co-variance based on permutation tests (df = 1,641; number of iterations: 100.000) for the difference in niche width between exotic and native species (i.e. identity) estimated by beta diversity (Shannon) and the percentage of variance explained in the CCA analysis. The frequency of occurrence is included as co-variable and interaction with species identity (exotic versus native). P-values were calculated using permutation tests (100.000 iterations).

Response variable		P-value
Beta diversity (Shannon)	Identity	0.86
	Frequency	<0.001
	Identity x Frequency	0.81
% var. CCA	Identity	0.12
	Frequency	<0.001
	Identity x Frequency	<0.001

We found that exotic and native species had different habitat preferences based on weighted indicator values that were calculated for each relevé. Compared to native species, exotic species occurred in shadier, warmer, more continental and more nitrogen-rich habitats (Fig. 7). Moisture and acidity values were not different between exotics and natives (Fig. 7; Table 3). Common exotic species were associated with higher temperatures, less common exotics with cooler temperatures, while for the natives this pattern was reversed. Furthermore, common exotic and native species were associated with a more nitrogen-rich environment than less common species and this pattern was stronger for exotic species (Table 3).

Table 3 Analyses of co-variance based on permutation tests (number of iterations: 100.000) for the difference between exotic and native species (i.e. identity) for Ellenberg values: light, temperature, continentality, moisture, pH and nitrogen. The frequency of occurrence of the species was included in the model as co-variable and as interaction with species identity. P-values were calculated using permutation tests (100.000 iterations).

Response variable		df	P-value
Light	Identity	1, 639	<0.001
	Frequency	1, 639	0.17
	Identity x Frequency	1, 639	0.29
Temperature	Identity	1, 608	<0.001
	Frequency	1, 608	0.34
	Identity x Frequency	1, 608	0.04
Continentality	Identity	1, 620	0.001
	Frequency	1, 620	0.12
	Identity x Frequency	1, 620	0.12
Moisture	Identity	1, 624	0.24
	Frequency	1, 624	0.84
	Identity x Frequency	1, 624	0.57
pH	Identity	1, 627	0.81
	Frequency	1, 627	0.09
	Identity x Frequency	1, 627	0.06
Nitrogen	Identity	1, 613	0.007
	Frequency	1, 613	0.03
	Identity x Frequency	1, 613	0.04

Discussion

Exotic plants occur in less diverse habitat than natives

Exotic plants occurred in habitat that was less diverse in terms of native species richness and Shannon diversity. One explanation for this observation could be that native species richness declined following exotic invasion, as was found in many other studies (Gaertner et al. 2009, Hejda et al. 2009). However, we did not find indications for this, since there was a positive correlation between the change in native and exotic species richness, which indicates that colonization by exotic species did not subsequently reduce native diversity. Moreover, it illustrates that environmental mechanisms, such as succession, equally affect native and exotic species richness (Levine and D'Antonio 1999). Our observations are in agreement with the findings by Daehler (2003), who showed that, based on 79 independent native-invasive plant comparisons, exotic invaders were not different from natives in terms of growth rates, competitive ability, or fecundity. At the same time, several meta-analyses on community effects of invasions reported significant reductions on native diversity (Gaertner et al. 2009, Hejda et al. 2009, Vilà et al. 2011). The reason of these contradicting results remain unclear, but it seems that local co-varying factors, including propagule pressure, resource availability and disturbance (Levine 2000, Meiners et al. 2004, Rejmánek et al. 2005), play an important role in defining the end result of exotic invasions (Daehler 2003, Rejmánek et al. 2005, Richardson and Pyšek 2006).

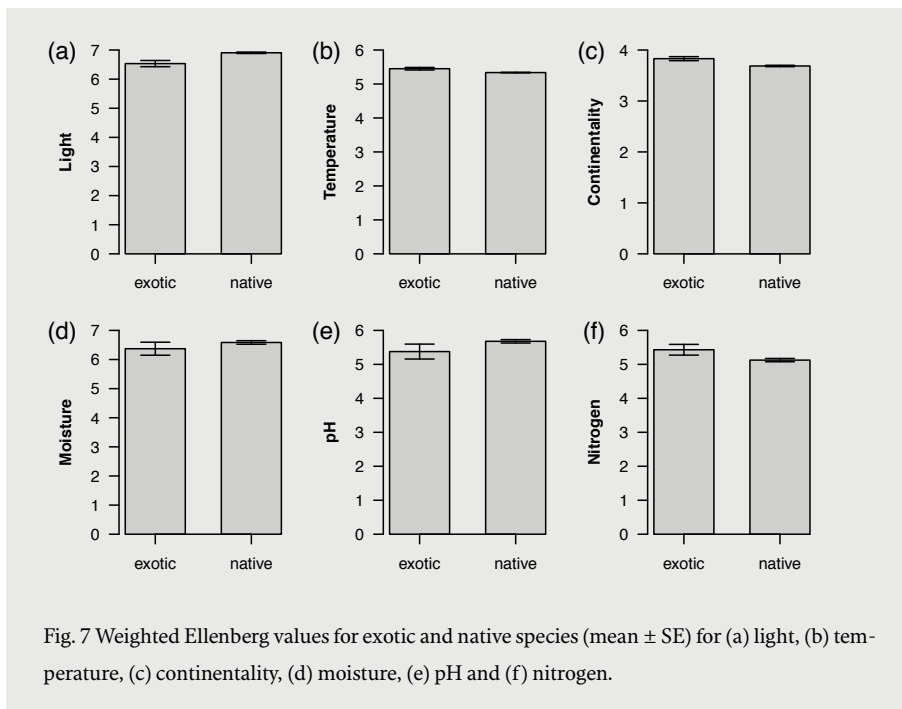


Fig. 7 Weighted Ellenberg values for exotic and native species (mean \pm SE) for (a) light, (b) temperature, (c) continentality, (d) moisture, (e) pH and (f) nitrogen.

Support for the diversity-resistance hypothesis

A second explanation for the observation that exotic species occur in less diverse habitats is based on the theory of diversity-resistance (Elton 1958). Weak support for the diversity-resistance hypothesis was provided by the negative relationship between the initial native species richness and the change in exotic species richness. Although the observed effect was very small, over longer time spans it may still be ecologically relevant. Our small- to medium scale observational study provides one of the few examples reporting a negative relationship between native and exotic species richness. Moreover, in contrast to experimental studies, the data covers a wide variety of habitat types, giving us more confidence to make generalisations.

We repeated the analysis with initial total species richness instead of initial native species richness and found a stronger, though still very small, effect of diversity-resistance. This could be due to the fact that exotic species that are already present in a community also contribute to diversity resistance. Therefore, rather than using the relationship between exotic and native species richness as evidential support for the diversity-resistance hypothesis (Davies et al. 2005, Fridley et al. 2007), we argue that it would be more correct to use the relationship between exotic and total (native and exotic) species richness instead.

While there was a positive relationship between native and exotic species richness, we found a negative relationship between the change in native and exotic canopy cover. This suggests that an increase in cover of exotic species reduces native cover, and vice versa. Apparently, factors that favour the expansion of cover of exotic species are different from the factors that favour the expansion of cover of native species. For instance, exotic invaders may show stronger responses to factors like climatic variability or nutrient enrichment than natives (Hobbs et al. 2007, Huenneke et al. 1990).

No support for the resource enrichment hypothesis

Increased availability of resources following disturbance, estimated by the bare ground cover, did not affect exotic species richness or cover. This deviates from predictions of the resource enrichment hypothesis (Davis et al. 2000), and from previous studies. For instance, Chytry et al. (2008), who also estimated the level of disturbance with bare ground cover, found that the proportion of exotics was positively related to the availability of bare ground. Similarly, Stohlgren et al. (2006) reported a positive relationship between variability in native cover and the expansion of exotic plant cover. We suggest two explanations for the absence of such an effect in this study: First, exotic and native species may have responded similarly to disturbance (Alpert et al. 2000, Hobbs and Huenneke 1992). Second, the availability of bare ground cover that we used may be not a suitable estimator for disturbance, when the relevant type of disturbance does not result in the increase

of bare ground cover. For instance, disturbance in the form of mowing or nutrient fluctuations may leave the canopy cover unaffected. Additional studies are needed that include other proxies for disturbance, such as variation in mowing frequency, grazing intensity and nutrient dynamics.

Invasive exotics have a wider niche than equally common natives

Niche width is often mentioned as an important predictor for invasion success, where species with wide niches are more likely to invade than species with narrower niches (Rejmanek 2000, Vazquez 2006). Reduced resource allocation to plant defence mechanisms, as predicted by the enemy release hypothesis (Keane and Crawley 2002), as well as high phenotypic plasticity are important factors that may explain wide niches of invasive species (Geng et al. 2007; Woods et al. 2009). Our analysis partly supported this theory; one of the two estimators of niche width that we used (the percentage of variance explained by the CCA) indeed showed a wider niche for common exotics than equally common natives, but the second method based on beta-diversity did not show any effect. The reason for this discrepancy is unclear and needs further exploration, but the two estimators may reflect different aspects of niche width.

Exotics have different niche than native species

For plant invaders, having a different niche is often equated to being successful (MacDougall et al. 2009). Our results indeed suggest that exotic species occur in habitats that differ from those of the natives: exotic species occur in habitat that is shadier, warmer, more continental and richer in nitrogen. These results are in accordance with the predictions based on the vacant niche hypothesis, i.e. that exotic species occupy niches that are unoccupied by natives. However, given the large anthropogenic impact on the landscape in the Netherlands, we think that invasive species could also be better in exploiting new niches created by human activities (MacDougall and Turkington 2005, Shea and Chesson 2002). For example, nitrogen deposition in terrestrial and aquatic ecosystems in The Netherlands are amongst the highest in the world (van Breemen and van Dijk 1988), providing opportunities for exotic plants that are able to exploit this enhanced resource more efficiently than native species. Furthermore, the temperature in the Netherlands has risen, on average, by 1.2°C since 1900 (KNMI, <http://www.knmi.nl>). This increase may have facilitated the colonization of thermophilic exotic species that were previously constrained by temperature. Currently, most exotic species in the Netherlands originate from Europe, of which the majority originates from southern Europe (<http://www.compendiumvoordeleefomgeving.nl>). Finally, since 1900, land

use has changed considerably and the land surface occupied by forest has increased by almost 37% between 1900 and 2008 (CBS Statline, <http://statline.cbs.nl>). This may have created new opportunities for shade-tolerant woody invaders, such as *Prunus serotina*, *Quercus rubra* and *Amelanchier lamarcki*, which are currently the three most common exotics in the Netherlands.

Recommendations and constraints

Long-term monitoring proves to be a powerful tool to increase our understanding on exotic invasions and their impact on local communities. At the same time, there are some limitations that should be taken into account. For instance, the current study only covered an average period of 7 years between repeated observations, which may be too short to detect delayed ecological effects (Crooks 2005, Strayer et al. 2006). The most common exotics in the Netherlands are woody and their effects are difficult to detect because of their long life-span compared to herbaceous plants. We therefore encourage the continuation of detailed monitoring efforts such as the one presented here, but at the same time we emphasize the necessity of experimental studies in order to gain more insight into the mechanistic processes that explain ecological impact of plant invasions.

Conclusions

Despite the abundant presence of exotic species they did not reduce native plant diversity during our study period. Rather, we found support for the diversity-resistance hypothesis, suggesting that species-rich habitat may function as a barrier against exotic invasion. Yet, the magnitude of this effect was small and its biological relevance remains unclear. The intensity of disturbance was not predictive for the frequency of exotic invasions; thus providing no support for the resource enrichment hypothesis. Finally, the vacant niche hypothesis was supported by the finding that exotic species occupy different niche-space in terms of nutrient- and light availability, continentality and temperature. Moreover, invasive exotics had a wider niche than equally frequent native.

Although in the current study we did not identify direct negative impact of exotic invasions on native plant diversity, the continuation of long-term monitoring efforts is essential to detect potential future effects of exotic invasions and to plan management strategies. ●

General discussion

This general discussion is centred around the two major aims of this thesis. The first aim was to identify ecological effects of plant invasions by focusing on herbivory, invertebrate communities, pollination and native plant diversity. The second aim was to provide knowledge for the improvement of environmental risk assessment systems. For this purpose we will propose and discuss three tools that could be used to predict ecological effects of introduced species.

Ecological effects of plant invasions

Are plant invaders good or bad neighbours?

Invasive plants can be both good and bad neighbours. In some situations, natives may benefit from their neighbours, for example in terms of increased pollination success (Ghazoul 2006, Molina-Montenegro et al. 2008), resource availability (Vitousek et al. 1987), productivity (Ehrenfeld 2003), or habitat heterogeneity (Crooks 2002). However, the majority of the published literature on the ecological effects of plant invasions report negative impacts on recipient environments (Levine et al. 2003, Levine and Rees 2004). For example, plant invasions have been shown to reduce the diversity of native plant and animal communities (Vilà et al. 2011). In addition, negative effects on pollination and herbivore intensity of native plants have been identified (Meiners 2007, Morales and Traveset 2009). In this thesis I focused on the effect of plant invasions on herbivory, invertebrate communities, pollination and native plant diversity in the Netherlands. Each of these aspects will be discussed in the next section, starting with the effect on pollination success.

Pollinator sharing

Whether and to what extent invaders will interact for pollinators with neighbouring plants will mostly depend on the overlap in their pollinator communities (Bjerknes et al. 2007). In Chapter 2 we demonstrated that two out of five native focal species studied (*Linaria vulgaris* and *Trifolium pratense*) shared almost no pollinators with the invader (*Brassica rapa*) and as a result, their pollination success remained unaffected by the presence of the invader. The three remaining native focal species (*Daucus carota*, *Leucanthemum vulgare*, and *Diplotaxis tenuifolia*) shared a considerable number of pollinators with the invader, but the effect on their pollination success varied considerably: *L. vulgare* received more visits, *D. tenuifolia* received less visits and *D. carota* visitation was unaffected. Several possible mechanisms have been proposed to explain these responses. First, relative flower attractiveness and reward (i.e. the attractiveness and rewards of flowers from invasive compared to native species) might determine whether competitive or facilitative effects will

occur (Brown et al. 2002, Chittka and Schurkens 2001, Brown and Mitchell 2001, Brown et al. 2002, Chittka and Schurkens 2001). For example, at the landscape level invaders may draw a large number of pollinators to patches containing both invaders and co-occurring native species. If flowers of invaders are more attractive than the native ones, native plants may not profit from the increased pollinator presence. To my knowledge no studies have explicitly tested the effect of relative flower attractiveness on pollination success of natives in relation to invasions.

The size and density of the invader's population is a second factor that may be involved in the pollination success of neighbouring natives. Larger and denser populations may attract larger pollinator populations and may locally increase visitation rates (Bjerknes et al. 2007, Hegland and Totland 2005, Mustajärvi et al. 2001). Nevertheless, evidence for this theory in relation to plant invasions is scarce and therefore requires additional studies in which different invader densities and population sizes are used (Muñoz and Cavieres 2008).

Is reproductive success affected?

Despite the fact that the visitation frequency for two out of five focal species was affected by the invader, none of them experienced reduced or increased seed set (Chapter 2), suggesting that the species were not pollinator limited. Besides reduced visitation frequency and resource availability, heterospecific pollen deposition can have major negative effects on the plant's reproductive success (Da Silva and Sargent 2011, Morales and Traveset 2009). In Chapter 2 of this thesis the pollen composition on stamens of *D. tenuifolia* were analysed in detail, with the goal to explain potential effects on the species' reproductive success. We observed that individuals that were placed next to the taxonomically related invader (*B. rapa*) contained significantly more pollen from the invader than the plants in control plots. This may be due to the fact that taxonomically related species deposit their pollen on the same body parts of their shared pollinators and therefore increase the total abundance of heterospecific pollen, as suggested by Bjerknes et al. (2007). Morales and Traveset (2009) found that the reproductive success of native species was more negatively affected when the native and invader were taxonomically related and suggest that heterospecific pollen deposition may be responsible for this outcome. However, our results do not support this theory, since the reproductive success of *D. tenuifolia* remained unaffected despite the fact that about one third of the pollen load originated from the closely related invader.

Consequences for invertebrate communities

From our meta-analysis (Chapter 4) we concluded that the abundance and richness of arthropod communities is generally reduced following plant invasion. This result

was congruent with those of our own two-year experiment, in which we found that invertebrates that were sampled in plots containing *Brassica napus* and *Lactuca serriola* were generally less abundant and had lower taxonomic richness compared to control plots (Chapter 3). In contrast, invertebrates in the surroundings of invasion plots were often more abundant and had a higher taxonomic richness, illustrating that invasions can (at least locally) be advantageous for invertebrate species.

The exact drivers for these differences in invertebrate communities are not entirely clear, but we proposed several possible mechanisms. In our meta-analysis we found that woody invaders caused a stronger reduction in invertebrate abundance and richness than herbaceous invaders. Among other factors, this may be explained by differences in palatability or habitat complexity (Haukioja and Koricheva 2000, Tews et al. 2004). Furthermore, the diversity of invaded plant communities is generally lower than in uninvaded communities (Mason et al. 2009, Vilà et al. 2011). Since plant and invertebrate are positively related (Crisp et al. 1998, Haddad et al. 2001, Siemann et al. 1998), a reduction in invertebrate diversity may be expected following plant invasion. The fact that we often found higher abundance and richness of invertebrates in the surroundings of invasion plots may have been caused by (a combination of) spillover from within the invasions plots and by positive edge effects: the invader may provide complementary resources to the system in the form of food or refuge (Blitzer et al. 2012, Ries et al. 2004). Finally, when resources are locally concentrated (resource concentration), herbivores, especially specialists can become more abundant, which may explain the increased abundance of some specialist herbivores (e.g. *Ceutorhynchus obstrictus*; Root 1973).

Consequences for herbivore pressure of natives

One of our goals was to find out whether changes in the (functional) composition of invertebrate communities would subsequently affect herbivore pressure to native co-occurring species. We expected that apparent competition would enhance herbivore pressure to neighbouring native plants that share herbivores with the invader. Despite the fact that herbivore abundance was often higher around invasion plots (Chapter 3), we did not find any effect on herbivory in two co-occurring native species. We have no direct explanation for this lack of effect, but it seems likely that the herbivores that increased in abundance due to the presence of the invader were not shared between the two target species. A better approach would be to monitor herbivore pressure of a larger set of co-occurring native plants.

In Chapter 2, we demonstrated that co-occurring natives may also be facilitated by the presence of the invader. Our target species *Diplotaxis tenuifolia* experienced significantly less damage to their fruits when placed along a population of *Brassica*

rapa than placed in nearby control habitat. There may be several, still speculative, explanations for this result that remain to be tested: (1) *Diplotaxis* plants were visually camouflaged by *Brassica*, (2) *Brassica* was preferred over *Diplotaxis*, (3) herbivores became saturated by the large amount of resources, or (4) volatiles from *Brassica* triggered defence responses in *Diplotaxis* (Barbosa et al. 2009, Gotthard et al. 2004).

Is native plant diversity affected by exotic invasions?

In Chapter 5 of this thesis we investigated the effect of exotic species on native plant diversity in the Netherlands using an extensive monitoring database spanning a period of 11 years. With this data we demonstrated that invasion of exotic species did not reduce native species richness and diversity, which is in contrast with previous findings (Vilà et al. 2011). However, at the same time, exotic plants occurred in habitat that was less diverse in terms of native species richness. This may be explained by the observation that exotic species more frequently invaded habitats that were low in species richness, providing support for the diversity-resistance hypothesis (Stohlgren et al. 1999). We also found that invasive exotics have a broader niche than equally common natives. Finally, we show that exotics are found in habitats that are shadier, warmer, more continental and more nutrient rich compared to native species. These niche differences may partly explain the success of exotic species in the Netherlands.

The results from the experimental studies performed in this thesis have yielded some important methodological and ecological insights on when a recipient ecosystem is likely to be affected by an invasive species. We now discuss how such ecological knowledge could potentially be used to improve the accuracy and performance of environmental risk assessment (ERA).

Environmental risk assessment

Environmental risk assessment is an important instrument to manage the risk of biological invasions and their potential economic and environmental impact (Gordon et al. 2008, Levine et al. 2003, Pimentel et al. 2005). Two important classes of ERA systems are operational: ERA systems for evaluation of genetically modified crops (EFSA 2010a, Raybould 2007) and risk assessment systems for 'conventional' species, i.e., non-GMO crops and ornamental species (e.g. Pheloung et al. 1999). Both systems have the objective to quantify the risk level of an introduction by predicting the potential environmental effects, be it of plants carrying a transgene

(either feral plants, or crop-wild hybrids), or an exotic plant species. Although the two systems use different terminology and methodology, in essence they are based on the same principles: risk estimation based on the consequences of *hazard* and the likelihood of *exposure*. Hazard is defined as the potential of an organism to cause harm to or adverse effects on human health and/or the environment, while exposure is defined as the likelihood of an adverse effect to occur (EFSA 2010a). The combination of the two factors will define the risk level. Whether a certain level of risk is acceptable cannot be determined purely scientifically, but will depend on political, economic and social criteria, and therefore goes beyond the scope of the ERA, and this thesis. ERA considers different environmental aspects, for example, whether evaluated species are interfertile with wild relatives, whether they have the potential to become agricultural pests or whether there are effects on human health. In the next paragraphs we focus on effects for the recipient ecosystem. More specifically, we will consider potential effects on pollination success, composition of invertebrate communities and, as a result from a side project (Van Hengstum, unpublished), the effect of toxins from the introduced plant on plant consumers.

Decision support

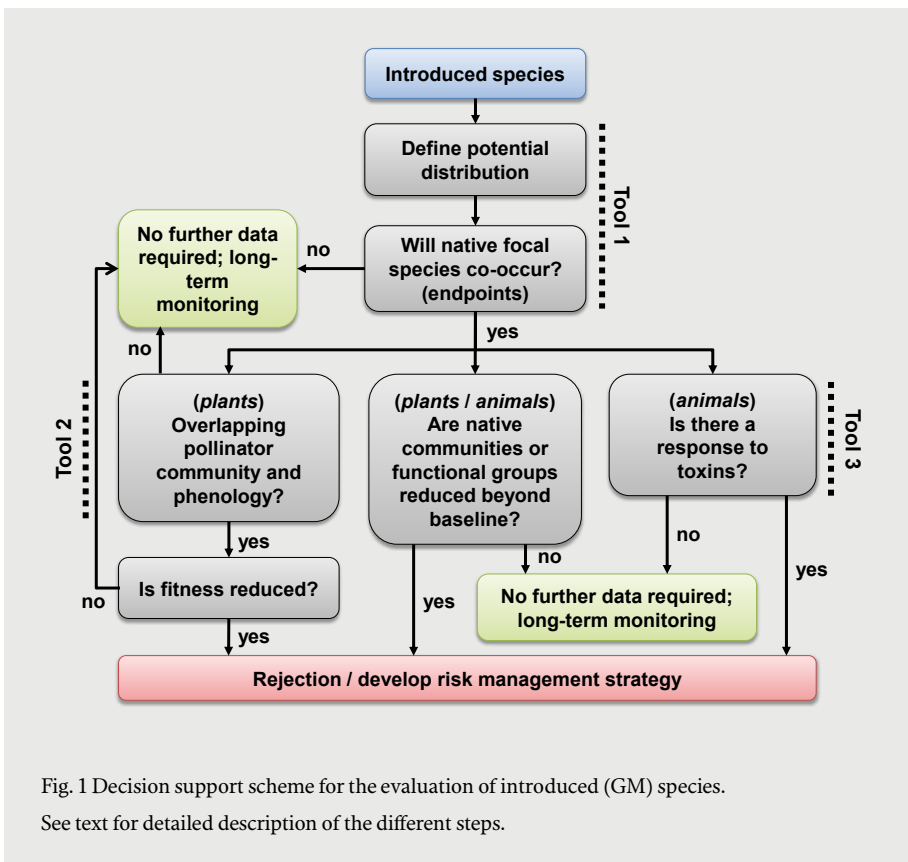
An essential step in the process of risk assessment is the identification of protection goals (or management objectives), i.e. environmental aspects that should be protected from potential adverse effects (Romeis et al. 2008, Wolt et al. 2010, EFSA 2010a). For example, a protection goal can be the conservation of biodiversity or ecosystem functions, such as pollination and decomposition. Protection goals may also be related to the protection of natural resources, including natural enemies that can function as pest control agents (EFSA 2010b). The next step is to define measurable assessment endpoints for each of the protection goals (EFSA 2010a, Wolt et al. 2010). For example, if your protection goal is to protect biodiversity the assessment endpoint would be species abundance and taxonomic richness (Table 1).

Currently, hazard and the likelihood of exposure are estimated by means of

Table 1 Examples of protection goals and possible endpoints and test systems (after EFSA 2010a)

Protection goal	Endpoint	Test system (or focal species)
Plant and animal diversity	Plant/animal species richness	Some representative species from each functional group
	Abundance of rare, endangered or protected species	A selection of rare or endangered species
Ecosystem functions	Pollination success	A selection of co-flowering plants
	Turnover of soil organic matter	Litter decomposition analysis
Natural resources	Abundance of natural enemies for pests	A selection of beneficial species

lab- and/or field experiments (Romeis et al. 2008, EFSA 2010a), which is a time consuming and costly procedure (see also this thesis). In the next sections of the discussion I will introduce a decision support scheme (Figure 1) that can help risk assessors to decide whether a potential introduction (an ornamental plant, or a GM crop with a particular transgene) is likely to affect the recipient ecosystem. At different steps in the decision support scheme I will introduce tools that could make risk assessment procedures more efficient and cost effective. The scheme is meant as a general aid both for the evaluation of GM and non-GM taxa, in addition to currently operating risk assessment systems.



The first step in this scheme is to determine the potential distribution of the introduced species. This can be achieved by means of niche modelling using Tool 1 (see below for a detailed description of the tools). Depending on the habitat requirements of the introduced species, this step will rule out a number of native species

that are unlikely to co-occur. From the remaining pool of species a selection of 'focal species' should be made, which can be based on criteria such as the species' vulnerability, rareness or conservation status (EFSA 2010, Table 1). These focal species will enter the next step of the decision support scheme where we will determine whether they are in any way affected in terms of (i) pollination/reproductive success of native plants (using Tool 2, see below), (ii) abundance and richness of co-occurring plant and animal communities and (iii) toxic response to chemical compounds (using Tool 3, see below).

If any of these factors is affected beyond a-priori established baselines, the introduction under evaluation will potentially affect the recipient ecosystem, which may lead to denial of permission, or restricted admission with management strategies to reduce the risk to an acceptable level, for instance by applying containment measures (EFSA 2010a). If no detrimental effects for native focal species are identified, no further data is required on these aspects of the ERA. However, long-term monitoring of flora and fauna is desirable in order to detect any delayed or unforeseen environmental effects (Chapter 5).

Tool 1: Niche modelling

Niche models (or species distribution models) are numerical tools that predict species' potential distributions based on linking known occurrence records to digital layers of environmental variables. They have been used for a wide range of applications, including the identification of biodiversity hotspots (e.g. Raes et al. 2009), effects of climate change (e.g. Engler et al. 2011) and to assess the invasive potential of species into new habitat (e.g. Thuiller et al. 2005). This latter purpose can also be applied in the context of risk assessment, because it can forecast whether the introduced species will co-occur with a native focal species of interest. In addition, niche modelling could be used to predict the range expansion of GMOs that have acquired increased abiotic tolerance, for instance for drought, soil salinity or frost (Bhatnagar-Mathur et al. 2008). By tweaking the fitted niche model parameters of these species one can artificially mimic the increase of their fundamental niche. The effect that the introduced gene has on the potential distribution of its carrier can then be predicted, so that habitats/species are identified that will be exposed to the GMO.

While niche modelling has proven to be a powerful tool in ecology, some major challenges remain to be addressed, especially when forecasting the distribution of (potentially) invasive species (Guisan and Thuiller 2005). Most importantly, niche models have the fundamental assumption that the current range of species is in equilibrium with its associated environment (Zimmermann et al. 2010). Especially for invasive species this can be an unrealistic assumption, because the very nature

of invasive species is that they have not reached their full realized niche yet. In consequence, the niche model may fail to capture the species full environmental potential (e.g. due to enemy release) leading to an underestimation of the potential niche. Another challenge for using niche models for risk assessment of (GM) plants is the lack of natural occurrence data. A possible, but elaborate approach would be to determine the species fundamental niche by means of (physiological) experiments returning the species abiotic habitat requirements, including the soil acidity, moisture and mineral content (Wright et al. 2006). Recent efforts to combine classical niche models with such mechanistic models have generated promising results (Kearney and Porter 2009). Finally, there are some general limitations of niche models. For example, to date most niche models do not incorporate dispersal limitations, local adaptation, meta-population dynamics and most importantly, biotic interactions (Araujo and Guisan 2006, Zimmermann et al. 2010). This latter aspect is especially important with respect to modelling invasive species, because invaders are likely to alter biotic interactions as they invade novel habitat (Keane and Crawley 2002, Morales and Traveset 2009).

Despite these limitations there seem to be few alternatives to predict what the potential spread will be after an introduction, and without such information the ecological impact will be hard to assess.

Tool 2: Assessing effects on pollinator communities

In Chapter 2 we demonstrated that invaders and co-flowering natives only interacted in pollination visitation if they had overlapping phenology and at least some degree of pollinator sharing. If one of these conditions is not met, the risk of exposure will be minimal and thus no direct adverse effects on pollination success are expected. This tool does not consider potential effects of invaders on the size and composition of pollinator communities on the landscape level, which could potentially also affect native species with non-overlapping phenology (e.g. Diekötter et al. 2010). In addition, the tool can also detect whether (locally) new pollinator taxa will possibly be introduced together with the invader.

To reduce the amount of resources that is currently required to experimentally determine whether pollinator communities (and phenology) of two species overlap, a pollination database is needed containing information regarding phenology and associated pollinator communities of native focal plant species that occur within a biogeographic region of interest. The main advantage of this approach is that entire native plant communities can be screened relatively easily and that much of this process can be automated once the system is in place. Filling this database with expert knowledge and observations will initially require a significant effort, especially for complex pollinator communities, but on the long-term this method

can save a lot of resources. The database can also be used for other purposes, for example, to determine the optimal composition of herbs with the goal to reach maximum pollinator diversity or optimal pollination success for crops. With the database in place, a risk assessor can infer the phenology and associated pollinator community of the species that is being evaluated. The next step would be a query of the database that returns those native focal species that have significant overlap in phenology and pollinator communities. Additional (experimental) trials would be required for those native focal species (Table 1) that have significantly overlapping phenology and pollinator communities to see if their fitness is reduced. Species with specialized pollination systems, which are thought to be more vulnerable to disruptions of plant-pollinator interactions than species with generalized pollination systems, could be given higher priority (Bjerknes et al. 2007).

Tool 3: Predicting toxin sensitivity

As a sideline to this thesis project we studied whether existing knowledge of how toxins affect different organisms could help to develop a tool to predict the potential effects of introduced species. Introduced (GM) plants may contain proteins/metabolites that are toxic to some plant consumers. An important component of risk assessment is to test whether native focal species (or non-target organisms) are affected by such new or altered plant metabolites. Currently, risk assessment is performed by exposing non-target species to different levels of toxins, or alternatively, by feeding them directly with plant material (EFSA 2010a). These conventional ecotoxicological methods are rather time-consuming and are often restricted to the evaluation of a few species.

We propose to develop an additional approach to assess the sensitivity of native focal species to toxins, based on their mode of action. The approach involves different steps: (1) identify the potentially toxic compound, (2) determine its mode of action, e.g. what is the chain of events leading to toxic reaction in the native focal species, and what key receptors are involved?, (3) identify the genetic sequence of (components of) key receptors, (4) determine in which organisms the receptor is also present, using DNA database queries. The goal is to predict the spectrum of species that are most likely also affected, so that time-consuming ecotoxicological studies can be avoided or made more targeted. The number of model species with full genome information is increasing rapidly, and it is quite feasible to screen non-model species on the presence of particular conserved DNA sequences.

As an example, *Bacillus thuringiensis* (Bt) transgenic crops produce crystal (Cry) proteins, which are toxic to many arthropod species, including many agricultural pests. To date, 137 different Cry toxins have been identified, each targeted to control a specific taxonomic group of (pest) organisms (Frankenhuyzen 2009). The cadherin

receptor, located in the arthropods intestinal epithelium, is thought to play a crucial role in mode of action of Cry toxins and represents an important link determining the arthropods' sensitivity to Cry toxins (Gahan et al. 2001). By combining known sensitivity of native focal species to different Cry toxin families with their associated receptor (cadherin) coding sequence, predictions can be made for native focal species that have not been experimentally tested. BLAST searches of the cadherin coding sequence of *Heliothis virescens* that is susceptible to the so-called *Cry 1Ac* toxin, returned a list of 14 species that are also known to be susceptible to the *Cry 1Ac* toxin, and 5 species with an unknown toxin susceptibility (van Hengstum et al., unpublished data, Frankenhyzen 2009). An additional challenge is introduced by Bt crops containing stacked Bt transgenes expressing different types of Cry toxins. Studies are required to find out how these different Cry toxins interact and how this affects toxicity for different arthropods, but again, knowledge of the working mechanism of the different variants may help to determine the potential spectrum of affected organisms.

Whether this tool can be used to detect nonlethal effects to toxins is questionable. For example, the effects of neonicotinoid pesticides on behaviour of honey bees, causing high mortality due to homing failure (Henry et al. 2012), was not anticipated despite extensive toxicological testing.

To conclude, the ecological insights gained from the experiments performed in this thesis, together with the tools that we propose to develop have the potential to further improve the scientific underpinning of consequences of putative invasions. They are not meant to replace current ERA procedures or create additional requirements, but hopefully this will reduce the risk that detrimental ecological effects to the environment come as a surprise. In this respect the knowledge can also serve as an aid in later phases after an introduction, rendering monitoring efforts better targeted. ●

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English summary

The Oxford dictionary describes the word *invasion* as “an unwelcome intrusion into another’s domain”. While this definition does not specifically refer to biological invasions, it is well applicable to plant and animal invasions, some of which have severely affected recipient environments. For instance, invasive species such as *Centaurea solstitialis* and *Euphorbia esula* are noxious agricultural pests in the US causing major economic losses. Others suppress native flora and fauna by competing for food or resources such as nutrients, light and space. Nowadays, there is also the concern that genetically modified organisms, in particular crops that are increasingly cultivated worldwide, can lead to invasions, either by the introgression of the transgene to wild relatives, or by the spread of crops as feral plants.

This thesis aims to increase our knowledge on ecological effects of plant invasions, particularly in relation to pollination ecology, herbivory, and native invertebrate and plant communities. Moreover, we aim to provide tools and insights that can be used for risk assessment systems, which have the purpose to reduce the risk of future invasions and their associated impact on the environment. In order to achieve these goals we used a combination of experiments and analyses of existing data.

We first investigated how the mass-flowering invasive model species *Brassica rapa* influences the insect visitation frequency to native co-flowering species (Chapter two). We observed that native species that share pollinators with the invader were affected in terms of visitation frequency, but not in seed set and seed production. This suggests that the seed set of these species is not constrained by the number of received flower visits. The flower visitation frequency of native species that did not share pollinators with the invader remained unaffected. In the same experiment we investigated the occurrence of infested seed pods of *Diplotaxis tenuifolia* growing in the presence or absence of *B. rapa*. We found that the number of infested seed pods was significantly lower for plants growing near invaders. This illustrates that native plants can also benefit from the invader’s presence.

In Chapter three we describe how plant invasions can alter local invertebrate communities and how this may affect the incidence of herbivory to co-occurring natives. For this purpose we created a two-year field experiment in which we introduced two invasive model species, *Brassica napus* (oilseed rape) and *Lactuca serriola* (prickly lettuce). Invertebrate abundance and taxonomic richness within invasion plots was generally lower than in control habitat, while along the edges of invasion plots we found the opposite pattern. The incidence of herbivory for two native focal species was not affected, despite shifts in the trophic composition of invertebrates.

A meta-analysis across 56 studies showed that invaded habitat generally contains a lower arthropod abundance and taxonomic richness than uninvaded habitat (Chapter four). Moreover, we showed that woody plant invaders have a more negative effect on arthropod abundance and richness than herbaceous species. The reasons for this are unclear, but it may be related to the palatability of the invader's plant tissues.

Finally, we analysed a dataset of more than 10.000 periodically monitored vegetation plots in the Netherlands and found that on average the habitat containing one or more exotic (non-native) species is less diverse than habitat containing only native species (Chapter five). A possible explanation is that exotic species more easily invade low diversity habitat, as described by the so-called diversity-resistance hypothesis. Furthermore, we demonstrated that the rate of colonization by invaders was not correlated to the availability of bare ground, suggesting that disturbance did not facilitate colonization of exotic plants. Finally, we found that invasive exotics have a broader niche than equally common natives and that exotic plants are found in habitats that are shadier, warmer, more continental and more nutrient rich compared to native species. We conclude that these niche differences may (partly) explain the success of exotic species in the Netherlands.

This thesis provides insights into some important direct and indirect effects that invasive species may have on native plant and animal communities. Moreover, we demonstrate how complex ecological interactions are, and that effects can vary in space and time. With this in mind, we present three tools that may be valuable for the development and improvement of risk assessment systems (Chapter six): one to predict spatial co-occurrence of invasive and native species, a second one to estimate the probability that pollination success of natives will be affected by the invader and a third to predict the sensitivity of animals to toxins produced by genetically modified plants. ●

Nederlandse samenvatting

Invasieve planten kunnen grote invloed hebben op de biodiversiteit en ecologie van geïnvadeerde gebieden. Sommige soorten concurreren met inheemse flora om licht, ruimte en water, maar ze kunnen ook op andere manieren invloed uitoefenen op ecosystemen. Zo zijn er bijvoorbeeld soorten die in staat zijn om sedimentatieprocessen te beïnvloeden of de chemische samenstelling van de bodem te veranderen. Sommige invasieve planten zijn onkruiden en veroorzaken problemen in de agrarische sector. In de Verenigde Staten zijn *Centaurea solstitialis* (Zomercentaurie) en *Euphorbia esula* (Heksenmelk) voorbeelden van sterk invasieve onkruiden. In Nederland zorgt onder andere *Hydrocotyle ranunculoides* (Grote waternavel) voor aanzienlijke economische schade door hele watersystemen te verstopen. Recentelijk is er ook bezorgdheid geuit over het risico en de mogelijke gevolgen van invasies van genetisch gemodificeerde gewassen, die als gevolg van verwildering of door uitkruising met wilde verwanten zouden kunnen ontsnappen en invasief kunnen worden.

Het doel van dit proefschrift is om de kennis over de ecologische effecten van planteninvasies te vergroten, voornamelijk op het gebied van bestuiving, vraat door insecten en andere organismen, (herbivorie), en veranderingen van (ongewervelde) dier- en plantengemeenschappen. Verder is het doel om 'gereedschappen' en nieuwe inzichten te verschaffen om het risico van invasies, en de hiermee samenhangende milieueffecten, te kunnen reduceren. Om deze doelen te bereiken hebben we gebruik gemaakt van veldexperimenten en analyse van bestaande onderzoeks- en monitoringgegevens.

De massaal bloeiende inheemse *Brassica rapa* (Raapzaad) gedraagt zich als een niet-inheemse invasieve soort en kon dus goed als modelsoort voor ons onderzoek gebruikt worden. Allereerst hebben we onderzocht hoe de massale aanwezigheid van Raapzaad in een plantengemeenschap de frequentie van bloembezoek door insecten aan tegelijk bloeiende inheemse planten beïnvloedt (Hoofdstuk 2). We constateerden dat de bezoekfrequentie van inheemse plantensoorten met (deels) dezelfde bestuivers als de invasieve soort zowel positief als negatief werd beïnvloed. Dit had echter geen effect op de zaadzetting en zaadproductie van die soorten. Dit suggereert dat de frequentie van bloembezoek geen beperkende factor was voor de zaadzetting van de onderzochte soorten. De bezoekfrequentie van inheemse soorten die geen bestuivers deelden met Raapzaad werd niet beïnvloed door de aanwezigheid van de invasieve soort. Tijdens hetzelfde experiment hebben we onderzocht of de massale (invasieve) aanwezigheid van Raapzaad invloed had op de mate van vraat (herbivorie) van de verwante inheemse plant *Diplotaxis tenuifolia* (Grote Zandkool). We vonden dat de vruchten van planten die in de buurt stonden

van Raapzaad minder vaak werden aangetast, wat aantoont dat een invasieve soort ook positieve effecten kan hebben door herbivoren van inheemse soorten af te leiden.

In het derde hoofdstuk hebben we onderzocht hoe planteninvasies invloed kunnen hebben op lokale gemeenschappen van ongewervelde dieren, en wat de gevolgen hiervan kunnen zijn op de intensiteit van herbivorie van aanwezige inheemse plantensoorten. Hiertoe hebben we een tweejarig veldexperiment gecreëerd waarin we een invasie door twee soorten, namelijk *Brassica napus* (Koolzaad) en *Lactuca serriola* (Kompassla), hebben nagebootst. Wij vonden dat zowel de aantallen individuen (abundantie) als de soortenrijkdom van ongewervelde dieren binnen de experimenteel geïnvadeerde proefvlakken over het algemeen lager was dan binnen controleproefvlakken, terwijl we aan de randen van de geïnvadeerde proefvlakken juist het tegenovergestelde vonden. Ook waren er verschillen tussen de twee typen proefvlakken in de verhoudingen tussen herbivoren, predatoren en detritivoren (de zogenaamde trofische samenstelling) binnen de ongewervelde dieren. Echter, vaak verschilde de trofische samenstelling ook tussen de twee onderzochte jaren. Ondanks deze verschillen werd de intensiteit van herbivorie voor de twee als referentie gebruikte inheemse modelplantensoorten, *Tragopogon pratensis* (Gele Morgenster) en *D. tenuifolia*, niet beïnvloed.

In een meta-analyse (Hoofdstuk 4) waarin we de uitkomsten van 56 eerdere studies hebben geanalyseerd bleek dat geïnvadeerd habitat over het algemeen een lagere taxonomische rijkdom en abundantie aan geleedpotigen (arthropoden) bevat dan niet-geïnvadeerd habitat. Bovendien vonden we dat dit effect voor houtige invasieve planten sterker was dan voor kruidachtige soorten. De reden hiervoor is nog niet duidelijk, maar het zou te maken kunnen hebben met het verschil in verteerbaarheid van plantenweefsel, bijvoorbeeld doordat houtige soorten meer afweerstoffen bevatten dan kruidachtige soorten.

Tot slot hebben we een dataset geanalyseerd die bestaat uit vegetatieopnamen van meer dan 10.000 periodiek opgenomen permanente proefvlakken in Nederland (Hoofdstuk 5). Deze dataset behoort tot het Netwerk Ecologische Monitoring (NEM). Uit onze analyse kwam naar voren dat habitat waarin één of meerdere niet-inheemse (exotische) soorten voorkomen een lagere diversiteit heeft dan habitat met alleen inheemse soorten. Historische analyse van de data suggereerde dat dit veroorzaakt wordt doordat exoten gebieden met een lage soortenrijkdom gemakkelijker kunnen invaderen, zoals geponeerd in de 'diversity resistance' hypothese. Verder hebben we aangetoond dat kolonisatie door exoten niet gecor-

releerd is aan de aanwezigheid van open grond, wat suggereert dat de mate van verstoring geen invloed heeft gehad op kolonisatie door exoten. Tenslotte vonden we dat invasieve exoten een bredere niche hebben dan inheemse soorten met een vergelijkbare abundantie. Bovendien komen exoten in vergelijking met inheemse soorten voor in habitat dat schaduwrijker, warmer, meer continentaal en voedselrijker is. Deze verschillen in nichekenmerken tussen exoten en inheemse soorten zouden een gedeeltelijke verklaring kunnen zijn voor het succes van exoten.

Dit proefschrift biedt inzicht in enkele belangrijke directe en indirecte effecten van invasies op inheemse planten- en dierengemeenschappen. Verder illustreren onze resultaten hoe complex ecologische interacties zijn, en hoe deze kunnen variëren in tijd en ruimte. Mede op grond van deze kennis presenteren we drie 'gereedschappen' die een waardevolle bijdrage kunnen leveren aan de verbetering van zogenaamde risicobeoordelingssystemen: protocollen die gebruikt worden om het risico van invasies en de mogelijke gevolgen ervan in te schatten en te beperken (Hoofdstuk 6). Deze drie gereedschappen zijn bedoeld om te voorspellen (i) of een invasieve en inheemse soort zullen voorkomen in hetzelfde gebied/habitat, (ii) of het bestuivingssucces van inheemse soorten zal worden beïnvloed door een invasieve soort, en ten slotte (iii) of dieren (voornamelijk insecten) gevoelig zullen zijn voor toxines die geproduceerd worden door sommige genetisch gemodificeerde gewassen.

Als we in de toekomst het aantal invasies willen beperken zouden overheden het proces van toelating van niet-inheemse soorten beter moeten reguleren. Risicobeoordelingssystemen moeten worden ingezet om soorten te beoordelen op hun invasie-potentie. Gereedschappen, zoals de drie bovenstaande voorbeelden, kunnen ervoor zorgen dat dit proces effectief en financieel haalbaar is. Zonder striktere regulering van niet-inheemse soorten zullen invasies, en de daarbij behorende potentiële ecologische en economische impact, een blijvend probleem vormen. ●

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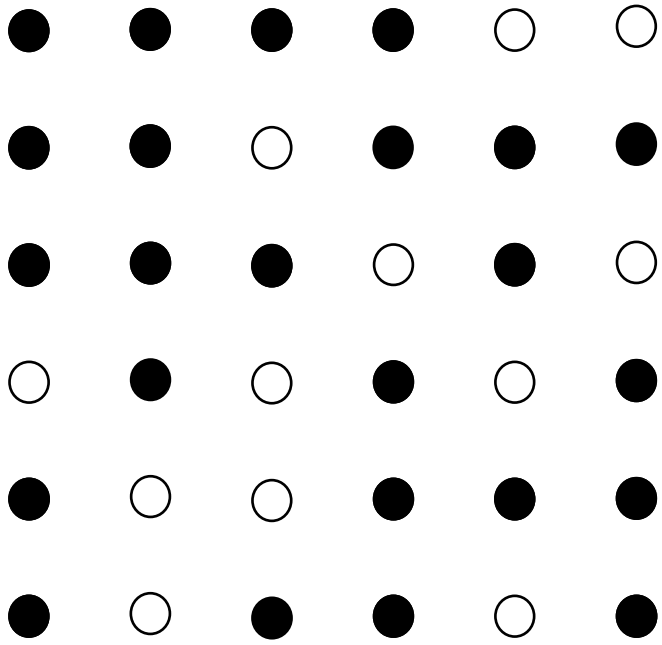
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