Testing the relative roles of competition and plant-soil feedback in explaining commonness and rarity of alien and native plant species

Dissertation submitted for the degree of Doctor of Natural Sciences

(Dr. rer. nat.)

Presented by

Gregor Simon Müller

at the

Universität
Konstanz

Faculty of Sciences

Department of Biology

Day of the oral examination: 9th of December 2016

Referee 1: Prof. Mark van Kleunen

Referee 2: PD Judy Simon

Summary

Why some species become common while others remain rare is a long-standing question in ecology. Yet, it has not lost any of its relevance. Particularly, the striking success of some invasive alien species has fuelled research interest in what drives such invasions. Understanding the underlying mechanisms that allow a species to become common is an important prerequisite to be able to predict and manage such phenomena. However, the success of some alien species may be driven by the same factors that help some native species achieve and maintain a wide distribution and high abundance.

Plant-soil interactions have been put forward as a potential mechanism that could explain why some species become common, while others remain rare. A growing number of studies acknowledge the role of plant-soil interactions in species performance, yet many questions remain unanswered. For example, competition may also play a role in explaining species success, and both factors may not act independently but work in concert to govern species performance. Moreover, the importance of plant-soil interactions relative to ecological factors like herbivores or disturbance has received little attention. Lastly, the question of whether alien and native species are similarly affected by these factors or if, for example, only common alien species generally profit from less negative plant-soil interactions (e.g. due belowground enemy release) is not fully clarified.

First, to disentangle the relative roles that plant-soil feedback and intraspecific competition play in plant performance, we conducted a multispecies greenhouse experiment with 30 common and rare alien and native plant species. We used a two-phase setup to first train the soils at increasing intraspecific frequency and then regrow the target species at increasing frequencies in these trained soils. Our results showed that increasing levels of intraspecific competition decreased plant performance regardless of

species origin or commonness. Moreover, alien species suffered from negative plant-soil feedback in soils trained by high conspecific planting levels, but natives did not. This suggests that alien species may not generally be released from belowground enemies. Furthermore, species commonness does not seem to be linked to differences in plant-soil interactions or magnitude of intraspecific competition.

Second, we tested the roles of plant-soil feedback and competition on species population-level performance in a two-year mesocosm study with annual two-species communities grown at high and low sowing frequency and treated with or without fungicide. We found that alien and native species similarly profited from fungicide treatment and that alien species, but not natives, showed a higher per capita performance at low sowing frequencies. This highlights the role that generalist pathogens may play affecting natives as well as aliens. Furthermore, aliens may profit from high per capita performance at initial establishment.

Lastly, we performed a field experiment testing how disturbance and above and belowground enemies influence species establishment success. We found a strong positive effect of disturbance on establishment success regardless of species origin or commonness, and only weak effects of belowground enemy release for alien species. Thus, we found strong evidence for biotic resistance of grassland towards incoming species, and only a minor mediatory role for soil-borne enemies in combination with disturbance for establishment success of alien species.

In summary, my studies show that alien species may not always be released from belowground enemies, and that species commonness is not generally linked to plant-soil interactions or intraspecific competition. Moreover, assessing the relative role of plant-soil interactions under realistic conditions is crucial for gaining insight into when and under what circumstances plant-soil interactions are important for species performance.

Zusammenfassung

Schon lange beschäftigt sich de Ökologie mit der Frage warum mache Arten häufig, andere hingegen selten sind. Nichtsdestotrotz hat diese Frage nichts an ihrer Aktualität eingebüßt. Insbesondre der Erfolg einiger invasiver nichtheimischer Arten hat in der Forschung die Suche nach den zugrundeliegenden Faktoren befeuert. Diese zugrundeliegenden Mechanismen zu verstehen, die es einer Art ermöglichen sehr häufig zu werden, ist eine entscheidende Voraussetzungen um das Phänomen invasiver Arten vorherzusagen und zu handhaben. Allerdings sind die Faktoren welche den Erfolg nichtheimischer Arten erklären womöglich die gleichen, die es auch heimischen Arten ermöglichen eine weite Verbreitung und eine hohe Abundanz zu erreichen.

Interaktionen zwischen Pflanze und Boden stellen einen potentiellen Mechanismus dar, der erklären könnte warum einige Arten sehr häufig auftreten und andere hingegen selten bleiben. Eine zunehmende Anzahl an Studien bestätigt die Rolle von Pflanzen-Boden Interaktionen für das Pflanzenwachstum, jedoch bleiben noch viele Fragen offen. So könnte zum Beispiel auch Konkurrenz ein wesentlicher Faktor für den Erfolg von Arten sein. Darüber hinaus beeinflussen beide Faktoren das Pflanzenwachstum möglicherweise nicht unabhängig voneinander sondern im Zusammenspiel. Des Weiteren wurde die relative Bedeutung von Pflanzen-Boden Interaktionen im Vergleich zu anderen ökologischen Faktoren wie Fraßfeinden und Störungseinflüssen nur wenig untersucht. Darüber hinaus ist auch die Frage, ob heimische und nichtheimische Arten von den genannten Faktoren gleichermaßen beeinflusst werden oder ob zum Beispiel nur häufige nichtheimische Arten generell von weniger hemmenden Pflanzen-Boden Interaktionen (z.B. aufgrund weniger natürlicher Feinde im Boden) profitieren, nicht abschließend geklärt.

Um den relativen Einfluss von Pflanzen-Boden Interaktionen und innerartlicher Konkurrenz auf das Pflanzenwachstum zu testen wurde ein Gewächshaus Experiment mit 30

häufigen und seltenen heimischen sowie nichtheimischen Arten durchgeführt. Es wurde ein zwei Phasen Design benutzt, in dem zunächst der Boden durch das Aufziehen von Pflanzen mit zunehmenden intraspezifischer Anzahl kultiviert wurde und anschließend die selben Arten wiederum mit zunehmender intraspezifischen Anzahl in diesem vorkultivierten Boden gezogen wurden. Die Ergebnisse zeigten, das mit zunehmender innerartlichen Konkurrenz das Pflanzenwachstum abnimmt, jedoch unabhängig von Herkunft und Häufigkeit der Arten. Außerdem, wurden nichtheimische Arten stärker durch Pflanzen-Boden Interaktionen, in Böden die zuvor von einer hohen Anzahl der selben Art kultiviert wurden, gehemmt als heimische Arten. Dies zeigt, das nichtheimische Arten nicht generell weniger von im Boden vorkommenden Schädlingen betroffen sind. Darüber hinaus konnte die Häufigkeit der Arten nicht durch Pflanzen-Boden Interaktionen oder Unterschieden im Ausmaß der innerartlichen Konkurrenz erklärt werden.

In der zweiten Studie wurde der Einfluss von Pflanzen-Boden Interaktionen und Konkurrenz auf das Populationswachstum in einer zweijährigen Mesokosmos Studie mit Pflanzengemeinschaften aus zwei jeweils einjährigen Arten, welche in hoher und niedriger dichte ausgesät, sowie mit oder ohne Fungizid behandelt wurden, getestet. Es zeigte sich, dass heimische und nichtheimische Arten gleichermaßen von der Behandlung mit Fungizid profitierten und das nichtheimische Arten einen etwas höhere pro Kopf Ertrag bei niedriger Aussaatrate erreichten als heimische Arten. Dies betont die Rolle von generalistischen Pathogenen welche heimische wie nichtheimische Arten gleichermaßen befallen. Darüberhinaus profitieren nichtheimische Arten vermutlich vom höheren pro Kopf Ertrag bei einer Neuetablierung.

Zuletzt wurde ein Feldexperiment durchgeführt in dem untersucht wurde wie Störung sowie über- und unterirdische Feinde den Etablierungserfolg von Arten beeinflussen. Es zeigte sich, dass Störung, unabhängig von Herkunft und Häufigkeit der Arten, einen starken positiven Einfluss auf den Etablierungserfolg hat wohingegen sich nur sehr schwache Anzeichen von geringerer Hemmung von nichtheimischen Arten durch weniger natürliche

Feinde im Boden nachweißen lies. Folglich konnten starke Anzeichen von ausgeprägter biotischer Resistenz von Grasland gegenüber einwandernden Arten nachgewiesen werden und dementsprechend nur eine geringe vermittelnde Rolle von bodenlebenden Pathogenen im Zusammenspiel mit Störung für den Etablierungserflog nichtheimischer Arten.

Zusammenfassend lässt sich sagen, das nichtheimische Arten nicht generell von einem geringeren Befall an bodenlebenden Feinden profitieren und das sich die Häufigkeit von Arten im Allgemeinen nicht mit Pflanzen-Boden Interaktionen oder innerartlicher Konkurrenz erklären lässt. Darüber hinaus ist es notwendig die relative Bedeutung von Pflanzen-Boden Interaktionen unter realistischen Bedingungen zu ermitteln um herauszufinden, wann und unter welchen Umständen Pflanzen-Boden Interaktionen für das Pflanzenwachstum von Bedeutung sind.

Table of Contents

General Introduction

Plant-soil feedback

Over the past few decades increasing awareness that interactions between plant and soil may play a key role for species performance has arisen in plant ecology (Ehrenfeld, Ravit & Elgersma 2005; Mordecai 2011; van der Putten *et al.* 2013). This concept termed plant-soil feedback can be defined as: "*Changes to soil properties that are caused by plants, which in turn influence performance of plants…*" (van der Putten *et al.* 2013). This definition includes biotic as well as abiotic aspects including chemical (e.g. pH) physical (e.g. soil structure, percolation) as well as biotic components (e.g. pathogens, herbivores but also mutualists). Consequently, the interplay between a multitude of factors and driving agents defines the net outcome of plant-soil feedback effects. Generally, plant-soil feedback is found to be predominantly negative (Kulmatiski *et al.* 2008) but may range from positive to neutral or negative and may also change over time (Diez *et al.* 2010; van der Putten *et al.* 2013). It is intriguing that, for sessile organisms as plants, spending their whole lifecycle at the same place, being strongly influenced by their surrounding environment also specifically including their rooting zone, the belowground perspective has until recently played only a minor role in research. However, research is about to catch up to fill knowledge gaps concerning the complex interplay between plants and their soil. Thus, there is a growing array of studies showing that plant-soil feedback can be important for individual growth, survival and reproductive output (Bever 1994; an der Putten & Peters 1997; Brandt *et al.* 2013; Maron *et al.* 2013; Maron *et al.* 2014; Xu *et al.* 2015) as well as for maintaining species diversity (Bever, Westover & Antonovics 1997; Mills & Bever 1998; Reynolds *et al.* 2003; Rodriguez-

Echeverria *et al.* 2013; Bever, Mangan & Alexander 2015) and mediating species coexistence (Packer & Clay 2000; Bever 2003; Bonanomi, Giannino & Mazzoleni 2005; HilleRisLambers *et al.* 2012; Aponte, Garcia & Maranon 2013; Brandt *et al.* 2013; Burns & Brandt 2014) or community productivity (Maron *et al.* 2011; Schnitzer *et al.* 2011). Especially pathogenic (e.g specifcally soil fungi, Oomycetes and bacteria) or herbivorous soil organisms (e.g nematodes) have been increasingly identified as drivers behind plant-soil feedback effects (Callaway *et al.* 2004; Reinhart *et al*. 2005; van der Putten *et al.* 2005; Mangan *et al.* 2010) Thus, while the general idea that pathogens may play a role for species performance is not new (see for example Hendrix & Crocker 1978) new approaches and efforts have been made and progress in molecular tools increasingly allow us to shed more light into the black box of soil, though the complexity of the soil system and the interplay of numerous factors still leaves many questions unanswered.

Plant- soil feedback and species success

A specific aspect, in which plant-soil feedback may play an important role, is species success or failure. Based on the framework of coexistence theory (Chesson 2000; HilleRisLambers *et al.* 2012) a prerequisite for stable coexistence of species is the presence of stabilizing mechanisms to mitigate relative fitness differences. One such potential stabilizing mechanism can be negative density-dependent plant-soil feedback. This means, if a species is locally rare it may suffer less from negative plan-soil feedback due to lower accumulation of species-specific pathogens. This advantage when rare allows the species to maintain high population growth. Once it has reached high intraspecific densities it may be increasingly self-limited, resulting in lower per capita performance and (e.g. allowing co-occurring species to maintain higher growth rates) and consequently population decline or at least limited growth. If, however, species differ in magnitude of this self-limitation by plant-soil feedback then this difference would allow for differences in abundance (i.e. common species suffer less or accumulate fewer pathogens) and thus may help explain why some species are common while others are rare.

Such weaker self-limitation due to plant-soil feedback for dominant species was found by MacDougall, Rillig & Klironomos (2011) in a study on six native and eight alien species in a savannah grassland. All species in their study showed stronger negative plant-soil feedback responses in conspecific compared to heterospecific soils (i.e. self limitation) allowing for coexistence, but differences in strength of self-limitation were reflected by respective abundances in the field. Moreover, alien species tended to have weaker negative plant-soil feedback than native species. Similarly, in a study on five rare natives and five common alien species Klironomos (2002) found that the invasive alien species profited form consistently positive soil-feedback compared to negative feedback of the rare native species. These results seem to be influenced by the presence or absence of closely related species of the studied aliens in the plant community (Anacker *et al.* 2014), however, it highlights the aspect of potential belowground enemy release in alien species (Reinhart *et al*. 2003; Callaway *et al.* 2004; Suding *et al.* 2013). Specifically, weaker self-limitation due to plant-soil feedback in alien plant species may be the result of a complete absence of some belowground antagonists that keep them in check in their home range (Reinhart *et al.* 2003; Mitchell & Power 2003). Consequently, the disproportional success of some alien species in their introduced range may, at least partly, be explained by weak negative or even positive plant-soil feedbacks.

Moreover, there are a large number of studies particularly in tropical forests that show for example highly density-dependent mortality of seedlings in proximity to adult trees. The hypothesis that this density-dependent enemy attack maintains species diversity is known as the Janzen-Connell hypothesis (Janzen 1970; Connell 1971). In a recent study Bagchi *et al.* (2014) could corroborate this hypothesis and found that species diversity was increased from seed to seedling transition in plots that remained untreated with fungicide or insecticide, pointing towards a role of natural enemies in maintaining species diversity and community composition. However, density-dependent enemy attack may not only influence species diversity; if differences in negative density-dependence arise between species they also influence relative species abundance. Comita *et al.* (2010) for example also found negative density-dependence in tropical tree species and furthermore observed differences in the strength of the density dependence for different species. Notably, negative density-dependence was negatively correlated with species abundance in the field, thus the most common species suffered least from negative density-dependence. Similarly, Mangan *et al.* (2010) showed that common species suffer less form negative density-dependent seedling mortality and could identify soil-borne pathogens as drivers behind this pattern. These studies show that soil-borne enemies can have substantial impacts on species success and therefore highlight the need to include a belowground perspective on plant performance.

The relative role of plant-soil feedback and and other factors

Testing the influence of a single factor as a potential driver behind species success is an important first step to screen its importance. However, it is also necessary to evaluate its relative role in relation to other factors that may contribute to species performance and eventually their abundance. For example, resource partitioning may also provide a stabilizing mechanism, such that high intrapecific densities should result in stronger competition (i.e self-limitation) than interspecific competition (Levine & HilleRisLambers 2009; HilleRisLambers *et al.* 2012). As outlined above, differences in magnitude of self-limitation between species may then ultimately help explaining differences in species relative abundance.

Furthermore, density-dependent enemy attack may act aboveground (e.g. hebivores) as well as belowground (e.g. soil-borne pathogens). Consequently, it is necessary to consider and integrate the above *and* belowground components of the role that natural enemies play for species performance (van der Putten *et al.* 2001; Agrawal *et al*. 2005) Finally, to get a clearer picture, it is important to simultaneously test for the impact of other factors like competition and plant-soil feedback to elucidate what role they play relative to each other (van der Putten & Peters 1997; Casper & Castelli 2007; Petermann *et al.* 2008; Dawson & Schrama 2016). This is because these factors may not act independently but may interact opening the possibility for synergies between them (Blumenthal 2006).

Research gaps

Though there has been much progress and a growing number of studies on the role and impact of plant-soil feedback on species performance and also species commonness, there remain aspects that have yet to be answered. For example, the finding by Klironomos (2002), that common alien species may profit form positive plant-soil feedback does not allow one to draw general conclusions on whether plant-soil-feedback effects work as a general mechanism behind species commonness. This is because the mechanism behind the success of species and whether it is linked to strength of plant-soil feedback effects may be the same for common natives as for common aliens. However, Klironomos (2002) only tested rare native species and invasive alien species, which confounds species commonness and species origin. Thus, it is necessary to test both groups, common and rare species of both native and alien origin (Thompson, Hodgson & Rich 1995; van Kleunen *et al*. 2010). Furthermore the relatedness of species (i.e sharing evolutionary history) may also play a key role in the effect of soil-borne pathogens on species (see Anacker *et al.* 2014). Consequently, a multispecies setup with quadruplets of related common and rare alien and native species across multiple families is suited to test for the generality of the underlying mechanism (van Kleunen *et al.* 2014).

Moreover, there is a need for more studies that directly assess fitness-related parameters (e.g. survival, reproductive output) and population-level performance (Dawson & Schrama 2016). Varying experimental protocols and differing measures of plant performance (e.g. the use of indices *versus* raw plant weight) may explain a large amount of variation in study outcomes and hamper interpretation of studies assessing plant-soil-feedback effects on species performance. (Kulmatiski *et al.* 2008; Brinkmann *et al.* 2010). Furthermore, so far studies on the effects of plant-soil feedback on species commonness are mostly based on correlations between soil-feedback and species abundance (Mangan *et al.* 2010; Comita *et al.* 2010 but see Harpole & Suding 2007). Thus, being able to link effects of plant-soil feedback to demographic processes would enhance our understanding and help to quantify and evaluate the drivers behind species success (Gurevitch *et al.* 2011).

Lastly, testing for the effects of multiple factors considering for example above- and belowground aspects under realistic conditions (e.g. in the field, including potential direct and indirect effects of resident species and respective soil communities) is necessary to help to validate findings based on greenhouse studies (Dawson & Schrama 2016).

Contribution of this thesis

In summary, my thesis aimed to disentangle the relative role that plant-soil feedback and competition play in explaining commonness and rarity of alien and native plant species. Therefore, I addressed the question using three approaches, ranging from a short-term greenhouse study to a long-term realistic field setup with an increasing degree of realism. First I used a greenhouse study with a two-phase setup, using increasing intraspecific frequencies of target plants, to test for differences in density-dependence and relative importance of the effects of competition and plant-soil feedback. Second, I used a two-year mesocosm study of annual common and rare alien and native species to test for the effects of intraspecific competition and soil-borne enemies (i.e. fungicide treatment) with a focus on population-level performance Third, I explored the roles of multiple factors (i.e. disturbance, soil-borne enemies and herbivores) on establishment success of common and rare alien and native species in a field study.

In the 1st chapter I tested if common and rare native and alien species are differentially affected by density-dependent plant-soil feedback or by intraspecific competition. Therefor, I used a multispecies greenhouse study on 15 alien and 15 native species in a two-phase setup. Thus, target species were grown at increasing intraspecific frequencies in a first phase to train the soil and were replanted again at increasing frequencies in trained soil of the different frequencies to disentangle the effects of competition and plant-soil feedback. Specifically, I asked if common species are less negatively affected by density-dependent plant-soil feedback than rare species, irrespective of origin and if common species are less self-limited by intraspecific competition than rare species, irrespective of origin. Moreover I asked if plant-soil feedbacks and intraspecific competition interact synergistically and thus increase the negative impact on per capita plant performance.

Fig. 1: Plant communities of the 1st experiment in the greenhouse

In the 2nd chapter I explored how population growth of a set of 20 annual alien and native common and rare species is affected by intraspecific competition or plant-soil feedback. I used a two-year mesocosm study, in which target species were grown under high and low intraspecific frequencies and under fungicide or water treatment in two species communities. I specifically asked if population growth of common species is less limited by intraspecific competition than for rare species and if population growth of common species is less limited by soil fungal pathogens than it is for rare species. Furthermore, I asked if effects of intraspecific competition and soil fungal pathogens on common and rare species interact and if alien species profit from belowground enemy release and thus show weaker effects of fungicide treatment or intraspecific competition than native species.

Fig. 2: Pots with two-species communities of the 2nd experiment in the botanical garden

In the 3rd chapter I used a multi-species field study to test for the effects of disturbance, soil-borne pathogens and herbivores on the establishment success of common and rare alien and native plant species. I tested whether a set of 20 different alien and native common and rare species differ in establishment success in relation to high and low disturbance, biocide or water control treatment as well as reduction of herbivores or not. Therefore I transplanted seedlings and sowed seeds in an existing grassland community and followed their establishment success and survival over two years. I specifically asked if disturbance increases establishment success of incoming species, and if that effect differs between alien and native or common and rare species. Furthermore, I asked if biocide treatment lowers establishment success of incoming species due to release from pathogen pressure on the resident community, and if this affects aliens more than natives, as alien species may lose their potential competitive advantage if enemy release is acting. Lastly, I asked whether release from herbivore pressure influences establishment success of the incoming species, and if this effect differs between alien and native or common and rare species and if the three factors disturbance, biocide treatment and herbivore reduction interact to affect plant establishment success.

Fig. 3: One site of the 3rd experiment with a seed plot (front) and open and closed net cages with seedlings (back).

Chapter I

Commonness and rarity of alien and native plant species – The relative roles of intraspecific competition and plant-soil feedback

Gregor Müller, Mark van Kleunen and Wayne Dawson

Oikos, in press; doi:10.1111/oik.02770

Abstract

The success of invasive alien and common native species may be explained by the same underlying mechanisms. Differences in intraspecific competition as well as differences in plant-soil feedback have been put forward as potential determinants of plant success. We teased apart the relative roles of competition and plant-soil feedback in a greenhouse experiment with 30 common and rare alien and native species from nine plant families. We tested whether plant biomass decreased less for common than rare species, regardless of origin, when grown at higher relative frequencies (1, 3 or 6 out of 9 plants per pot) in a community and in soil previously conditioned by the same species at different frequencies (0, 1, 3 or 6 out of 9 plants per pot) in an orthogonal design for these two factors. Plant survival decreased slightly, but non-significantly, for all species when grown in soil previously occupied by conspecifics. Among surviving plants, we found a decrease in biomass with increasing intraspecific competition across all species (regardless of origin or commonness), and alien species were more negatively affected by previous high plant frequency than native species, but only marginally significantly so. Our findings suggest that, while intraspecific competition limits individual biomass in a density-dependent manner, these effects do not depend on species origin or commonness. Notably, alien species but not natives showed a decrease in performance when grown in soil pre-conditioned with a higher frequency of conspecifics. In conclusion, soil-borne pathogen accumulation might be weak in its effects on plant performance compared to intraspecific competition, with neither being clearly linked to species commonness.

Introduction

Why are some species common while others are rare? This short question has puzzled ecologists for decades (Preston 1948). With the emergence of invasion ecology in times of globalization (Mack *et al.* 2000; Bradley *et al.* 2010), the question "what determines species commonness" has gained further interest. Numerous theories and approaches that tackle this challenging question have been developed (Kunin & Gaston 1993; Mitchell *et al.* 2006; Gaston 2011). However, general rules and the driving mechanisms behind sometimes striking differences in species success have not been clearly identified. The mechanisms explaining why some alien species successfully spread and occupy large areas at high abundances in the introduced range might be the same as those explaining high abundance in their native range or that similarly allow some native species to obtain a wide distribution and a high abundance. This possibility has recently received growing attention by ecologists (Thompson, Hodgson & Rich 1995; van Kleunen *et al.* 2010). Thus, commonness and rarity of alien and native species might represent ‘two sides of the same coin’ (Jeschke & Strayer 2008).

Differences in density-dependent enemy attack or resource partitioning have been put forward to explain plant species success (Adler, HilleRisLambers & Levine 2007; MacDougall, Gilbert & Levine 2009). We specifically focus on two processes in our study; plant-soil feedback and intraspecific competition, and potential interactions between the two processes. Plant-soil feedback has recently been proposed as a potential mechanism that could explain plant species commonness and rarity (Klironomos 2002; MacDougall, Rillig & Klironomos 2011; van der Putten *et al.* 2013). Since plants influence their community of soil biota and these in turn influence plant performance, such host-specific plant-soil feedback may be an important regulator of plant species abundance. In particular, differences among species in accumulation of soil-borne pathogens, or a low susceptibility to or even the absence of such pathogens could lead to the dominance or high abundance of common native and alien species. Especially invasive alien species might have left their soil pathogens behind, allowing them to gain advantage over resident native species. An absence of soil-borne pathogens would be in line with the Enemy Release Hypothesis (ERH) (Keane & Crawley 2002), which so far has mainly been tested with regard to aboveground enemies (Mitchell & Power 2003; Liu & Stilling 2006).

Based on the theoretical framework of species coexistence, self-limitation of species by intraspecific competition should be stronger than limitation by interspecific competition providing a stabilizing mechanism that allows for species coexistence as lined out by (Chesson 2000). However, species might differ in the magnitude of the difference between self-limitation and limitation by other competitors (Comita *et al.*

2010). In other words, common species might be less self-limited in terms of intraspecific competition than rare species.

If plant-soil feedback acts in a density-dependent manner, with more negative effects of soil biota when conspecifics grow at high density, then plant-soil feedback and self-limitation might operate simultaneously and may interact to determine plant performance. The relative importance of the two mechanisms in explaining success of alien and native species has yet to be tested. Consequently, testing both factors simultaneously may shed light on whether an interaction between plant-soil feedback and intraspecific competition takes place. For example Petermann *et al.* (2008) stated that negative plant-soil feedback might hamper the competitive ability of plants (see also Kardol *et al.* 2007) leading to the possibility that plant-soil-feedback effects become more important than self-limitation by intraspecific competition in regulating plant species abundances.

To test the roles of plant-soil feedback and intraspecific competition in explaining species success, we used a greenhouse experiment with 30 herbaceous plant species, including 13 native and 17 alien species, respectively, that vary in commonness within Germany. We grew the species at different intraspecific frequencies and in soils previously occupied by the same species at differing frequencies, and analysed individual plant performance. We asked the following questions: 1) Are common species less negatively affected by density-dependent plant-soil feedback than rare species, irrespective of origin? 2) Are common species less self-limited by intraspecific competition than rare species, irrespective of origin? 3) Do plant-soil feedbacks and intraspecific competition interact synergistically and thus increase the negative impact on per capita plant performance?

Materials and Methods

In order to be able to generalize results beyond a few study species (van Kleunen *et al.* 2014) we conducted a multi-species greenhouse experiment using 30 different target species from nine different families (Table I.1). The chosen target species represented taxonomic groups, which ideally contained at least one common native, one rare native, one common alien and one rare alien plant species. As a proxy for the degree of commonness of each species, we used the number of 130-km^2 grid cells occupied by the species in Germany (maximum 3000) extracted from the FloraWeb database (FloraWeb, Bundesamt für Naturschutz, last accessed 16th February 2014). We also aimed to choose species that occur in similar habitats, excluding habitat specialists (excluding also woodland and riverine species), and which are not simply rare due to their geographic distribution overlapping only marginally with the borders of Germany. Alien species were defined as being introduced to Germany after 1492. Another three species from families different to the ones of the target species, *Phleum pratense* (2558 grid cells), *Galium album* (2463 grid cells), and *Ranunculus acris* (2985 grid cells), were selected to represent a common background community typical for grasslands in Germany. The experiment consisted of two growing phases. Phase one was used as a soil-conditioning phase to build up a potentially species-specific microbial community. Phase two was then used for testing the effect of increasing intraspecific competition under simultaneous influence of the pre-trained soil microbial community at different frequencies, allowing for an orthogonal study design with respect to phase 1 frequencies (i.e. plant-soil feedback) and phase 2 frequencies (i.e. intraspecific competition.

Table I.1: The 30 study species and their respective commonness (measured as number of ~130 km^2 grid cells occupied in Germany (out of 3000 maximum), (FloraWeb, Bundesamt für Naturschutz)) listed by family and origin. Year of introduction of alien species in Germany as found in Krausch (2003) and year of naturalization (FloraWeb, Bundesamt für Naturschutz) are displayed. The percentage of pots per species (out of 36 pots maximum) that was not affected by mortality in phase 2 is also shown. Species in bold font were represented in all treatment combinations.

Family	**Alien**				**Native**		
	Species	**Grid cells**	**Year introduced (naturalized)**	**%of pots**	**Species**	**Grid cells**	**% of pots**
Apiaceae	***Anethum graveolens***	576	- (-)	94.4%	***Daucus carota***	2942	88.8%
	Foeniculum vulgare	297	- (-)	86.1%	*Oenanthe lachenalii*	104	75%
Asteraceae	*Artemisia verlotiorum*	168	- (1920)	33.3%	***Achillea millefolium***	2741	80.5%
	Aster novi-belgii	1530	1686 (18th cent.)	88.9%	*Gnaphalium luteoalbum*	562	44.4%
	Solidago canadenis	2660	1648 (1736)	83.3%	***Lactuca saligna***	119	88.8%
					Senecio jacobaea	2773	61.1%
Brassicaceae	*Cardaria draba*	1513	- (1728)	36.1%	*Cardamine pratensis*	2923	41.6%
	Lepidium heterophyllum	98	- (-)	86.1%	***Lepidium graminifolium***	86	88.8%
Caryophyllaceae	***Cerastium tomentosum***	1296	1594 (16th cent.)	80.5%	***Silene latifolia***	2893	80.5%
	Gypsophila paniculata	122	1757 (-)	86.1%			
Geraniaceae	*Geranium macrorrhizum*	146	1588 (-)	27.7%	***Geranium lucidum***	103	80.5%
	Geranium pyrenaicum	2134	17th cent. (1800)	91.6%	***Geranium pratense***	1844	66.6%
Lamiaceae					*Salvia pratensis*	1694	41.6%
Plantaginaceae	*Linaria dalmatica*	21	- (-)	63.8%	*Pseudolysimachion spicatum*	559	63.8%
	Veronica persica	2863	- (1805)	91.6%	***Veronica chamaedrys***	2913	80.5%
					Digitalis lutea	127	47.2%
Scrophulariaceae	***Scrophularia vernalis***	105	18th cent. (1768)	58.3%			
Phrymaceae	*Mimulus guttatus*	694	1812 (1830)	36.1%			

Experimental setup phase 1

We planted a total of nine plants per pot in a 3x3 square (Fig. I.1). For the target species we planted zero, one, three or six plants per pot. The remaining plants planted were the three community species. These were planted using the following frequencies: pots with zero target plants contained six *Phleum pratense*, two *Galium album* and one *Ranunculus acris* plants; pots with one target plant contained five *Phleum pratense,* two *Galium album* and one *Ranunculus acris* plants; pots with three target plants contained four *Phleum pratense*, one *Galium album* and one *Ranunculus acris* plants; pots with six target plants contained two *Phleum pratense*, and either one *Galium album* or one *Ranunculus acris* plants chosen at random. We chose these frequencies in order to maintain a ratio of approximately 1:2 of forbs to grasses among the community species. We replicated each frequency level nine times in the first phase resulting in 36 pots for each target species including the nine "community" pots, which did not contain a target plant. The total number of pots in the first phase consequently was 1080.

Prior to the start of the first growing phase, we collected approximately 250 L of soil from six grassland areas in the vicinity of the University of Konstanz (List of species occurring at the site is given in Supplementary material Appendix I.1 Table A1). We systematically took 5-15 samples (10 cm deep) per area along transects with a spacing of approximately 10 m between each sampling point. We pooled the samples and homogenised the soil by sieving through a 5 mm mesh to remove roots, stones and other plant material. This soil was then used as a soil inoculum for each pot.

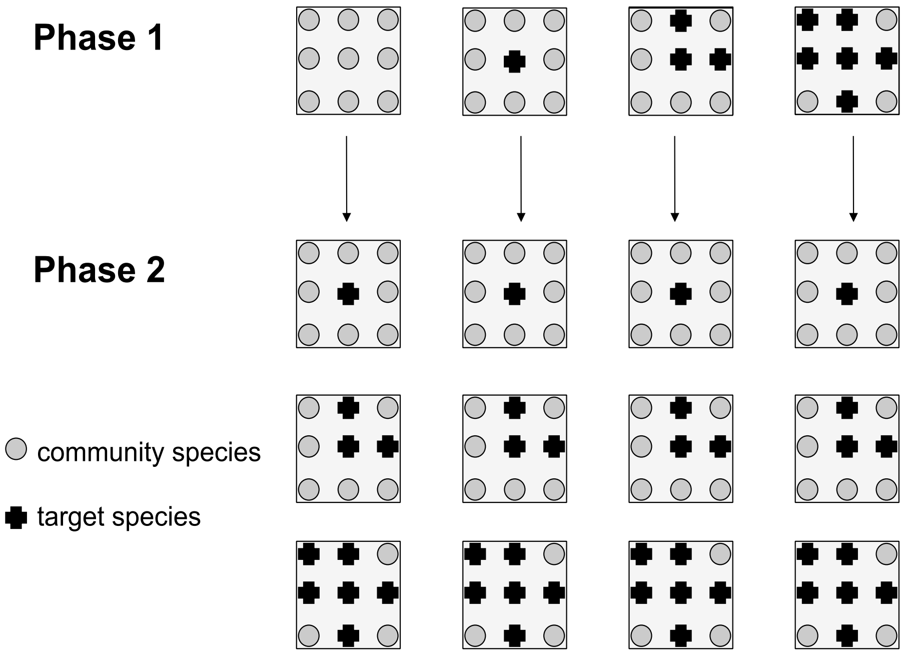


Fig. I.1: Schematic illustration of the experimental setup. Phase 1 represents the soil-conditioning phase. Phase 2 represents varying intraspecific competition levels in preconditioned soil of phase 1. (Replicated three times for each species)

The species were germinated in a growth chamber (temperature = 15°C/20°C, 12h/12h darkness/light, light level = 150μmol $m^{-2}s^{-1}$, relative humidity = 90%) on a 1:1 sand:vermiculite substrate. The substrate was not sterilized prior to the germination of seedlings, however, incubated agar plates that were inoculated with the substrate showed no visible signs of contamination. We planted the species in 4.2-L square pots (16 cm x 16 cm x 16.4 cm) filled with ~4 L mixed substrate. The substrate consisted of 250 ml field soil inoculum, mixed with 1:1 vermiculite:sterile sand and 20 g 8-9 month slow release fertiliser pellets (Osmocote^{TM} [NPK = 18:6:12]). Because of the large size of the experiment, we planted the species over a staggered period between the 27th of August and the 7th of September 2012. We replaced dead plants within a period of two weeks after initial planting. The pots were blocked into the three greenhouses (1 replicate in each) and were randomized within the greenhouses. We set the mean temperature to 21°C during the day and 16°C at night. Lights were switched on for 14 h per day but only if natural light levels fell below 100 μmol/s/m^2. After six weeks, we moved a randomly chosen subset of the pots into a fourth greenhouse compartment with the same growing conditions in order to provide sufficient space for continued growth. We watered the plants once every three days to levels that ensured no lack of water availability.

We harvested the aboveground biomass of plants of the first phase after a growing period of 8 weeks. We then dried (72 h at 80°C), and weighed the biomass and calculated the per capita aboveground biomass (total target biomass divided by number of target plants in the pot). After the harvest, we sieved and homogenised the substrate in the pots through a 5 mm mesh, removed roots and rhizomes from the soil, and put the substrate back in its original pot. All pots were then returned to their original greenhouse compartments and stored at an air temperature of 5-8°C until they were required for the second phase of the experiment.

Experimental setup phase 2

We germinated the plants for the second phase in January 2013 under similar growth conditions as the seedlings for phase 1, and planted them again staggered from the 28th of January to the 10th of February 2013. We planted the target species always in pots that previously contained the same species or only community plants in the following manner: target species in frequencies of one, three and six out of nine were planted in pots previously containing zero, one, three, or six conspecific plants (i.e. in phase 1). This resulted in three pots per target species for each combination of planting frequency in the second phase and planting frequency of the first phase (Fig. I.1). Thus, we achieved a fully orthogonal design. We filled the remaining positions in the pots again with community species in the same way as in phase 1. Because of variation in substrate volumes due to loss from pots during sieving, we placed 1 L of 1:1 vermiculite and sterile sand mixture at the bottom of each pot and refilled the remainder with the substrate of phase 1.

Since *Oenanthe lachenalii* germinated in insufficient numbers, we only planted this species at phase-2 frequencies of one and six out of nine plants in pots with soil of one and six plants in phase-1. Thus we finally had a set of 29 species with 36 pots each, representing three replicates of all respective combinations of phase-1 and phase-2, and one species with twelve pots, making a total of 1056 pots in phase 2. We kept the plants under the same greenhouse conditions as in phase 1, and applied the same watering regime. To reduce mortality after planting, we delayed additional lighting until the 25th of February. We then increased lighting from five to eight and finally to 14 hours in a two-day stepwise interval. We replaced dead plants within a period of two weeks from initial planting.

After a growth period of 10 weeks, we harvested the plants, again in a staggered manner following their planting sequence (8th-19th of April 2013). We dried and weighed the aboveground biomass of all plants following the same procedure as in phase 1. We counted and recorded the number of surviving plants one week before the harvest.

Analyses

Due to high mortality of target plants in phase 2, we split the statistical analysis into an analysis of survival, and, for the subset of plants that survived, an analysis of aboveground biomass. We used the proportional data on survival of target plants per pot to analyse probability of survival per target. Survival was analysed using a generalised linear mixed model with binomial error distribution in the lme4 package (Bates *et al.* 2014) in the software R Version 3.1.1. We used the optimizer "bobyqa" and set the maximum number of iterations to 100000 to achieve model convergence. Species nested in family and greenhouse compartment were included as random effects. Initial phase-1 planting frequency (0, 1, 3, 6 out of 9 plants per pot, i.e. plant-soil feedback effect), initial phase-2 planting frequency (1, 3, 6 out of 9 plants per pot, i.e. intraspecific competition), commonness as a continuous variable (number of grid cells occupied by the species in Germany; centred on the mean and scaled by the standard deviation), origin and all respective interactions were included as fixed effects in a four-way interaction model. We also added total biomass per pot in phase 1 as a covariate (centred to the mean and scaled by the standard deviation). We used stepwise backward model selection *via* likelihood-ratio tests to obtain a minimum model and to test for significance of interactions. We performed multiple pairwise comparisons to test for differences among levels of planting frequency in phase 2 using the 'multcomp' package (Hothorn, Bretz & Westfall 2008).

For the biomass analysis, we only used the subset of pots in which all target plants survived, which resulted in a data set of 719 pots (out of 1056 pots). All 30 species were represented in this subset. We used linear mixed effect models in the lme4 package to analyse per capita aboveground biomass. Per capita aboveground biomass (in grams) was natural log-transformed prior to analysis to achieve normality of the residuals. The fixed and random effects were the same as in the model used for the analysis of survival. Similarly we used stepwise backward model selection *via* likelihood-ratio tests to assess significance of the model terms. The ‘multcomp’ package was used to test for differences among levels of phase-1 and phase-2 planting frequencies. Furthermore, to ensure that our results were not affected by species that were absent in some treatment combinations, we analysed a subset of the data excluding those species (see Table I.1). The analysis was performed in the same way as for the complete data set. To assess whether relationships between per capita biomass and commonness were non-linear, we also performed the same analysis with a discretized commonness variable (rare; intermediate; common – based on clear groupings evident in the supplementary material Appendix I.1 Fig. A4). However, per capita performance was not significantly explained by this discrete measure of commonness and hence, the minimum model remained the same (data not shown).

Results

Survival response

Analysis of survival showed that none of the model terms was significant (Supplementary material Appendix I.1 Table A2). Increasing phase-1 planting frequency resulted in slightly lower survival for all plant species, however, this effect was only marginally significant and accounted only for a 3% lower survival probability between the different planting frequencies (Fig. I.2).

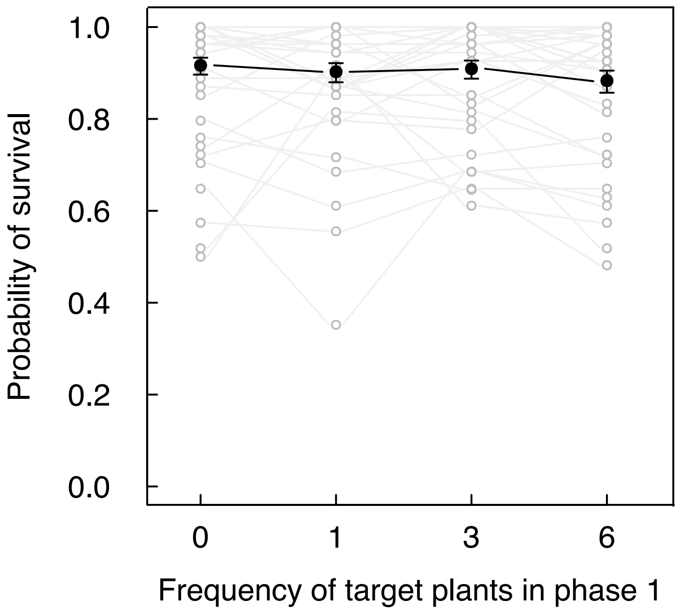


Fig. I.2: Probability of survival of target plants (± SE) of 30 species grown in soils of target plant frequencies of 0, 1, 3 and 6 out of 9 plants in phase 1 (averaged across phase-2 frequencies). Black dots display means across 30 species. Grey dots indicate means for each of the 30 species.

Biomass responses

The minimum model for per capita biomass retained phase-2 planting frequency as a significant main effect and a significant interaction between species origin and phase-1 planting frequency (Table I.2, Supplementary material Appendix I.1 Table A3). Per capita biomass of the target species was reduced by increasing intraspecific plant frequency in phase 2 (Fig. I.3). Multiple comparisons between phase-2 planting frequencies revealed that pots with six target plants in phase 2 showed a significant reduction in per capita biomass compared to pots with one target plant in phase 2 (mean difference = -0.209, 95% CI= -0.376 ; -0.041, P = 0.004, Fig. I.3). Reductions in per capita biomass of pots with six target plants compared to pots with three target plants in phase 2 (mean difference = -0.150, 95% CI = -0.324 ; 0.017, P =0.146, Fig. I.3) and of pots with three targets compared to pots with one target in phase 2 were not significant (mean difference = -0.058, 95% CI = -0.214 ; 0.097, P = 0.956, Fig. I.3).

Multiple comparisons between levels of phase-1 planting frequencies showed a marginally non-significant reduction in per capita biomass of alien species when grown on phase-1 soils containing six target plants compared to phase-1 soils containing no target plants (mean difference = -0.249, 95% CI = -0.518 ; 0.01, P = 0.092, Fig. I.4a). Notably, native species showed no significant reduction of per capita biomass when grown in soils previously occupied by six conspecifics compared to pots with no conspecifics in phase 1 (mean difference = -0.028, 95% CI = -0.304 ; 0.247, P = 1.000, Fig I.4b). Comparisons between the control and one, three or six plants in phase 1 were not significant for either aliens or natives (Supplementary material Appendix I.1 Fig A5). The covariate of total biomass per pot in phase 1 had no significant effect on per capita biomass in phase 2 (Table I.2). Our analysis showed that the relationship between species commonness and per capita plant performance did not significantly differ according to phase 1 planting frequency (Table I.2; Supplementary material Appendix I.1, Fig. A4), suggesting that density-dependent plant-soil feedback effects are not related to species commonness. The results for the analysis of the species that were represented in all treatments led to the same minimum model as for when all 30 species were included (Supplementary material Appendix I.1 Table A6), indicating that our findings were not driven by underrepresented species in our data set. To the contrary, this analysis revealed a more significant effect of phase 1 planting frequencies for alien species, and non-significant effects for native species (Supplementary material Appendix I.1 Fig. A7).

Table I.2: Results for each step of the stepwise backward model selection via likelihood-ratio tests for the linear mixed effects model of per capita aboveground biomass. The main effects 'Origin' and 'Phase-1 frequency' were not tested, as the interaction of both terms was significant and therefore was retained in the minimum model.

Term	χ^2	**df**	**p**
4-way-interaction			
Phase1 frequency *x* Phase2 frequency *x* Origin	1.006	6	0.985
3-way-interactions			
Phase1 frequency *x* Phase2 frequency *x* Origin	2.350	6	0.884
Phase2 frequency *x* Origin *x* Commonness	1.169	2	0.557
Phase2 frequency *x* Phase2 frequency *x* Commonness	5.439	6	0.488
Phase1 frequency *x* Origin *x* Commonness	6.622	3	0.084
2-way-interactions			
Origin *x* Commonness	0.000	1	0.993
Phase1 frequency *x* Phase2 frequency	1.263	6	0.973
Phase2 frequency *x* Origin	0.589	2	0.744
Phase2 frequency *x* Commonness	0.555	2	0.757
Phase1 frequency *x* Commonness	2.121	3	0.547
Phase1 frequency *x* Origin	9.309	3	**0.025**
Main effects			
Commonness	0.029	1	0.864
Total biomass Phase1	0.947	1	0.330
Phase2 frequency	11.862	1	**<0.001**
Phase1 frequency	-	-	-
Origin	-	-	-

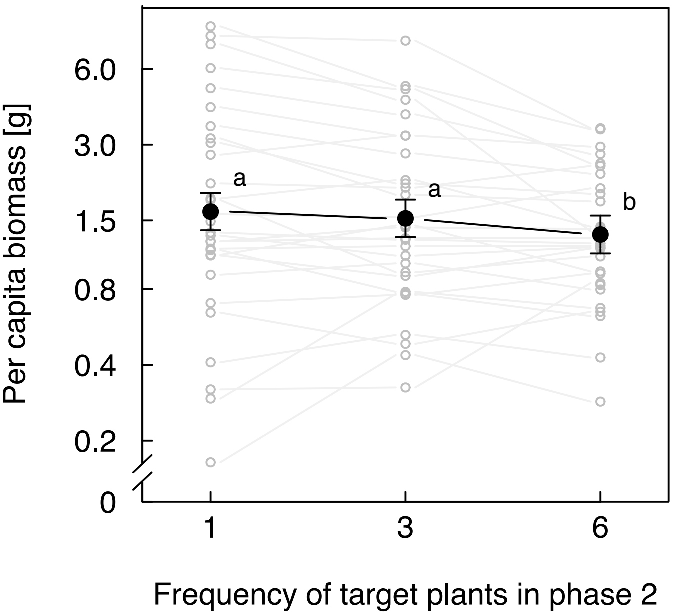


Fig. I.3: Least-square mean per capita biomass (± SE) of 30 species grown at target plant frequencies of 1, 3 and 6 out of 9 plants (averaged across phase-1 frequencies). Black dots indicate means across the 30 target species. Grey dots display means for each of the 30 target species. Letters indicate significant differences between planting frequencies. (Y-axis is displayed on natural log scale).

Discussion

In this study, we tested if differences in intraspecific competition and plant-soil feedback could explain commonness of alien and native species. We found that per capita plant performance declined with increasing intraspecific plant frequency, regardless of origin and commonness, which suggests that alien and native, and rare and common species are affected similarly by intraspecific competition. Negative plant-soil feedback effects were relatively weak, and alien but not native species showed a reduction of performance when grown in soils previously occupied by the same species at high frequencies. Furthermore, we did not find evidence for interactions between density-dependent soil effects and intraspecific competition.

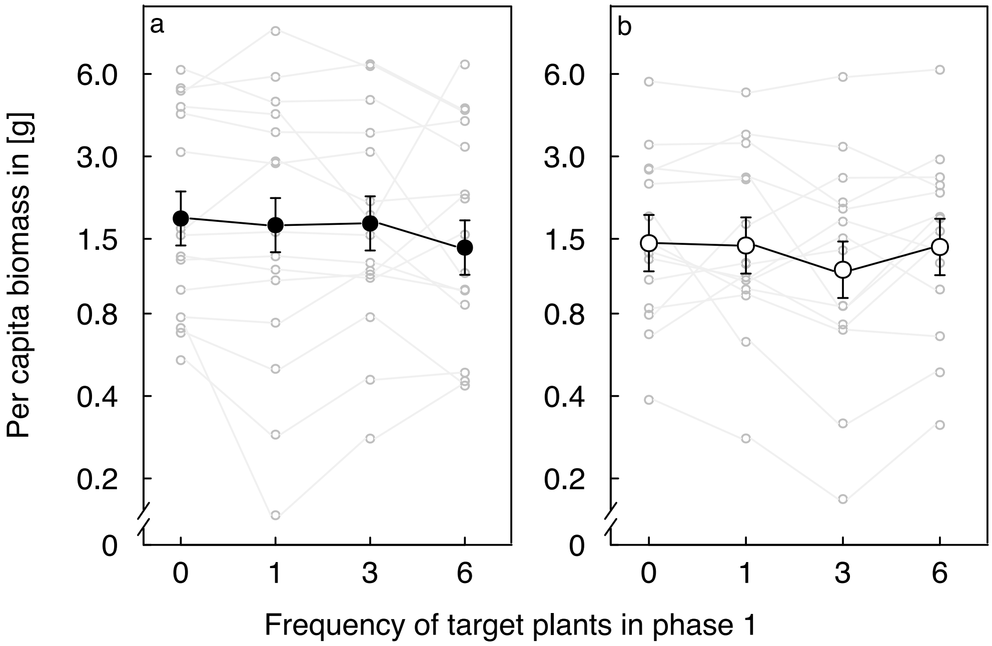


Fig. I.4: Panel a) displays least-square mean per capita biomass (± SE) of 17 alien species grown in soil previously occupied by 0, 1, 3 or 6 target plants of the same species in phase 1 (averaged across target plant frequencies in phase 2). Black dots display means across the 17 target species. Grey dots indicate the respective means for each of the 17 target species. Panel b) displays least-square mean per capita biomass (± SE) of 13 native species grown in soil previously occupied by 0, 1, 3 or 6 target plants of the same species in phase 1 (averaged across target plant frequencies in phase 2). Filled white dots display means across the 13 target species. Grey dots indicate the respective means for each of the 13 target species. (Y-axis is displayed on natural log scale in both panels)

In contrast to expectations, our study shows that growing plants in soil previously occupied by the same species at increasing frequencies did not lead to a stronger reduction of performance of rare species compared to common species. However, alien plant species showed reduced per capita biomass when grown in soil conditioned by the highest conspecific plant frequency compared to control community soil, whereas native species were generally not affected by phase-1 planting frequencies. In a previous study Klironomos (2002) found evidence for consistently positive plant-soil feedback for common alien species compared to a consistently negative feedback of rare native species. In a comparable experiment, Reinhart (2012) found a prevalence of negative plant-soil feedbacks for the focal species in semiarid grasslands, but did not find a clear pattern explaining species abundances with regard to effects of soil biota. Our findings similarly point towards a limited role of plant-soil feedback in explaining species commonness, and plant-soil feedback effects may rather be largely context dependent (Bezemer *et al.* 2006; Reinhart 2012).

An explanation for our findings, specifically the absence of a significant and concordant pattern in reduction of plant performance by plant-soil interactions, likely lies in the experimental approach that we used. We did not test plant performance in pre-trained soils in comparison to sterilized soils, which is an often-used method in plant-soil feedback experiments and which leads to stronger feedback effects (van der Putten & Peters 1997; van der Putten, Klironomos & Wardle 2007; MacDougall, Rillig & Klironomos 2011; but see Brinkman *et al.* 2010). Instead we used a set of common grassland species as a neutral community, creating a much more realistic soil-microbiome control. Consequently, the 'neutral' community could have accumulated already a high density of pathogens so that native species experienced a 'ceiling-effect', such that an increase in frequency of a target species does not lead to a substantial further reduction of biomass due to the presence of pathogens. Similarly, Maron *et al.* (2014) found that negative soil effects for species grown in soil with their native soil biota seem to develop largely independently of study species presence. This would indicate that generalists among soil pathogens might play an important role. We also found a marginally non-significant trend that phase 1 planting frequencies cause mortality in phase 2, albeit with a very small effect size that cannot fully explain greater mortality rate observed in the second phase of the experiment compared to the first. Mortality also did not differ according to species origin or commonness. This, combined with the overall greater mortality in the second phase may further indicate the accumulation of generalist pathogens within the whole experiment.

The limited role of plant-soil feedback that we find in our study does not mean that for a particular species in a particular context, plant-soil-feedback effects are not important. There are a number of studies that find negative feedback effects on plant performance (Kulmatiski *et al.* 2008) that are likely driven by species-specific pathogens (see e.g. Bezemer *et al*. 2006; Kardol *et al*. 2007; Petermann *et al*. 2008; Reinhart 2012). However, the complexity of plant-soil interactions and their dependence on multiple factors (i.e soil type, soil-legacy history, presence of competitors) often results in large variation among species and study systems (Kulmatiski *et al.* 2008), thus preventing clear, general patterns from being observed among species.

An alternative explanation for our results may be that pot limitation (Poorter *et al.* 2012) has magnified the effect of competition relative to plant-soil-feedback effects, so that effects of the soil biota were overridden by competition for root space. However, growing plants in larger pots would have reduced the potential for plants to compete, and plants may also experience intense belowground competition in natural communities (Casper & Jackson 1997). Thus, we consider the conditions under which competition and plant-soil feedback can be detected in our experiment to be reasonably realistic.

Nevertheless, alien species in our study showed a reduction in performance when grown in soil previously occupied by conspecifics at high frequencies. These findings may be explained by the nature of the interactions between alien species and their new soil biota. Alien species might on the one hand not be affected by some of the soil-borne pathogens of their new range and might even have left some of their co-evolved enemies of their native range behind. On the other hand they might be naïve towards some of their soil-borne enemies in the new range resulting in accordingly strong detrimental effects (Parker, Burkepile & Hay 2006; Parker & Gilbert 2007; Verhoeven *et al*. 2009). Verhoeven *et al.* (2009) argue that 'novelty' can be claimed for both sides of the interaction, the plant as well as the pathogen. Consequently there can be a mismatch that leads to enemy release, but also a mismatch that leads to biotic resistance, which may explain the unexpected divergent plant-soil feedback effects on alien and native species in our study.

However, since the selected species in our experiment have been present in Germany for at least two centuries, alien species and their respective pathogens might have already adapted, resulting in the reduced performance of the alien species that we observe in our experiment. For example, Diez *et al.* (2010) found that the negative plant-soil-feedback effect of alien species in New Zealand increased with increasing residence time. In contrast, Speek *et al.* (2015) did not find such a pattern among alien species in a multi-species study in the Netherlands. These contrasting findings reflect the complexity of plant-pathogen interactions, such that changes in the effects of interactions over time are unlikely to be consistent.

We found that an increase in current conspecific frequency led to a strong reduction in per capita biomass. This finding is in line with the predictions of coexistence theory, namely that intraspecific competition should affect species performance more negatively than interspecific competition (Chesson 2000). Despite its clear theoretical underpinning, empirical proof of this concept has been equivocal (Goldberg & Barton 1992; Gurevitch *et al.* 1992; but see Levin & HilleRisLambers 2009). Our study supports the idea of stronger self-limitation, but we did not find evidence for any differences in density-dependent intraspecific competition between alien and native species, or in relation to commonness. However, it is important to note that interspecific effects could have an influence on the performance of the focal species in species mixtures, as other studies show (Bezemer *et al*. 2006; Kardol *et al.* 2007). Kardol *et al*. (2007) for example reported that a selection of early successional plant species showed differential responses towards heterospecific soil inocula, ranging from positive to negative. In our case this specifically means that interspecific effects of the community species, e.g *Phleum pratense*, may influence our results besides intraspecific effects of the targets. Due to the design of our study, which aimed to entail a realistic grassland background community, we cannot fully assess this role of interspecific effects. Nonetheless, Blank (2010) also reports on stronger effects of intraspecific competition compared to interspecific competition for a set of native and alien species, but highlights that alien species might gain advantage over natives by better capitalizing on nutrients in highly fertile soils (see also Dawson, Fischer & van Kleunen 2012). This may also explain the higher per capita performance of aliens compared to natives in our study, since plants were grown with addition of slow-release fertilizer and should therefore not have been limited in nutrient supply. Furthermore, Duralia & Reader (1993) tested if abundance of three prairie grasses is explained by competitive ability in a replacement series experiment, and found only weak evidence for a relationship between commonness and competitive ability. Despite the theoretical importance of density-dependent self-limitation in regulating species abundance and coexistence, we found no evidence that it covaries with commonness of either alien or native species.

Although plant-soil feedback has been proposed as a mechanism that could drive species success (Klironomos 2002; van der Putten *et al.* 2013), we found no evidence that commonness is explained by differences in density-dependent plant-soil feedback. A meta-analysis on the effects of plant-soil feedback by Kulmatiski *et al.* (2008) showed that there is a general signal for a reduction in plant performance due to plant-soil feedback. However, Kulmatiski *et al.* (2008) as well as van de Voorde, van der Putten & Bezemer (2012) and Brinkman *et al.* (2010) raise the point that varying experimental protocols and a bias towards simplified greenhouse studies, each with a limited but different set of target species and life forms, may account for a considerable amount of variation in study outcomes. Another aspect in studies on plant-soil feedback is that only net outcomes of plant-soil interactions are measured (e.g. biomass), however, this overall performance results from potentially multiple antagonistic (e.g. pathogens) and mutualistic (e.g mycorrhiza) interactions and physical properties of the soil (Reinhart & Callaway 2006; van der Putten *et al.* 2013). Disentangling the relative contributions of antagonistic and mutualistic soil organisms under controlled conditions will provide important insights into the underlying mechanisms. Moreover, transferring these insights to manipulative experiments under realistic field conditions with a focus on population dynamics covering the whole life cycle of a study organism (Maron, Horvitz & Williams 2010; Flory & Clay 2013) may lead the way to a better understanding of plant-soil interactions.

A specific aspect that arises by studying the role of plant-soil interactions in driving species commonness is the two-way nature of species commonness in this relationship. On the one hand, commonness may be the outcome or in part be influenced by plant-soil interactions, while on the other hand the commonness of a species is also a factor that determines the chances for pathogens and other soil biota to encounter (and potentially adapt to) their host or *vice versa*. Hence, commonness is a dependent and independent variable at the same time. Furthermore the chances of encountering a pathogen (or a host) also depend on dispersal abilities of both parts of the pathogen-plant interaction, which adds spatial and temporal dimensions to plant-soil interactions (Adler & Muller-Landau 2005). Considering these spatial and temporal dimensions may help to improve our understanding of whether plant-soil feedbacks drive the patterns of commonness we observe in both alien and native plant species.

Conclusions

Our study suggests that plant-soil feedback plays little role in determining the success of common native and alien species compared to rare natives and aliens, at least for a number of species from several families in Central Europe. Furthermore, species responses to increasing intraspecific frequency are not linked to commonness and origin, suggesting that intraspecific competitive ability does not in general drive alien and native plant species commonness or rarity. Contrary to the notion of enemy release, we also found that alien species suffer more from plant-soil feedback than native species. Rather than being released from soil-borne pathogens, alien species may suffer more from novel belowground interactions; alternatively, adaptation of pathogens to the alien species may have already taken place, resulting in negative feedback. Thus, enemy release needs to be interpreted as a potentially dynamic process since a static interpretation may not capture the evolutionary aspects in the plant-pathogen interaction. While we found no clear role for plant-soil feedback in explaining current commonness of alien and native species, future studies should consider whether plant-soil feedback is linked to dynamic changes in species commonness over time.

Acknowledgements

G.M. and W.D. were funded by the Deutsche Forschungsgemeinschaft (Project DA 1502/1-1). We thank O.Ficht, Tilman Rönneburg, Sina Glöckner and Anna Gorenflo for horticultural assistance and practical help. Thanks also go to the International Max Planck Research School for Organismal Biology for supporting G.M.

Appendices

Appendix I.1 Table A1. Species present in the field sites from which the soil inoculum was taken. Survey for presence/absence of species was conducted on 48 plots (3x4m) with 3 quadrats (50cm x 50cm) per plot for another experiment on the same site. Species that are either target or community species in our experiment are in bold font.

Species present on field sites (soil inoculum)

Achillea millefolium
Agrimonia eupatoria
Ajuga reptans
Alopecurus pratensis
Bellis perennis
Campanulas rotundifolia
Cardamine flexuosa
Cardamine pratensis
Carex flacca
Centaurea jacea
Cerastium fontanum
Cerastium hirsutum
Cirsium olearaceum
Colchicum autumnale
Convolvulus arvensis
Daucus carota
Festuca rubra
Filipendula ulmeria
Galium album
Geum rivale
Geum urbanum
Glechoma hederacea
Heracleum sphondylium
Hieracium spp.
Holcus lanatus
Knautia arvensis
Lathyrus pratensis
Lotus corniculatus
Luzula campestris
Lysimachia nummularia
Medicago lupulina
Phleum pratense
Pimpinella saxifraga
Pimpinella major
Plantago lanceolata
Poa pratensis
Potentilla reptans
Ranunculus acris
Ranunculus repens
Rhinanthus alectorolophus
Rumex acetosa
Rumex obtusifolius
Taraxacum officinale
Tragopogon pratensis
Trifolium pratense
Trifolium repens
Veronica chamaedrys
Vicia cracca
Vicia sepia

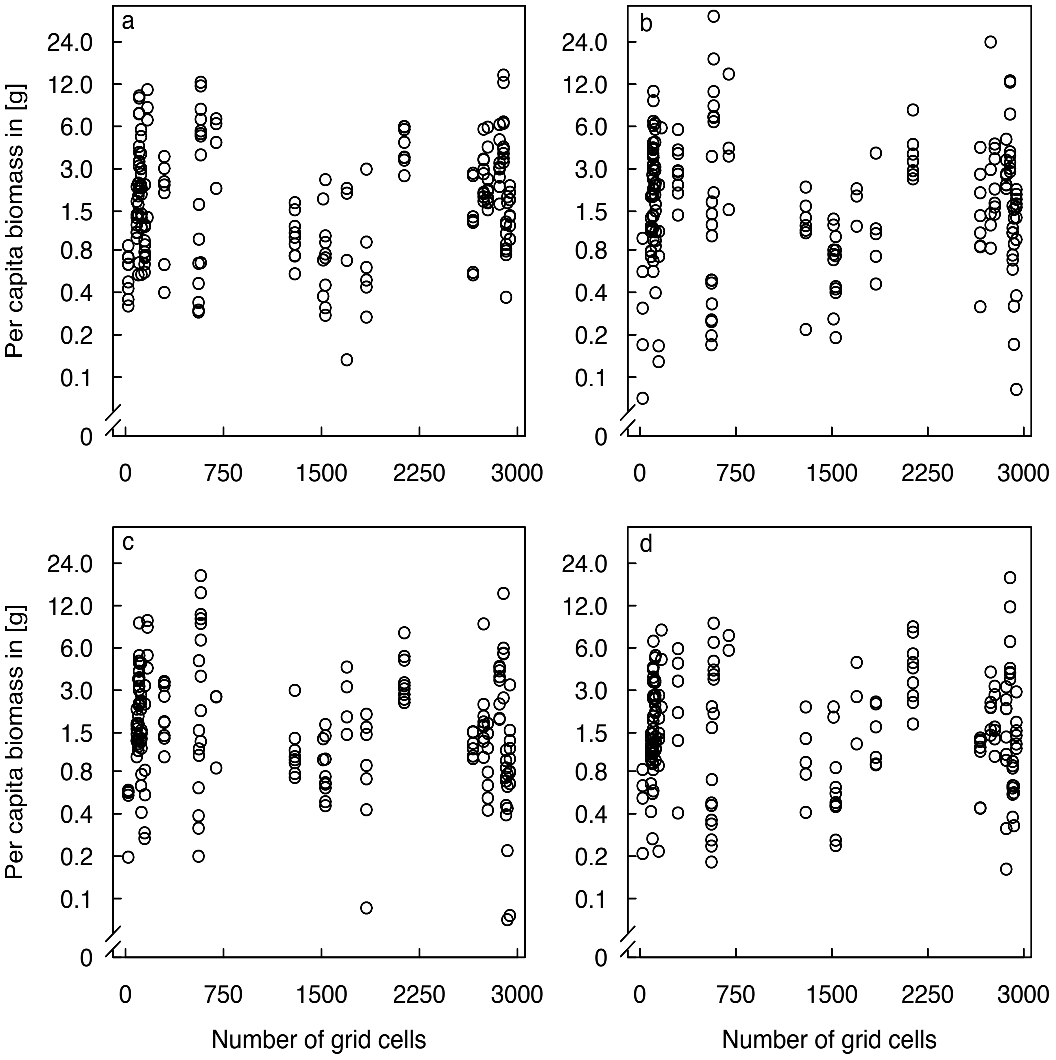
Appendix I.1 Table A2. Results for each step of the stepwise backward model selection via likelihood-ratio tests for the generalized linear mixed effects model of survival.

Term	χ^2	**df**	**p**
4-way-interaction			
Phase1 frequency *x* Phase2 frequency *x* Origin *x* Commonness	3.817	6	0.701
3-way-interactions			
Phase1 frequency *x* Phase2 frequency *x* Origin	5.894	6	0.435
Phase2 frequency *x* Origin *x* Commonness	1.832	2	0.399
Phase1 frequency *x* Phase2 frequency *x* Commonness	7.166	2	0.305
Phase1 frequency *x* Origin *x* Commonness	4.405	3	0.220
2-way-interactions			
Phase1 frequency *x* Phase2 frequency	3.596	6	0.731
Phase2 frequency *x* Commonness	2.088	2	0.352
Origin *x* Commonness	1.229	1	0.267
Origin *x* Phase2 frequency	4.136	2	0.126
Phase1 frequency *x* Origin	6.169	3	0.103
Phase1 frequency *x* Commonness	4.585	3	0.204
Main effects			
Total biomass Phase1	0.022	1	0.880
Origin	0.273	1	0.603
Phase2 frequency	2.053	2	0.358
Commonness	1.347	1	0.245
Phase1 frequency	7.039	3	0.070

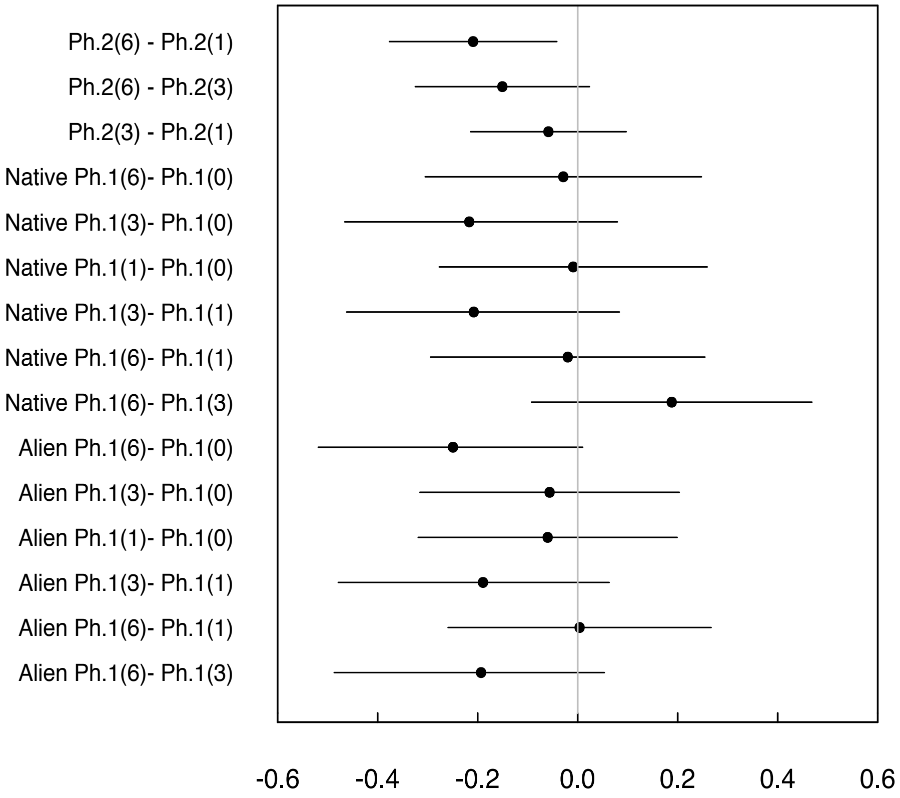
Appendix I.1 Table A3. Minimum linear mixed effects model for per capita biomass of 30 alien and native plant species grown at different frequencies in phase 1 (0, 1, 3, 6) and different frequencies in phase 2 (1, 3, 6).

Parameters	**Estimate (Std. error)**	**t-value**
Fixed Effects		
Intercept [alien, Ph.1(0), Ph.2 (1)]	0.666 (0.224)	2.965
Ph.2 frequency (3)	-0.061 (0.054)	-1.141
Ph.2 frequency (6)	-0.209 (0.058)	-3.602
Ph.1 frequency (1)	-0.055 (0.089)	-0.624
Ph.1 frequency (3)	-0.041 (0.089)	-0.464
Ph.1 frequency (6)	-0.244 (0.091)	-2.666
Origin (native)	-0.207 (0.306)	-0.677
Origin (native): Ph.1 frequency (1)	0.036 (0.128)	0.280
Origin (native): Ph.1 frequency (3)	-0.182 (0.129)	-1.411
Origin (native): Ph.1 frequency (6)	0.212 (0.131)	1.620
Random Effects	**Std. deviation**	
Family	0.148	
Species nested in family	0.796	
Greenhouse	0.041	

Appendix I.1 Fig A4. Relationship between per capita biomass for the 30 target species and commonness measured in grid cells for the different levels of phase 1 planting frequencies. Panels display a) phase 1 planting frequencies of 0, b) for phase 1 frequencies of 1, c) for phase 1 frequencies of 3 and d) for phase 1 planting frequencies of 6 target plants.



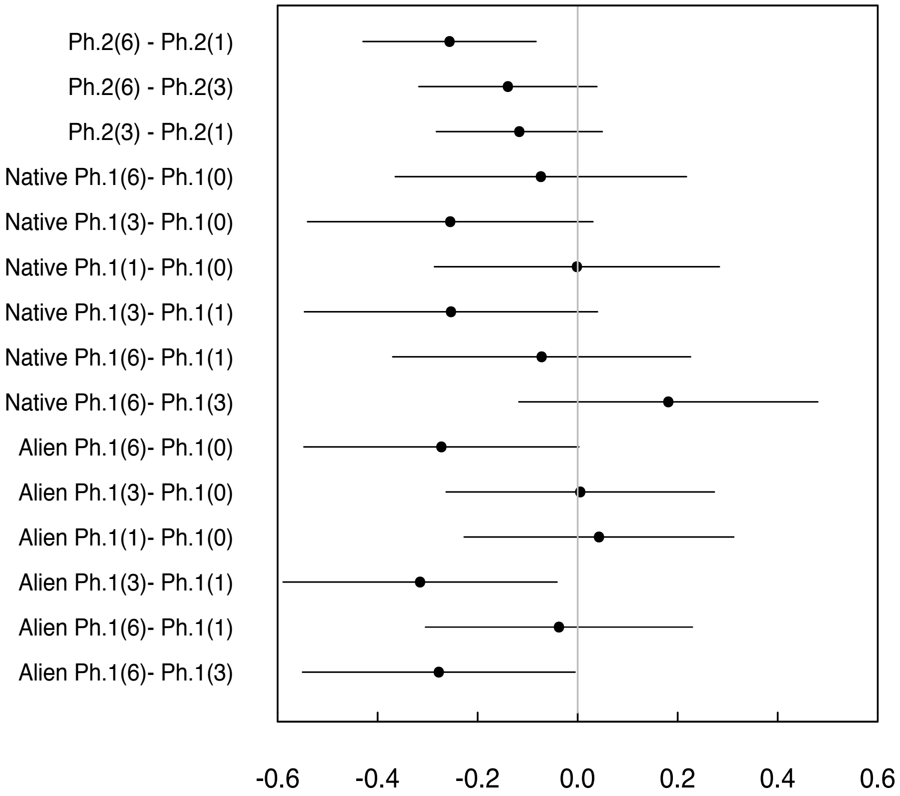
Appendix I.1 Fig A5. Estimates and 95% confidence intervals of differences between levels of phase 1 and phase 2 frequencies in per capita biomass (natural log transformed) using multcomp "single-step" correction procedure for multiple testing.



Appendix I.1 Table A6. Results for each step of the stepwise backward model selection via likelihood-ratio tests for the linear mixed effects model of per capita aboveground biomass *for species represented in all treatments.* The main effects 'Origin' and 'Phase-1 frequency' were not tested, as the interaction of both terms was significant and therefore was retained in the minimum model.

Term	χ^2	**df**	**p**
4-way-interaction			
Phase1 frequency *x* Phase2 frequency *x* Origin *x* Commonness	2.218	6	0.896
3-way-interactions			
Phase1 frequency *x* Phase2 frequency *x* Origin	2.061	6	0.899
Phase2 frequency *x* Origin *x* Commonness	1.094	2	0.578
Phase2 frequency *x* Phase2 frequency *x* Commonness	8.943	6	0.176
Phase1 frequency *x* Origin *x* Commonness	6.459	3	0.091
2-way-interactions			
Phase2 frequency *x* Origin	0.134	2	0.934
Origin *x* Commonness	0.011	1	0.916
Phase1 *x* Phase2 frequency	2.404	6	0.879
Phase2 frequency *x* Commonness	1.068	2	0.586
Phase1 frequency *x* Commonness	2.407	3	0.492
Phase1 frequency *x* Origin	11.70	3	**0.008**
Main effects			
Commonness	0.353	1	0.552
Total biomass Phase1	0.872	1	0.350
Phase2 frequency	18.322	1	**<0.001**
Phase1 frequency	-	-	-
Origin	-	-	-

Appendix I.1 Fig A7. Estimates and 95% confidence intervals of differences between levels of phase 1 and phase 2 frequencies in per capita biomass (natural log transformed) *for the model including only species represented in all treatments* using multcomp “single-step” correction procedure for multiple testing.



Chapter II

Alien species profit from weaker effects of self-limitation but not from belowground enemy release

Gregor Müller, Mark van Kleunen and Wayne Dawson

Journal of Ecology (submitted)

Abstract

1. Interactions between plants and soil have recently gained growing attention, and it has been proposed that plant-soil interactions can play a major role in explaining species success. Moreover, differences in magnitude of self-limitation among species would also allow for differences in species abundances. However, the relative roles that these two mechanisms and their interaction play in determining species success or failure, has rarely been investigated.

2. To disentangle the roles of self-limitation due to intraspecific competition and soil-borne pathogens in explaining species success, we used a mesocosm experiment in which we grew a set of 20 annual common and rare species of alien and native origin from three different families over two generations. Species communities were initially sown at two frequency levels (high/low) and were regularly treated with or without fungicide. We recorded the number of (reproductive) individuals, the number of seeds produced as well as the number of offspring surviving in the 2^{nd} generation to test for the effects of intraspecific frequency and pathogens on population-level performance.

3. We found that per capita population growth was generally higher at lower initial sowing frequencies, while alien species disproportionally profited from weaker effects of self-limitation compared to natives. Moreover, fungicide treatment led to a higher number of surviving offspring in the second generation for both alien and native species.

4. *Synthesis:* We conclude that annual alien species might gain an advantage from weaker effects of self-limitation under low sowing frequencies. This may allow them to establish and help explain their success in invading plant communities and spreading in their introduced range. Notably, alien species similarly profited from fungicide treatments as native species. While this contradicts the enemy release hypothesis, it shows that alien species may similarly be attacked by generalist pathogens.

Introduction

While invasive species have received growing research attention over the last few decades, the question what makes an alien species successful still remains partly unanswered. The interplay of biological processes that allows a species to reach and maintain a high abundance, to spread and often achieve a wide distribution (Gaston 2003), may not only be applicable to aliens but also to native species (Thompson, Hodgson & Rich 1995; van Kleunen *et al.* 2010). Consequently, common alien and common native species may rely on the same drivers for their success, and similarly, rare natives and rare aliens could be limited by the same factors.

Recently, interactions between plants and soil (i.e. plant-soil feedback), specifically pathogenic and mutualistic biotic interactions, have been proposed to play a potentially important role for plant performance (Mordecai 2011; van der Putten *et al.* 2013). It has been shown that plant-soil feedback can mediate species coexistence (Packer & Clay 2000; Bever 2003; Bonanomi, Giannino & Mazzoleni 2005, HilleRisLambers *et al.* 2012; Aponte, Garcia & Maranon 2013; Brandt *et al.* 2013; Burns & Brandt 2014), and therefore can be important for maintaining species diversity (Bever, Westover & Antonovics 1997; Mills & Bever 1998; Reynolds *et al.* 2003; Rodriguez-Echeverria *et al.* 2013; Bever, Mangan & Alexander 2015). Moreover, species range expansions (Eppinga *et al.* 2006; van Grunsven *et al.* 2007; but see Levine *et al.* 2006; Diez *et al.* 2010) or their spread in a non-native range, and thus their potential to invade can also be influenced by plant-soil interactions, specifically due to belowground enemy release (Reinhart *et al*. 2003; Callaway *et al.* 2004; Suding *et al.* 2013; but see Beckstead & Parker 2003). In the same vein, plant-soil feedback has been put forward as an important factor explaining differences in species abundance (Klironomos 2002; MacDougall, Rillig & Klironomos 2011; McCarthy-Neuman & Ibanez 2013, but see Reinhart 2012). For example, Mangan *et al.* (2010) showed in a combined shade-house and field study that the presence of soil biota of adult trees reduced the performance of conspecific tree seedlings but not of heterospecific seedlings. Moreover, species that showed a stronger negative feedback were also found to be less common as adults in the forest community, linking individual performance with abundance. Mangan *et al.* (2010) also found that the reduction in performance could largely be attributed to belowground enemies and not to mammalian herbivores and foliar pathogens. Thus species-specific differences in the strength of plant-soil feedback may ultimately lead to differences in species commonness. So far, there have mainly been theoretical (Bever, Westover & Antonovics 1997; Bonanomi, Giannino & Mazzoleni 2005) and correlative assessments (Mangan *et al.* 2010; Klironomos 2002) of the role of plant-soil feedback in explaining species commonness. Most experimental studies on plant-soil feedback focused on individual plant performance. So, while there are studies on e.g. above ground enemies on population dynamics (Munzbergova 2005; Royo, Bates & Lacey 2008; Maron, Horvitz & Williams 2010), there is a lack of studies that experimentally test how soil biota affect reproductive success and population-level performance in alien and native species. Studies on such demographic processes are, however, urgently needed to better understand the success of invasive species (Gurevitch *et al.* 2011).

Another mechanism that could govern differences in species relative abundance in a community is the degree of self-limitation due to intraspecific competition. To allow for stable species coexistence in a community, the classical conceptual model assumes that a species experiences higher intraspecific than interspecific competition (Chesson 2000; Adler 2011; Chisholm & Muller-Landau 2011; HilleRisLambers *et al.* 2012). Consequently, if species differ in the degree of self-limitation (i.e. the strength of negative impact of intraspecific competition relative to interspecific competition), they should differ in abundance. For example, if one species suffers less from frequency-dependent intraspecific competition than another, it should achieve a higher relative abundance in the community. Such a case has been described by Comita *et al.* (2010), who found that rare species in a tropical rainforest suffered more from conspecific neighbours than common species did. Thus asymmetries in self-limitation may be an important factor explaining species commonness.

Differences in plant-soil feedback and in magnitude of intraspecific competition, as well as their roles for species relative abundances and commonness, may also interact (Casper & Castelli 2007; Petermann *et al.* 2008; Hodge & Fitter 2013; McCarthy-Neumann & Ibanez 2013). However, the importance of plant-soil feedback in relation to other processes such as competition is not well understood (Casper & Castelli 2007). Therefore, a combined approach testing both factors simultaneously is needed to shed light on the interplay between competition and plant-soil feedback effects on population dynamics. This will allow us to better understand the roles of these mechanisms in determining species commonness.

To test for the roles of plant-soil feedback and intra- *versus* interspecific competition in explaining species commonness, we conducted a two-year (i.e. two generations) multi-species mesocosm experiment. We used 20 different annual species in five quadruplets of taxonomically related common and rare species of alien and native origin. We grew these 20 target species in two-species communities consisting of the respective target species and the common grass species *Bromus hordeaceus*, and applied a fungicide treatment (fungicide/water control) and a sowing frequency treatment (high/low) in an orthogonal design. This allowed us to test the effect of intra- *versus* interspecific competition as well as the influence of soil-borne pathogen accumulation (specifically fungi and oomycetes). Furthermore, as we recorded reproductive output (seeds) and number of offspring (survivors in the 2nd generation), we were able to assess the effects of soil-biota and differences in self-limitation on population-level performance.

We asked the following questions: 1) Is population growth of common species less limited by intraspecific competition than it is for rare species? 2) Is population growth of common species less limited by soil fungal pathogens than it is for rare species? 3) Do effects of intraspecific competition and soil fungal pathogens on common and rare species interact? 4) Do alien species profit from belowground enemy release and thus show weaker effects of fungicide treatment or intraspecific competition than native species?

Methods

Target species

In order to be able to generalize results beyond a few study species (van Kleunen *et al.* 2014), we conducted a multi-species mesocosm experiment in a common garden using 20 annual species from four different families (Table II.1). The chosen target species represented five taxonomic groups that contained one common native, one rare native, one common alien and one rare alien plant species. As a measure for the degree of commonness of each species, we used the number of 130-km^2 grid cells occupied by the species in Germany (maximum 3000) extracted from the FloraWeb database (FloraWeb, Bundesamt für Naturschutz, accessed 17th March 2016). Common species occurred in at least 600 grid cells (median = 2048.5), and rare species occurred in fewer than 600 grid cells (median = 177.5). We aimed to choose species that occur in similar habitats, excluding habitat specialists, and species that are not rare simply due to their geographic distribution overlapping only marginally with the borders of Germany.

Table II.1. The 20 target species, listed by status of origin (alien, native; extracted from BiolFlor database (Klotz, Kühn & Durka 2002)) and family, and their respective commonness, measured as the number of ~130 km^2 grid cells occupied in Germany out of a maximum of 3000 grid cells (FloraWeb, Bundesamt für Naturschutz).

Alien			**Native**		
Species	**Grid cells**	**commonness**	**Species**	**Grid cells**	**commonness**
Asteraceae					
Artemisia annua	*151*	rare	*Bidens tripartita*	2706	common
*Conyza canadensis**	*2898*	common	*Filago pyramidata*	44	rare
Galinsoga parviflora	*2542*	common	*Lactuca saligna**	119	rare
Guizotia abyssinica	*200*	rare	*Sonchus oleracaeus*	2932	common
Brassicaceae					
Brassica nigra	*677*	common	*Alyssum alyssoides*	1220	common
Calepina irregularis	*52*	rare	*Capsella bursa-pastoris*	2981	common
Lepidium densiflorum	*571*	rare	*Draba muralis*	276	rare
Lepidium virginicum	*849*	common	*Erysimum repandum*	209	rare
Caryophyllaceae					
Silene gallica	*155*	rare	*Holosteum umbellatum*	1555	common
Silene dichotoma	*629*	common	*Silene conica**	214	rare

* Species failed to reproduce in the 1st season, and was excluded from further analysis

Experimental setup

In this two-year mesocosm experiment (2013-2014), we used a two-species community approach in which we sowed each of the target species along with *Bromus hordeaceus.* The latter is a common annual or biennial grass species in Germany (occurring in 2928 out of 3000 grid cells) and was used as a matrix species to represent a simple grassland community. The experiment was located outside in the botanical garden of the University of Konstanz (47°41’31.49’’N, 9°10’46.09’’E). The two-species communities were sown into 35-L pots (31cm height x 38 cm diameter), each of them placed approximately 50 cm apart and filled with Rasenerde Top Dressing (ökohum GmbH, 88518 Herbertingen, Germany) with 500 ml soil inoculum mixed in to ensure the presence of soil biota, including pathogens and mycorrhizal fungi. For the soil inoculum, we collected approximately 200 L soil from a grassland area in the vicinity of the University of Konstanz. We systematically took 5-10 samples (10 cm deep, 20 x 20 cm wide) along transects with a spacing of approximately 5 m between each sampling point. We pooled the samples and homogenised the soil by sieving through a 5 mm mesh to remove stones, roots and other plant material. The sieved soil was then used as a soil inoculum for each pot.

The experimental setup consisted of a sowing frequency of the target species (high/low) and a fungicide treatment (fungicide application/water control). We replicated each treatment combination for each target species four times resulting in four blocks of each set of replicates. This sums up to a total number of 320 pots (20 species x 2 treatments x 2 levels x 4 replicates = 320 pots). The two levels of the intraspecific frequency treatment for the target species were either high (200 seeds of the target and 50 of *B. hordeaceus*) or low (50 seeds of the target and 200 of *B. hordeaceus*). Thus all pots received the same total number of 250 seeds.

For the fungicide treatment, we applied either fungicide or water as a procedural control. We used a mixture of two fungicides: Fonganil Gold® (Syngenta Agro GmbH, 63477 Maintal, Germany) and Cercobin FL® (BASF, 67056 Ludwigshafen, Germany). The active ingredient in Fonganil Gold® is metalaxyl-m (comparable to Ridomil Gold®), and in Cercobin FL® thiophanat-methyl (compareable to Cleary`s 3336®). Metalaxyl-m and thiophanat-methyl are both systemic agents that are taken up through the root system and accumulate in the plant tissue. Metalaxyl-m acts against soil-borne pathogens, such as *Pythium* and *Phytophthora* species (Oomycota). Thiophanat-methyl acts against *Sclerotinia sclerotiorum* and *Fusarium* spp. by suppressing the respiration capacities of the fungi (Hirschfeldt *et al.* 2009), and is also used for protection against mildew and rot in stone fruit storage. We used a mix of both fungicides containing concentrations of 0.013% of Fonganil Gold® and 2ml/L of Cercobin FL® (following the manufacturer’s recommendations). We applied 300 ml of this solution to every pot in the fungicide treatment using squeeze bottles. The other half of the pots received 300 ml of water as a control treatment. The fungicide was applied three times in the first growing season (21st June 2013, 24th July 2013 and 11th September 2013) and three times in the 2nd growing season (20th May 2014, 25th of July 2014 and 5th September 2014). The three applications aimed to cover the early seedling development, flowering and seed ripening phases.

The seeds were sown on the 20th and 21st of May 2013. Pots were watered when necessary throughout the growing season. During the seed-ripening phase of the 1st season, seeds of the species were collected daily and stored in separate paper bags per pot for re-sowing in the following season. We harvested and counted all reproductive and non-reproductive individuals separately per pot during the 8th – 17th of December 2013. Individuals of species with early seed set (*Capsella bursa-pastoris, Draba muralis,* *Holosteum umbellatum*) were marked with tooth picks during the seed collection phase to ensure a precise recording in case plants started to decay quickly after seed set.

To test for potential side effects of the fungicide on arbuscular mycorrhizal fungi, we additionally took soil and root samples with a soil corer (5 cm diameter) (for details, see Appendix S2) on the 7th of September 2013. The collected root samples were stained and mycorrhizal structures (arbuscules, vesicles and hyphae) of *B. hordeaceus* and target-species roots were counted, using a microscope, at 100 intersections.

In the 2nd season (2014), we sowed the species with the seeds produced during the 1st season (2013) on the 16th and 17th of April 2014. *Bromus hordeaceus* did not produce seeds in the 1st season and was sown in the respective 1st year densities (200 or 50 seeds per pot). Similar to the 1st season, we harvested and counted all plant individuals at the end of the growing season (29th October – 11th November 2014).

Statistical analysis

To analyse the total number of plants in the 1st season, we used a negative binomial generalized linear mixed model (nbinom) with a log link function from the glmmADMB package (Fournier *et al.* 2012) in the software R, Version 3.2.0 (R Core Team 2015). We used a negative binomial model instead of a Poisson model, as the latter was not adequate due to overdispersion of the data. We used the total number of individuals in the first season as the dependent variable. To account for taxonomic relatedness among species and non-independence of replicates within species, we included species nested in family as random effects. Initially, we also included block as a random effect, but this led to non-converging models. Therefore, to account for variation due to position of the pot in the botanical garden, we included block as a fixed effect. Sowing frequency treatment (high/low), fungicide treatment (with/without), species origin (alien/native), species commonness (common/rare) and all respective interactions were included as fixed effects in a four-way interaction model. Stepwise backward model selection *via* likelihood-ratio tests was used to obtain a minimum adequate model, and to test for significance of interactions and main effects. To test for differences between levels of the terms in significant interactions or fixed effects, we performed multiple pairwise comparisons using the 'multcomp' package (Hothorn, Bretz & Westfall 2008).

As we had to exclude pots in which the species did not reproduce in the 1st season for further analysis (number of seeds, number of offspring per reproductive individual; see below), we analysed and report here on the results for the same data set for all analyses. Thus, we excluded three species (the common alien *Conyza canadensis*, and the rare natives *Lactuca saligna* and *Silene conica* did not reproduce at all*;* 3x16 = 48 pots; another 20 pots of various species also had to be excluded due to a lack of seed production), as well as two pots in which by accident the wrong species had been re-sown in the second year (320 - 48 - 20 - 2 = 250 pots remaining in total). Moreover, results of the analysis with the full data set including all pots and species for the total number of plants, as well as for proportion of flowering individuals in the 1st season (see below) did not differ from the results of the smaller subset (results not shown).

To test for effects of the treatments, and commonness and status of the target species on proportion of flowering individuals in the 1st season, we again used a negative binomial model as described above. However, we used the number of flowering individuals in the 1st season as the dependent variable and, to make it proportional, the total number of individuals in the 1st season as an offset. Similarly, to analyse the number of seeds produced per reproductive individual in the 1st season, we used the same model as described above but with total number of seeds produced per pot in the 1st season as the dependent variable and the number of reproductive individuals in the 1st season as an offset.

Lastly, to test for effects of the treatments, and commonness and status of the target species on population growth, we analysed the number of offspring in the 2nd season per reproductive individual in the 1st season with a negative binomial model having number of surviving plants in the second season as the dependent variable and the number of reproductive individuals in the first season as an offset. All procedures for statistical tests for the mentioned analysis were the same as described above for the other variables.

Results

Total number of individuals, proportion of flowering individuals and number of seeds produced per reproductive plant in the 1st season

The minimum adequate model explaining the total number of plants in the 1st season contained a significant three-way interaction involving sowing frequency, species origin and commonness (Appendix II.S1 Table A1, Table A2). Alien, as well as native species showed higher numbers of individuals at high sowing frequencies with the strongest effect for rare native species (mean difference = 0.736 SE = ±0.062, P <0.001) followed by rare alien (mean difference = 0.659, SE = ±0.051, P <0.001) and common alien species (mean difference = 0.658 SE = ±0.051, P <0.001) and the weakest effect for common native species (mean difference = 0.499 SE = ±0.049, P <0.001). All other pairwise comparisons were not significant.

The median for proportion of flowering individuals in the 1st season was 65.47%, and the median number of seeds per reproductive individual in the first season was 123.3. The minimum adequate models explaining the proportion of flowering individuals and the number of seeds produced both retained no significant fixed term (Appendix II.S1 Tables A3 and A4). This indicates that the proportion of flowering individuals and the number of seeds per reproductive individual was similar in the different treatments and for the different status groups.

Number of offspring in the 2nd season per reproductive plant in the 1st season

The median number of offspring in the 2nd season per reproductive individual in the 1st season was 2.10. The minimum adequate model explaining the number of offspring contained a significant two-way interaction involving sowing frequency and species origin, and the significant main effect of fungicide (Table II.2, Appendix II.S1 Table A5). Overall, target species produced significantly more offspring per capita when sown at low initial frequencies compared to high initial sowing frequencies (Table II.2). However, as indicated by the significant frequency x origin interaction (Table II.2), the strength of the frequency effect was stronger for alien (mean difference = 0.996, SE = ±0.226, P <0.001, Fig. II.1, Appendix II.S1 Figure A2) than for native species (mean difference = 0.205, SE = ±0.253, P = 0.810), implying weaker self-limitation in alien species at lower initial frequency. The treatment of plants with fungicide led to a significantly higher number of offspring per capita (mean difference = 0.377, SE = ± 0.153, p = 0.013, Fig. II.2, Appendix II.S1 Figure A2) compared to the control treatment. This higher performance in number of offspring in response to fungicide occurred across species origins and commonness as well as initial sowing frequencies, as none of the interactions of fungicide treatment with frequency, origin or commonness was retained in the minimum model.

Table II.2. Minimum generalized linear mixed effects model for number of offspring per reproducing individual in the 2^{nd} season for 9 alien and 8 native rare and common plant species under high and low initial sowing frequencies and fungicide or water control treatment.

Parameter	**Estimate (SE)**	**z-value**	**p-value**
Fixed effects			
Intercept	2.206 (0.525)	4.195	<0.001
Frequency (high)	-1.004 (0.227)	4.415	<0.001
Fungicide (no)	-0.359 (0.155)	-2.311	0.021
Origin (native)	-1.080 (0.734)	-1.47	0.141
Block (B)	0.137 (0.218)	0.626	0.531
Block (C)	0.087 (0.218)	0.401	0.688
Block (D)	0.169 (0.226)	0.748	0.454
Frequency (high) : Status (native)	0.718 (0.341)	2.104	0.035
Random effects	**Std. deviation**		
Family	<0.001		
Species nested in family	1.424		

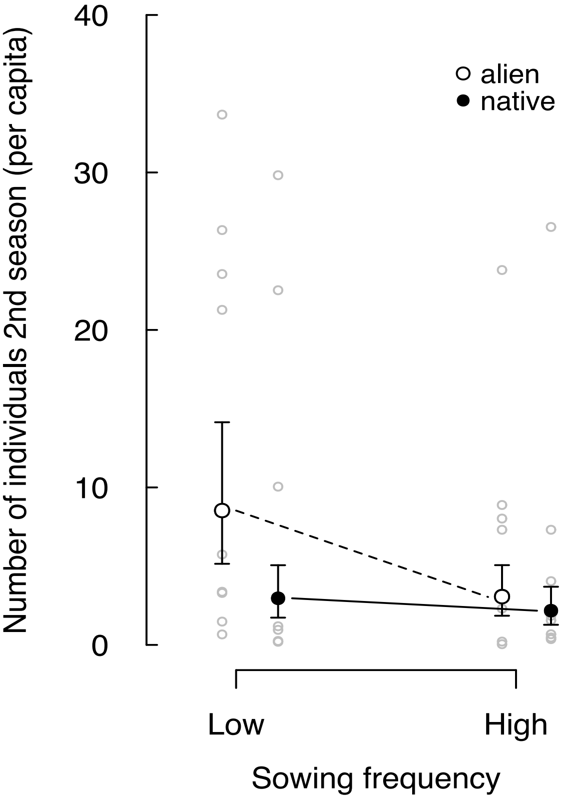


Fig.II.1: Number of offspring in the 2^{nd} season per reproductive individual in the 1^{st} season (± SE) of 9 alien (5 rare and 4 common) and 8 native (3 rare and 5 common) species under high and low initial sowing densities. Filled black dots (natives) and open white dots (aliens) display means across species for the respective groups, while grey dots indicate raw data means for each of the species.

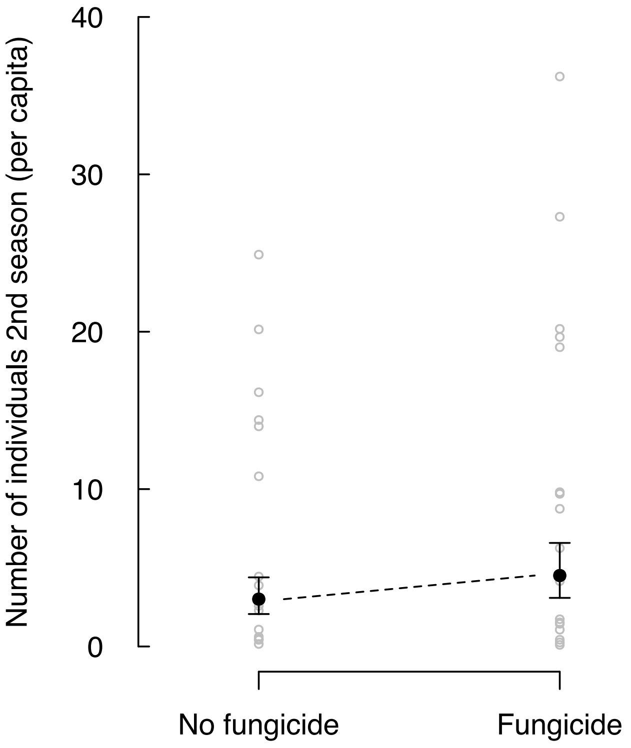


Fig.II.2: Number of offspring in the 2nd season per reproductive individual in the 1st season (± SE) of 17 plant species (5 rare and 4 common alien, and 3 rare and 5 common native species) under fungicide treatment and water control treatment. Filled black dots display means across all species, while grey dots indicate raw data means for each of the species.

Discussion

In this study, we tested if differences in commonness of both alien and native plant species can be explained by differences in magnitude of self-limitation due to intraspecific competition and/or effects of soil-borne pathogens. In our two-year mesocosm experiment, we found that alien species had a higher number of offspring per capita at low levels of intraspecific competition, indicating weak self-limitation, whereas natives did not show a significant difference between high and low initial sowing frequencies. Furthermore, we could show that fungicide treatment led to a higher number of offspring regardless of species origin or commonness compared to the water control treatment. This indicates that, in contrast to the predictions of the enemy release hypothesis (Keane & Crawley 2002), both alien and native species profited from pathogen release. This suggests that the influence of soil-borne pathogens may be mainly driven by generalists instead of species-specific pathogens, at least in our study.

Our initial high and low sowing frequencies resulted in respective differences in plant densities in the 1st season with only small differences between alien and native common and rare species. Regarding the effect sowing frequency on the number of offspring per capita, we found that only alien and not native species had a significantly higher number of offspring per capita at lower initial sowing frequencies. Notably, these findings were not caused by differences in the number of seeds produced in the 1st season and therefore must have arisen in the 2nd season. A higher per capita growth rate at low frequencies is in line with expectations of coexistence theory (Chesson 2000; Adler, HilleRisLambers & Levine 2007; HilleRisLambers *et al.* 2012). However, contrary to our expectations, we did not find differences between common and rare species, irrespective of status of origin. The significant difference for alien species but not for native ones indicates that aliens might rely on this advantage of higher population growth rates when being initially rare to overcome barriers of biotic resistance (Levine, Adler & Yelenik 2004), or to rapidly colonise available space over natives. This may allow them to successfully establish in plant communities in the new range.

In contrast to expectation, population growth of common species was not less limited by intraspecific competition than population growth of rare species. The lack of such a difference for alien species may be linked to the choice of study species. We included common and rare alien species, however, this entails only aliens that managed to establish in Germany, and thus alien species that have been introduced but failed to establish were not tested. Consequently, the alien species used in our experiment might already represent a subsample of more successful aliens. This could, however, not explain the lack of a difference in intraspecific frequency effect between common and rare native species. So, in our study, regulation of species abundances seems not to be linked to differences in magnitude of effects of intraspecific competition.

In contrast to our findings, Comita *et al.* (2010) found a negative correlation between degree of self-limitation and species abundances in a tropical rainforest. Yet, several other studies show substantial variation in direction and magnitude of intraspecific density dependence, and its contingency on biotic and abiotic factors (Goldberg & Barton 1992; Wassmuth *et al.* 2009; Blank 2010; Collins, Hart & Molofsky 2010; Leger & Espeland 2010; Mangla *et al.* 2011; Yan *et al.* 2015; Bachelot *et al.* 2016). Furthermore, the life stages at which intraspecific density dependence is measured can play an important role, as shown by Leger & Espeland (2010). In their study on three native and three alien annual grassland species, they found consistently negative density-dependence in individual plant growth (juvenile to adult). However, if all stages of the life cycle (germination to seed production) were taken into account, only one exotic species still showed stabilizing density dependence (see also Lortie & Turkington 2002). In another notable study on two native and two alien species, Harpole & Suding (2007) reported strong stabilizing density-dependent effects due to intraspecific competition, and a positive correlation between species mean individual growth rates and their abundance in experimental communities. However, their results indicate that one of the alien species in their study, *Bromus hordeaceus* (which was the native matrix species in our study), has the potential to competitively exclude all other species. This contradicts the coexistence of these species in the field and indicates that other factors, such herbivores, pathogens or dispersal play an important role in coexistence. Hence, while we found a difference between alien and native species in per capita growth rate at low frequencies, species abundances ultimately seem to be linked to factors other than differences in intraspecific competition.

Despite well worked-out theoretical models on species coexistence due to stronger intraspecific than interspecific limitation (e.g. Chisholm & Muller-Landau 2011), experimental studies on differences of this effect between species, and to what extent this can explain their abundances, are surprisingly scarce. Particularly, more experimental studies are still needed, with a focus on population-level performance to underpin the theoretical advances (but see Harpole & Suding 2007; Chu & Adler 2015).

Regarding the effects of the fungicide treatment, we found that species profited from experimental belowground enemy release (i.e. fungicide application). However, contrary to the prediction of the enemy-release hypothesis, species profited regardless of whether they are alien or native. While there are studies that show that alien species might disproportionally profit from natural soil-borne enemy release compared to natives, likely explaining their success (Klironomos 2002; Agrawal *et al.* 2005; Kulmatiski *et al.* 2008; de la Pena *et al.* 2010; Zuppinger-Dingley *et al.* 2011; Liu *et al* .2015), evidence for the generality of these findings remains mixed. For example, Chiuffo *et al.* (2015) did not find differences in plant-soil feedback between nine native and nine alien ruderal plants. Similarly, Birnbaum *et al.* (2014) analysed soil fungal communities of five native and five alien *Acacia* species in Australia and showed that fungal communities did not differ greatly between native and alien species. Furthermore, Anacker *et al.* (2014) extended the analysis of the study of Klironomos (2002) by incorporating the phylogeny of the studied plant community and found that, contrary to the original results, alien and native species did not differ in their plant-soil feedback response. Moreover, Nijjer, Rogers & Siemann (2007) tested the performance of the woody alien species *Sapium sebiferum* in soil collected near conspecifics and soil collected near heterospecifics in the introduced range, and found lower survival of *S. sebiferum*, but not of native species, in respective conspecific soils. Additionally, they conducted experiments in which the soil was sterilized or treated with fungicides, which showed that fungal pathogens were the likely driver behind plant-soil feedback effects of conspecific soil on the alien *S. sebiferum* plants. Consequently, while interactions between a plant and its soil microbial community have the potential to strongly affect plant performance, a clear general pattern of soil-borne enemy release for alien plants is not identifiable.

The fact that native as well as alien species profited from fungicide application in our study might be related to the presence of generalist pathogens with a broad host range. The large number of *Pythium* species, which include the entire spectrum from specialists to generalists, the wide distribution of the genus and their known impact on plant performance (Hendrix & Campell 1973; Reinhart *et al.* 2010; Crocker, Karp & Nelson 2015) makes *Pythium* a potential candidate genus that may explain negative effects of soil pathogens on alien as well as native species. This is corroborated by our findings, as the fungicide we used (Cercobin FL®) acts particularly against *Pythium*. However, differences in virulence of pathogens and susceptibility of plants, and therefore impacts on plant performance may still occur even for rather generalist pathogens (Crocker, Karp & Nelson 2015) and the occurance of specialist pathogens in our experiment can not be ruled out. Consequently, the balance between generalist and specialist pathogens, as well as variation in their plant-species specific virulence, rather than the presence or absence *per se* may be important for explaining when alien species profit from enemy release (Halbritter *et al.* 2012).

Plant-soil interactions do not only involve antagonistic interactions but also mutualistic ones with for example arbuscular mycorrhizal fungi (van der Heijden *et al.* 1998, Rillig 2004; Rillig & Mummey 2006). The fungicides that we used in our experiment to reduce soil pathogen pressure could also have had undesired non-target effects. However, we can exclude side effects of the fungicide on mycorrhization of our target and matrix species.

The target species belong to three families, and two of them, the Brassicaceae and Caryophyllaceae, are widely accepted to be non-mycorrhizal (Harley & Harley 1987; DeMars & Boerner 1996). For species of the third family, the Asteraceae, we did not find differences in the degree of mycorrhization between fungicide and water control treatments (Appendix II.S2). Nevertheless, we cannot completely rule out that there were side effects of the fungicide treatment on other soil properties or plant growth.

Several studies have shown a correlation between plant-soil feedback effects and abundance or frequency of species (Klironomos 2002; Mangan *et al*. 2010; MacDougall, Rillig & Klironomos 2011; Liu *et al.* 2015; Xu, Wang & Yu 2015). However, we did not find that species commonness or sowing frequency interacted with the effects of fungicide on population-level performance. In a study on semiarid grassland, Reinhart (2012) found predominantly negative soil-feedbacks for the studied species, but they also could not find a link between species commonness and their soil-feedback responses. Cases in which dominant species are subject to strong negative feedbacks (Nijjer, Rogers & Siemann 2007, Andonian *et al*. 2011) also contradict the notion that less negative feedback responses may be the driver behind a species success. Consequently, while effects of soil-borne pathogens can explain rarity or commonness in some systems (e.g. Klironomos 2002, but see Anacker *et al*. 2014; MacDougall, Rillig & Klironomos 2011), these effects alone might not always be sufficient to explain the differences in species success. Other factors, such as aboveground enemies (Gripenberg *et al.* 2014; Bagchi *et al.* 2014; Fricke, Tewksbury & Rogers 2014), abiotic conditions (Hendrix & Campell 1973; Newton *et al.* 2010; Larios & Suding 2015; LaManna *et al*. 2016,) or the identity and diversity of the co-occurring plant community (Annacker *et al*. 2014; Hantsch *et al.* 2014), may largely alter plant-soil feedback effects, and may thus explain the diverging results for the effects of soil biota on species abundances. Therefore, incorporating multiple factors and testing their roles across different species and ecosystems is needed to foster our understanding of the complex nature of plant-soil interactions (Dawson & Schrama 2016).

Conclusions

In summary, we could show that alien species may be able to gain an advantage when rare due to low self-limitation, likely allowing them to initially establish. The mechanism behind the lower self-limitation in alien than in native species is not clear, but as it was not affected by the fungicide treatment, it is unlikely to be caused by differences in the accumulation of species-specific pathogens. Our study also suggests that species commonness and rarity is not linked to differences in intraspecific competition (i.e. self-limitation). Plant-soil feedback effects could also not explain species success in our study, and affected alien and native species to similar degrees. Nevertheless, our findings still allow for single species to be successful due to belowground enemy release or a lower degree of self-imitation. However, a general advantage to alien species from enemy release seems unlikely. Consequently, approaches that identify the biotic agents, their respective impact on aliens as well as natives (Crocker, Karp & Nelson 2015) and differences between soil communities of the native and non-native range (Birnbaum *et al*. 2014) are necessary to identify when soil-borne pathogens can explain differential success of alien and native species. Moreover, more population-level experimental studies are needed to underpin and evaluate existing theoretical models and shed light on the respective importance of multiple drivers of species success and abundance (Dawson & Schrama 2016).

Acknowledgements

G.M. and W.D. were funded by the Deutsche Forschungsgemeinschaft (Project DA 1502/1-1). We thank Otmar Ficht, Sina Glöckner, Timo Scheu and Marisa Braun for horticultural assistance and practical help. Thanks also go to the International Max Planck Research School for Organismal Biology for supporting G.M.

Appendices

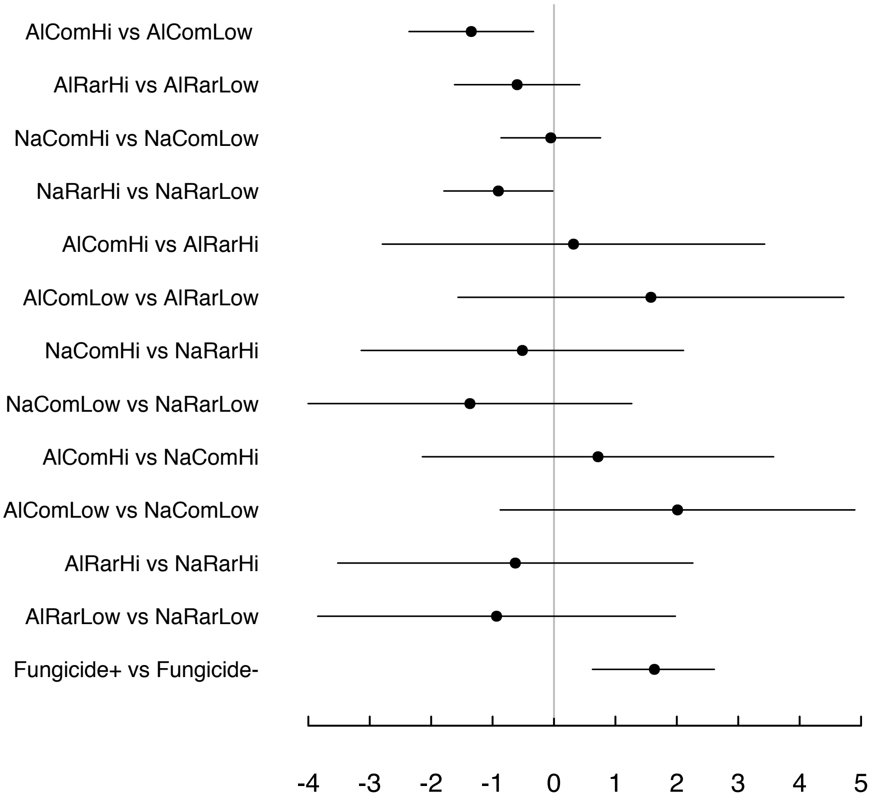
Appendix II.S1 Table A1. Results for each step of the stepwise backward model selection via likelihood-ratio tests for the generalized linear mixed effects model for number of offspring per individual in the 2nd season. The main effects and lower order interactions that were contained in a higher order significant interaction were not tested, as the interaction was retained in the minimum model. Block was kept in the model due to study design.

Term	χ^2	**df**	**p**
4-way-interaction			
Density x Fungicide x Origin x Commonness	2.18	1	0.139
3-way-interactions			
Fungicide *x* Origin *x* Commonness	0.12	1	0.729
Density *x* Fungicide *x* Commonness	0.16	1	0.689
Density *x* Fungicide *x* Origin	0.3	1	0.583
Density *x* Origin *x* Commonness	4.5	1	**0.033**
2-way-interactions			
Fungicide x Origin	0.44	1	0.507
Fungicide x Commonness	1.24	1	0.265
Density x Fungicide	2.88	1	0.089
Density x Origin	-	-	-
Density x Commonness	-	-	-
Origin x Commonness	-	-	**-**
Main effects			
Density	-	-	-
Fungicide	5.74	1	**0.016**
Commonness	-	-	**-**
Origin	-	-	-
Block	-	-	-

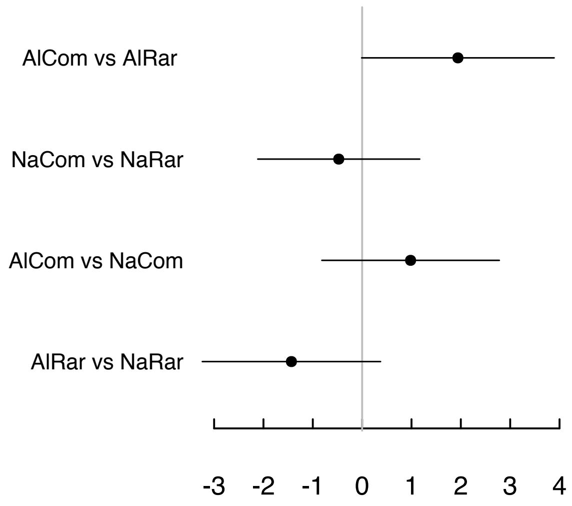
Appendix II.S1 Table A2. Results for each step of the stepwise backward model selection via likelihood-ratio tests for the generalized linear mixed effects model for number of seeds produced per individual in the 1st season. The main effects that were contained in significant interaction were not tested, as the interaction was retained in the minimum model. Block was kept in the model due to study design.

Term	χ^2	**df**	**p**
4-way-interaction			
Density x Fungicide x Origin x Commonness	0	1	1
3-way-interactions			
Density x Origin x Commonness	0	1	1
Fungicide *x* Origin *x* Commonness	0.5	1	0.479
Density *x* Fungicide *x Origin*	0.68	1	0.409
Density *x* Fungicide *x* Commonness	3.64	1	0.056
2-way-interactions			
Fungicide x Origin	0.02	1	0.887
Density x Origin	0.02	1	0.888
Density *x Commonness*	0.66	1	0.416
Fungicide x Commonness	1.1	1	0.294
Density x Fungicide	1.26	1	0.261
Origin x Commonness	4.62	1	**0.031**
Main effects			
Density	0.04	1	0.841
Fungicide	0.02	1	0.887
Origin	-	-	-
Commonness	-	-	-
Block	-	-	-

Appendix II.S1 Fig A1. Plot with estimates of differences between means and respective 95% confidence intervals for all pairwise comparisons of the terms included in significant interactions in the model for number of offspring per individual in the 2nd season.



Appendix II.S1 Fig A2. Plot with estimates of differences between means and respective 95% confidence intervals for all pairwise comparisons of the terms included in significant interactions in the model for number of seeds produced per individual in the 1st season.



Appendix II.S2 - Additional experiments to test for the effects of the applied treatments

Mycorrhiza analysis

To test for side effects of the biocide treatment on mycorrhization of plants we also collected root samples. Since only the species from the Asteraceae family are mycorrhizal species we focused on grass roots for the analysis of mycorrhizal structures. However, due to the species growing in mixed communities resulting in intermingled roots, it was not possible to collect samples for all pots that were suitable for mycorrhiza anylsis (sample size = 161 root samples). Roots were collected on the 7th of September 2013 and washed and stored in 50% alcohol until further processed for root staining in the following week. For root staining, we washed the roots and heated them at 80°C in a 10% KOH solution in a water bath until the roots became transparent. After that, the roots were heated again for five minutes in a 5 % vinegar, 5 % ink solution (Parker Quink Black, NWL France Services, Boulogne, France) to stain mycorrhizal fungal structures. We mounted the stained roots on glass slides, and analysed them by counting mycorrhizal fungal structures (vesicles, arbuscules and hyphae) at 100 intersections per sample under a microscope at 100x magnification (Zeiss Axioscope, Carl Zeiss, Jena Germany).

We analysed the number of intersections containing mycorrhiza fungal structures or not, using binomial generalized linear mixed models in the lme4 package. The model contained the fixed factors fungicide and sowing density as well as the interaction between both. Species nested in family and block were added as random effects. We used likelihood ratio tests to determine the significance of the model terms.

We did not find an significant effect of the fungicide on the mycorrhization of the grass roots (χ^2 = 0.152, df = 1, P = 0.696). There was a significant effect of sowing density on mycorrhization (χ^2 = 51.885, df = 1, P < 0.001) that showed that grass roots had a higher degree of mycorrhization when grown at low grass sowing density (mean= 0.814, SE = ±0.076) compared to high grass sowing density (mean= 0.559, SE = ±0.074; note that high grass sowing density corresponds to low target density and vice versa)

Chapter III

Alien and native plant establishment in grassland communities is more strongly affected by disturbance than above- and below-ground enemies

Gregor Müller, Lena Horstmeyer, Tilman Rönneburg, Mark van Kleunen and Wayne Dawson

Journal of Ecology, 104 (5). 1233-1242

Abstract

1. Understanding the factors that drive commonness and rarity of plant species, and whether these factors differ for alien and native species, are key questions in ecology. If a species is to become common in a community, incoming propagules must first be able to establish. The latter could be determined by competition with resident plants, the impacts of herbivores and soil biota, or a combination of these factors.

2. We aimed to tease apart the roles that these factors play in determining establishment success in grassland communities of 10 alien and 10 native plant species that are either common or rare in Germany, and from four families. In a two-year multi-site field experiment, we assessed the establishment success of seeds and seedlings separately, under all factorial combinations of low versus high disturbance (mowing vs mowing and tilling of the upper soil layer), suppression or not of pathogens (biocide application) and, for seedlings only, reduction or not of herbivores (net-cages).

3. Native species showed greater establishment success than alien species across all treatments, regardless of their commonness. Moreover, establishment success of all species was positively affected by disturbance. Aliens showed lower establishment success in undisturbed sites with biocide application. Release of the undisturbed resident community from pathogens by biocide application might explain this lower establishment success of aliens. These findings were consistent for establishment from either seeds or seedlings, although less significantly so for seedlings, suggesting a more important role of pathogens in very early stages of establishment after germination. Herbivore exclusion did play a limited role in seedling establishment success.

4. *Synthesis*: In conclusion, we found that less disturbed grassland communities exhibited strong biotic resistance to establishment success of species, whether alien or native. However, we also found evidence that alien species may benefit weakly from soil-borne enemy release, but that this advantage over native species is lost when the latter are also released by biocide application. Thus disturbance was the major driver for plant species establishment success and effects of pathogens on alien plant establishment may only play a minor role.

Introduction

Disentangling the determinants that allow some species to establish successfully and spread while other species fail to do so is an important question in ecology. Germination and seedling survival are important steps in a plant’s life cycle, and can have substantial impacts on plant population dynamics and consequently on species commonness. Establishment is a crucial step for a non-native species, in order to colonize new habitat and spread in a new range. While only few introduced species actually establish self-sustaining populations (Williamson & Fitter 1996), and most of those remain at low density (Ortega & Pearson

2005), some alien species possess the potential to dominate communities and reduce plant diversity (Vilà *et al.* 2011), and impact ecosystem processes (Liao *et al.* 2008; Vestergard, Ronn & Eklund 2015). Consequently, an improved understanding of what drives plant species establishment can help to explain patterns of community assembly (Seastedt & Pysek 2011) and dynamics of range expansions (Engelkes *et al.* 2008).

It has been proposed that the mechanisms explaining invasion success of alien species and commonness of native species may be the same, allowing both sets of species to achieve and maintain high abundances and a wide distribution (Thompson, Hodgson & Rich 1995: van Kleunen & Richardson 2007; Jeschke & Strayer 2008; van Kleunen *et al*. 2010). For example, Dawson, Fischer & van Kleunen (2012) found that invasive alien and common native species do not respond fundamentally differently to nutrient addition and competition. Furthermore, invasive species can have similar characteristics as common native species do, such as short life cycles, fast germination and growth, superior dispersal abilities and high reproductive effort (Grotkopp & Rejmanek 2007; van Kleunen, Weber & Fischer 2010; Dawson, Fischer & van Kleunen 2011). There is also ample evidence that removal of resident plants by disturbance can lead to greater recruitment of incoming species due to reduced competition or release of nutrients (Lozon & MacIsaac 1997; Hierro *et al*. 2006; Questad & Foster 2008; Myers & Harms 2009; MacDonald & Kotanen 2010; Maron *et al*. 2012; Kempel *et al*. 2013, but see Moles *et al*. 2012). For example, Meyers & Harms (2009) found in a meta-analysis on 28 studies that disturbance increases opportunities for species recruitment. Similarly, Hierro *et al*. (2006) identified disturbance as an important driver for success of *C. solstitialis* in its non-native range, while MacDonald & Kotanen (2010) found that disturbance increased establishment of *Ambrosia artemisiifolia* in its home range. These findings underline the role that disturbance can play for alien and native plant establishment success.

Although the drivers of commonness of natives and invasiveness of aliens may be partly the same, it is frequently argued that introduced non-native species may have gained an advantage over resident native species through leaving behind natural enemies (the "enemy release" hypothesis, Keane & Crawley 2002; Colautti *et al.* 2004). If the inhibitory effect of an interaction with pathogens or herbivores is relaxed for an alien species in its introduced range, such enemy release may explain the disproportional success of some species in their introduced range (Klironomos 2002; Mitchel & Power 2003; Agrawal *et al.* 2005; Liu and Stiling 2006). In contrast, generalist antagonists in the introduced range may contribute to the biotic resistance of native communities against invaders (Elton 1958; Levine, Adler & Yelenik 2004; Parker, Burkepile & Hay 2006; Parker & Gilbert 2007 Pearson, Potter & Maron 2012). The "biotic resistance" hypothesis proposes that the community of local herbivores, pathogens and competitors provide resistance against invading species, specifically hindering establishment and suppressing growth of species that are not adapted to their mode of predation, infection or competition (Maron & Vilà 2001; Levine, Adler & Yelenik 2004). In particular, enemy release and biotic resistance may be caused by above *and* below-ground interactions (Agrawal *et al.* 2005; Morriën, Engelkes & van der Putten 2011; Vestergard, Ronn & Ekelund 2015), and while often considered separately, they may also act simultaneously to drive establishment success of alien and native plant species, but with different relative importance.

Despite increasing awareness of potential effects of multiple interacting factors such as enemy release, biotic resistance and disturbance on invasion success (Blumenthal 2006), there are few studies that test the relative importance of different factors experimentally (Hierro *et al*. 2006; Morriën, Engelkes & van der Putten 2011; Kempel *et al*. 2013; Maron *et al*. 2013). In addition, we are not aware of any study that simultaneously assessed the relative roles of belowground enemies (e.g. soil fungi), aboveground enemies (e.g. invertebrate herbivores) and disturbance in explaining establishment success of alien and native species in semi-natural communities.

In this study, we provide a novel test of the effects of disturbance, pathogens and herbivores on establishment success of 10 alien and 10 native herbaceous plant species sown from seed and planted as seedlings into grassland communities in southern Germany. Half of the species are considered common and the other half are rare in Germany. Specifically, we asked the following questions: 1) Does disturbance increase establishment success of incoming species, and do its effects differ between alien and native or common and rare species? 2) Does biocide treatment lower establishment success of incoming species due to release from pathogen pressure on the resident community, and does this affect aliens more than natives, as alien species may lose their potential competitive advantage? 3) Similarly, does release from herbivore pressure influence establishment success of the incoming species, and does this effect differ between alien and native or common and rare species? 4) Do the three factors disturbance, biocide treatment and herbivore reduction interact to affect plant establishment success?

Materials and methods

Target species

In order to be able to generalize results beyond a few model species (van Kleunen *et al.* 2014), we conducted a multi-species field experiment using 20 different target species (biennial or perennial) from four different families (Table III.1). The chosen target species represented taxonomic quadruplets that contained one common native, one rare native, one common alien and one rare alien plant species. As a proxy for the degree of commonness of each species, we used the number of 130-km^2 grid cells occupied by the species in Germany (maximum 3000) extracted from the FloraWeb database (FloraWeb, Bundesamt für Naturschutz, last accessed 8th June 2015). We also aimed to choose species that occur in similar habitats, excluding habitat specialists and species that are not simply rare due to their geographic distribution overlapping only marginally with the borders of Germany.

Field sites and experimental setup

The experimental setup consisted of five sites located in meadows surrounding the University of Konstanz, Germany. Four sites were located in Hockgraben, a local park that has been managed for its conservation value as a meadow landscape and is fertilized and mown annually. The fifth site was located in a meadow next to the Limnological Institute of the University of Konstanz (Appendix III.S1 Table A1 in Supporting Information).

Each site consisted of 12 plots of 12 m^2 (4 m x 3 m) each, arranged in a four-by-three grid and separated by 2 m wide paths (Appendix III.S1 Fig. A1). The grid of plots was surrounded by a one-metre wide boundary, resulting in a total site area of 360 m^2 (15 m x 24 m). Four of the 12 plots per site were used to test establishment success from seed ("seed-experiment plots"), and the other eight plots were used to test establishment success of pre-reared seedlings ("seedling-experiment plots") (see "Seed experiment" and "Seedling experiment" subsections for details). This approach allowed us to assess whether the drivers of establishment success differ between the two early life stages. The positions of the seed and seedling plots were chosen randomly within each site.

Table III.1. The study species and their respective commonness, measured as the number of ~130 km^2 grid cells occupied in Germany out of a maximum of 3000 grid cells (FloraWeb, Bundesamt für Naturschutz), listed by family and origin.

Family	**Alien**			**Native**		
	Species	**Commonness**	**Grid cells**	**Species**	**Commonness**	**Grid cells**
Asteraceae						
	Aster novi-belgii	common	1530	*Achillea millefolium*	common	2741
	Solidago canadenis	common	2660	*Senecio jacobaea*†	common	2773
	Aster lanceolatus	rare	702	*Eupatorium cannabinum*†	common	2778
	Solidago graminifolia	rare	43	*Aster amellus*	rare	493
				Achillea nobilis	rare	299
Brassicaceae						
	Diplotaxis tenuifolia	common	1168	*Cardamine pratensis*	common	2923
	Lepidium heterophyllum	rare	98	*Lepidium graminifolium*	rare	86
Caryophyllaceae						
	Cerastium tomentosum	common	1296	*Silene latifolia*	common	2893
	Gypsophila paniculata	rare	122	*Silene viscosa*	rare	9
Onagraceae						
	Oenothera biennis	common	2591	*Epilobium tetragonum*	common	2468
	Oenothera glazioviana	rare	879	*Epilobium dodoneii*	rare	136

†*Senecio jacobaea* was only used in the seed experiment, and *Eupatorium cannabinum* was only used in the seedling experiment.

Seed experiment

Every seed-experiment plot consisted of 160 subplots, distributed in two sets of five rows of 16 subplots, with a 50 cm wide path in between the two sets of five rows and a 40-45 cm gap to the edge of the plot (Appendix III.S1 Fig A2). Each subplot consisted of a ring cut from PVC tubes, with a height of 1 cm and a diameter of 5 cm. The ring was fixed in the ground with two nails. The centres of two neighbouring rings were 21 cm apart within the row, and 20 cm apart between two adjacent rows.

We randomly selected eight subplots per plot for each species, and sowed eight seeds of the respective species into each ring. A total of 1,280 seeds per species were sown across the whole experiment, resulting in 25,600 seeds in total. When the random selection resulted in more than two subplots of the same species next to each other, one or more of them were moved, to avoid an aggregation of one particular species in an area. Sowing took place from the 14th to the 16th of April 2014 (Appendix III.S1 Table A2).

The seed experiment consisted of a factorial design with a disturbance treatment (high/low) and a biocide treatment (biocide/water control). In each site, we applied each of the four treatment combinations to one of the four seed-experiment plots. For the low-disturbance treatment, plots were mown to 5 cm sward height (Appendix III.S1 Table A2). For the high-disturbance treatment, we tilled the plots after mowing and before the sowing of seeds (Appendix III.S1 Table A2) with a motorised rotary tiller to a depth of approximately 5-7 cm, and compacted the soil afterwards with a soil compactor, thus disturbing the local plant community and creating patches of open ground. For the biocide treatment, we treated plots alternatingly with Previcur Energy® and Fenomenal® (Both: Bayer CropScience AG, Monheim, Germany). Previcur Energy® acts against downy mildew and *Pythium* species, and propamocarb and fosetyl-aluminium are the active ingredients. Fenomenal® acts against soil-borne pathogens like *Pythium* and *Phytophthora* species (Oomycota), and fosetyl-aluminium and fenamidone are the active ingredients. Propamocarb and fosetyl are both systemic agents that are taken up through the root system and accumulate in the plant tissue, providing a curative effect and preventing infection for three to eight weeks. Previcur Energy® is used in agriculture and horticulture to treat pathogenic *Pythium* species and downy mildew in a wide range of vegetables and ornamental plants from different families. Due to the systemic mode of the biocides, they may also have an effect on aboveground foliar pathogens (downy mildews). We applied one of the biocides alternatingly every six weeks during the growing season (see Appendix III.S1 Table A2). We followed the manufacturers' recommendations for the dosages of both biocides. For Previcur Energy®, we used 36 ml of the biocide dissolved in 36 L of water for each biocide plot. For Fenomenal®, we used 12 g dissolved in 16 L of water for each biocide plot receiving biocide treatment. Control plots received equivalent amounts of water instead. We surveyed the plots from the 2nd to the 17th of June 2014 in the first season for successful germination and establishment of the species (Appendix III.S1 Table A2).

Seedling experiment

We reared seedlings of all species in a greenhouse of the Botanical Garden of the University of Konstanz, starting with sowing on the 17th of March 2014 (Appendix III.S1 Table A2). Each tray (29 cm × 47 cm × 6 cm) contained roughly 500 seeds of one species in a standard substrate of peat and clay (Einheitserde Classic Profisubstrat Typ VM, Einheitserde- und Humuswerke Gebr. Patzer GmbH & Co. KG, Sinntal-Jossa, Germany). The light regime in the greenhouse was 12 h light/12 h dark. Temperatures were first between 18°C at night and a minimum of 20°C during the day. Then, to allow the plants to adjust to outside conditions, the temperatures were lowered on the 2nd of April to 7°C at night and external day temperatures during the day. One week later, the trays were placed outside until seedlings were transplanted. The plants were watered daily until transplanting into the field sites.

From the 17th to the 29th of April 2014 (Appendix III.S1 Table A2), we planted each of the eight seedling-experiment plots per site with 160 seedlings (20 species, 8 individuals per species) and marked the seedling positions with coloured wooden sticks. We used the same setup as for the seed experiment (Appendix III.S1 Fig. A2). With eight plots at each of five sites, a total of 6,400 plants were planted (320 per species). We used the same species as in the seed experiment, with one exception (Table III.1). *Senecio jacobaea* showed signs of a disease infection when the seedlings were reared in the greenhouse, and therefore we replaced it with another common native Asteraceae *Eupatorium cannabinum*. In the analysis of the seedling-experiment, we finally used 5,839 plants, as 561 plants had to be excluded due to damage or mortality before all treatments were set up and all initial plant height measurements had been done.

The seedling experiment entailed the same disturbance and biocide treatments as the seed experiment described above. Additionally, because seedling survival may depend on invertebrate herbivores (molluscs and arthropods), we included a herbivore-reduction treatment, leading to a factorial design of the three treatments with eight plots per site. We assigned treatment combinations to plots, such that each treatment factor was represented in every row and column (if possible), including the plots used in the related seed experiment (Appendix III.S1 Fig. A1).

For the herbivore-reduction treatment, we built 1.8 m high cages with insect netting around each reduction plot. Because netting affects light levels, we built similar cages with insect netting containing large slits (see below) that allowed herbivores access as a control treatment. We anchored the wooden frames of the cages in the ground with metal base spikes, and stapled white insect netting (mesh size 0.8 mm x 0.8 mm, HADI Gartenbau, Marschacht, Germany) tightly on all sides and the top of the cages. For closed cages, we buried the insect netting c. 5 cm into the ground and secured it with nails into the ground. We made 80-cm wide closable doors in the netting by using Velcro®. In order to further reduce the presence of herbivores in the closed cages, we installed a yellow sticky trap (3.5m x 0.15m, IVOG® Midiroll, Sauter und Stepper, Ammerbuch, Germany) in the middle path at a height of c. 80 cm. In order to control emerging gastropods in the closed cages, we installed beer traps in two corners of each closed cage, and renewed them when necessary. Additionally, we placed a small amount of molluscicide (2 g Schneckenkorn Spiess-Urania, active ingredient metaldehyde, Spiess-Urania Chemicals GmbH, Hamburg, Germany) in the closed cages, immediately after they were built. For the open cages of the control plots, we had openings without netting instead of doors, and we left the lower 40 cm on all sides completely open. Furthermore, we cut a slit of 20 cm width on each side at a height of 100 – 120 cm, and removed the outer 30 cm of the two short sides of the ceiling. We prioritized the building of the closed cages of the herbivore-reduction plots, which took place between the 1st and 10th of May. The control plots received their open cages subsequently until the 20th of May.

We assessed survival of the seedlings (presence/absence) during three surveys; a first survey starting on the 5th of May 2014, a second survey in August 2014 and a final survey after the winter in April 2015 (Appendix III.S1 Table A2). During each survey, we additionally measured the height (highest point of the plant to the nearest 0.5 cm) and counted the number of fully expanded leaves of the target plants. Furthermore, we conducted additional experiments to test the effect of biocide on the resident plant community and to assess potential side effects of the biocide treatment on the mycorrhization of the plants. Moreover, we also tested for the effectiveness of the herbivore reduction cages (detailed information in Appendix III.S2 in Supporting Information). To assess the effects of the disturbance treatment on competitor removal we additionally recorded percentage cover of plants and bare ground in a 20 x 20 cm square centered on each target position from the 23rd to the 27th of June 2014 (Appendix III.S2). We also took soil samples in all plots of both the seed and seedling experiment to test for effects of the disturbance treatment on nutrient availability on the 22th of July 2014 (Appendix III.S2).

Statistical analysis

For the seed experiment, establishment success was quantified as the proportion of seeds that resulted in successfully established plants per subplot. Establishment success was analysed using a generalised linear mixed model of the beta-binomial family in the glmmADMB package (Fournier *et al.* 2012) in the software R 3.2.0 (R Core Team 2015). To account for taxonomic non-independence of species and for non-independence of the plots within each site, species nested in family and site were included as random effects. Disturbance (high/low), biocide (with/without), species origin (alien/native), species commonness (common/rare) and all interactions were included as fixed effects in a four-way interaction model. We used stepwise backward model selection *via* likelihood-ratio tests to obtain a minimum model, and to test for significance of interactions and main effects. We performed multiple pairwise comparisons to test for differences among levels of the terms in significant interactions or fixed effects using the ‘multcomp’ package (Hothorn, Bretz & Westfall 2008).

For the seedling experiment, we used generalised linear mixed effects models in the lme4 package (Bates *et al.* 2014) to analyse establishment success (i.e. survival). We used the optimizer “bobyqa” and set the maximum number of iterations to 100,000 to achieve model convergence. We analysed establishment success in the first year (2nd survey) and after the winter (3rd survey, Appendix III.S1 Table A2). Disturbance, biocide, herbivore reduction, species commonness and species origin were included as fixed terms in the model, as well as all possible interactions. Additionally, we included initial plant height and natural-log transformed number of leaves (both centred on the mean and scaled by the standard deviation) measured in the first survey after planting to correct for initial size differences. Species nested in family and plot nested in site were included as random effects. As for the seed experiment, we used stepwise backward model selection *via* likelihood-ratio tests to obtain a minimum model and to assess significance of the model terms. We also performed multiple pairwise comparisons to test for differences among levels of the terms in significant interactions or fixed effects using the 'multcomp' package.

Additionally, we analysed growth using the data on number of leaves and plant height from the first and second survey. We multiplied number of leaves with plant height to obtain a proxy for accumulated biomass of the plants (and to compensate for differences between growth forms e.g. rosette and non-rosette plants). We used this proxy to calculate the relative change in plant size, derived from the calculation for relative growth rate: Relative change in plant size = (ln(leaves * height 2nd survey) – ln(leaves * height 1st survey)) / (days 2nd survey- days 1st survey). Relative change in plant size was analysed using a linear mixed model with the same terms as the analysis described above. Similarly, we also used likelihood-ratio tests to assess significance of model terms and multiple pairwise comparisons to test for differences of levels among significant model terms.

Results

Seed experiment

The minimum model for establishment success from seeds retained a significant three-way interaction between disturbance, species origin and commonness, and a significant two-way interaction between biocide treatment and species origin (Table III.2, Appendix III.S1 Table A3). Multiple comparisons showed that disturbance promoted establishment success of all groups of species. However, as indicated by the significant disturbance x origin x commonness interaction (Table III.2, Appendix III.S1 Table A3), the magnitude of the disturbance effect depended on origin and commonness of the species. Among common species, the disturbance effect was stronger for natives (mean difference = 1.024, SE = ±0.108, P <0.001, Appendix III.S1 Fig. A3) than for aliens (0.776, SE = ±0.128, P <0.001), whereas, among rare species, it was stronger for aliens (1.850, SE = ±0.178, P <0.001) than for natives (0.782, SE = ±0.108, P <0.001, Fig. III.1). The establishment success for alien species from seeds under biocide treatment was lower than under the control treatment (-0.430, SE = ±0.109, P < 0.001), but similar for native species (0.004, SE = ±0.076, P = 1, Fig. III.2). However, the effect size of this difference is relatively small with 1.2 % lower probability of establishment for aliens under biocide treatment.

Table III.2. Minimum generalized linear mixed effects model for probability of establishment success from seeds of 20 alien and native rare and common plant species under high and low disturbance treatment and biocide or water control treatment.

Parameters	**Estimate (Std. error)**	**t-value**	**p-value**
Fixed Effects			
Intercept	-2.390 (0.368)	-6.49	<0.001
Disturbance (low)	-0.755 (0.127)	-5.95	<0.001
Biocide (yes)	-0.303 (0.092)	-3.30	<0.001
Origin (native)	0.596 (0.495)	1.20	0.228
Commonness (rare)	0.040 (0.495)	0.08	0.935
Disturbance (low) : Origin (native)	-0.269 (0.167)	-1.61	0.107

Biocide (yes) : Origin (native)	-0.302 (0.117)	2.58	0.009
Disturbance (low) : Commonness (rare)	-1.029 (0.212)	-4.84	<0.001
Origin (native) : Commonness (rare)	-0.150 (0.695)	-0.22	0.829
Disturbance (low) : Origin (native): Commonness(rare)	1.271 (0.262)	4.85	<0.001
Random Effects	**Std. deviation**		
Family	<0.001		
Species nested in family	0.757		
Site	0.230		

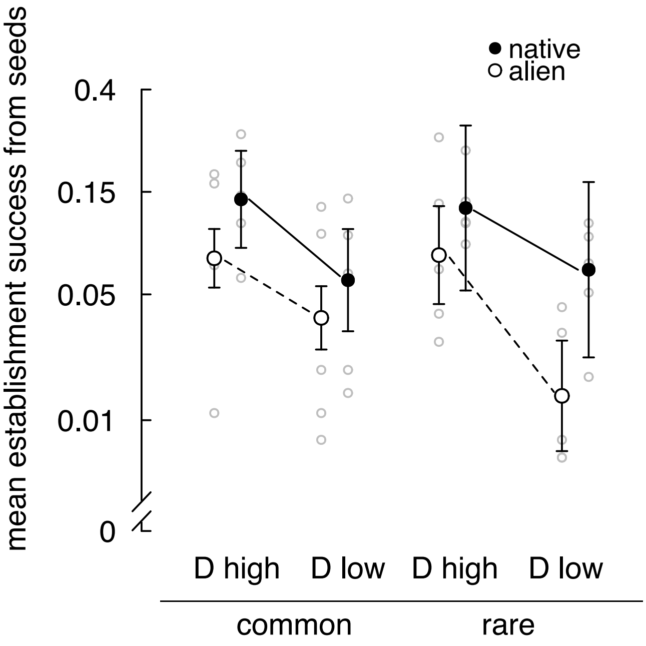


Fig.III.1: Probability of establishment success from seeds (± SE) of 20 alien and native common and rare species under high and low disturbance treatment. Black dots (natives) and open white dots (aliens) display means across species for the respective groups, while grey dots indicate raw data means for each of the species. (Note: y-axis on logit scale)

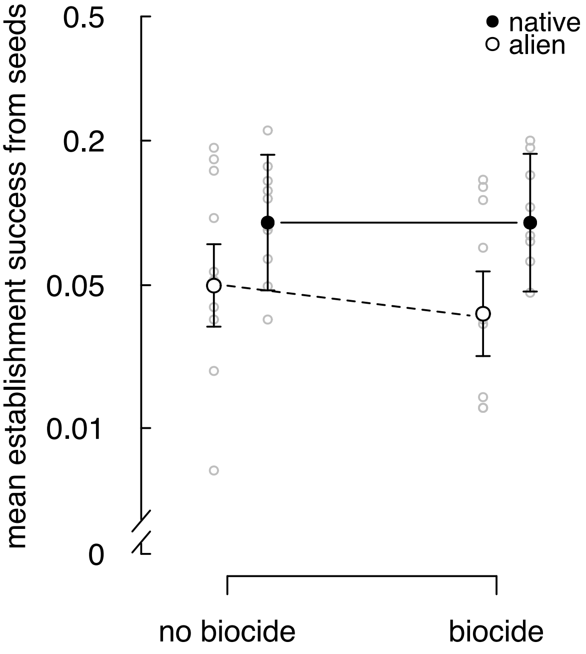


Fig.III.2: Probability of establishment success from seeds (± SE) of 10 alien and 10 native species under biocide or water control treatment (across species commonness). Black dots display means for native species and open white dots display means for alien species across species across biocide treatments. Grey dots indicate raw data means for each of the species. (Note: y-axis on logit scale)

Seedling experiment

The minimum model for establishment success from seedlings in the first growing season retained a significant 3-way interaction between biocide treatment, disturbance treatment and species origin (Table III.3, Appendix III.S1 Table A4). Initial number of leaves was kept as a significant covariate in the model, indicating that larger seedlings had a higher probability of successful establishment (Table III.3, Appendix III.S1 Table A4). Overall, establishment from seedlings tended to be increased in the disturbance plots (Fig. III.3), but as indicated by the significant biocide x disturbance x origin interaction, the strength and significance of the disturbance effect differed between the native and alien species, dependent on the biocide treatment. When plots were treated with the water control, the alien species did not benefit significantly from disturbance (0.584, SE = ±0.345, P = 0.460, Appendix III.S1 Fig. A4) whereas the native species did (0.989, SE = ±0.340, P = 0.028, Fig. III.3). However, when plots were treated with biocide, both the alien species (mean difference =1.558, SE = ±0.348, P < 0.001) and the native species (1.380, SE = ±0.341, P < 0.001) benefitted similarly from disturbance (Fig. III.3).

Table III.3. Minimum generalized linear mixed effects model for probability of establishment success of seedlings in the first growing season of 20 alien and native rare and common plant species under high and low disturbance treatment and biocide or water control treatment as well as open and closed cages.

Parameters	**Estimate (Std. error)**	**t-value**	**p-value**
Fixed Effects			
Intercept	1.020 (0.651)	1.568	0.116
Biocide (yes)	0.343 (0.345)	0.994	0.320
Disturbance (low)	-0.528 (0.343)	-1.538	0.124
Origin (native)	0.064 (0.543)	0.118	0.906
Leaves	0.436 (0.050)	8.684	<0.001
Biocide (yes) : Disturbance (low)	-0.857 (0.486)	-1.761	0.078
Biocide (yes) : Origin (native)	-0.199 (0.195)	-1.021	0.307
Disturbance (low) : Origin (native)	-0.404 (0.189)	-2.136	0.032
Biocide (yes) : Disturbance (low) : Origin (native)	0.562 (0.268)	2.097	0.036
Random Effects	**Std. deviation**		
Family	0.863		
Species nested in family	1.175		
Site	0.417		
Plot nested in site	0.700		

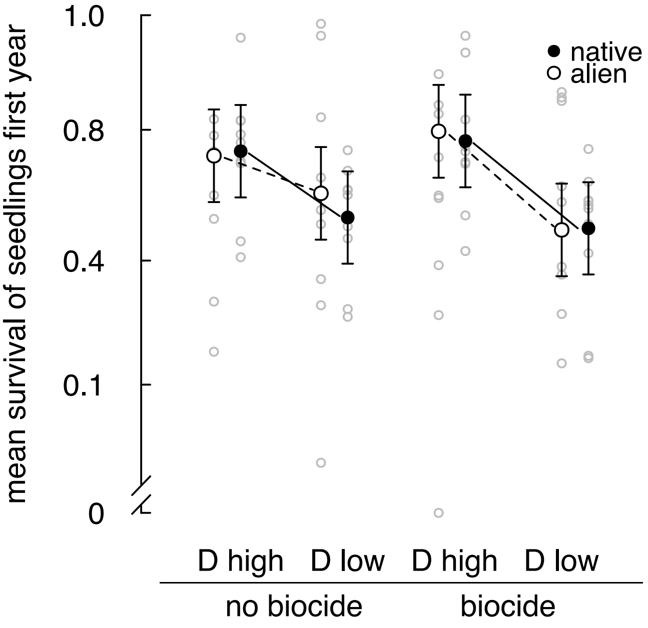


Fig.III.3: Probability of establishment success from seedlings (± SE) of 10 alien and 10 native species under high and low disturbance and biocide or water control treatment in the 1st growing season. Black dots display means for native species for the respective groups and open white dots display means for alien species (across species commonness). Small grey dots indicate raw data means for each of the species. (Note: y-axis on logit scale)

The minimum model for establishment success of seedlings in spring of the 2nd growing season contained a significant 3-way interaction between disturbance treatment, species commonness and species origin, another between biocide, herbivory and origin, and the significant main effects of initial height and number of leaves (Appendix III.S1 Table A5 and Table A6). Multiple comparisons showed that disturbance promoted establishment success of all groups of species. However, as indicated by the significant disturbance x origin x commonness interaction (Appendix III.S1 Table A5 and A6), the magnitude of the disturbance effect depended on origin and commonness of the species. Among common species, the disturbance effect was stronger for aliens (mean difference = 2.355, SE= ±0.277, P <0.001, Appendix III.S1 Fig. A5) than for natives (1.584, SE = ±0.248, P <0.001), whereas, among rare species, it was stronger for natives (3.480, SE= ±0.483, P <0.001) than for aliens (2.232, SE = ±0.0.306, P <0.001, Fig. III.4). As indicated by the significant biocide x herbivory x origin interaction (Appendix III.S1 Table A5 and A6), responses of alien and native species to the herbivore-reduction treatment depended on biocide application. While establishment success of native species in herbivory-reduction cages was slightly higher in plots without biocide, alien species showed a slightly increased establishment success in herbivory-reduction cages only in plots treated with biocide. However, none of these effects was significant when we corrected for multiple tests (Appendix III.S1 Fig. A5).

The minimum model for relative change in plant size in the first season retained a significant 4-way interaction between disturbance, herbivory, commonness and species origin (Appendix III.S1 Table A7 and A8), as well as a 3-way interaction between biocide treatment, disturbance and herbivory (Appendix III.S1 Table A7 and A8), and another between biocide treatment, herbivory and species origin (Appendix III.S1 Table A7 and A8). The high disturbance treatment showed an overall positive effect on plant species size across all other treatments, with only little variation between common and rare or alien and native species (Appendix III.S1 Fig. A7). This clear effect of disturbance is corroborating the results from the survival models. Furthermore, rare native species performed worse than rare alien species in closed cages under high disturbance treatment (Appendix III.S1 Fig. A7).

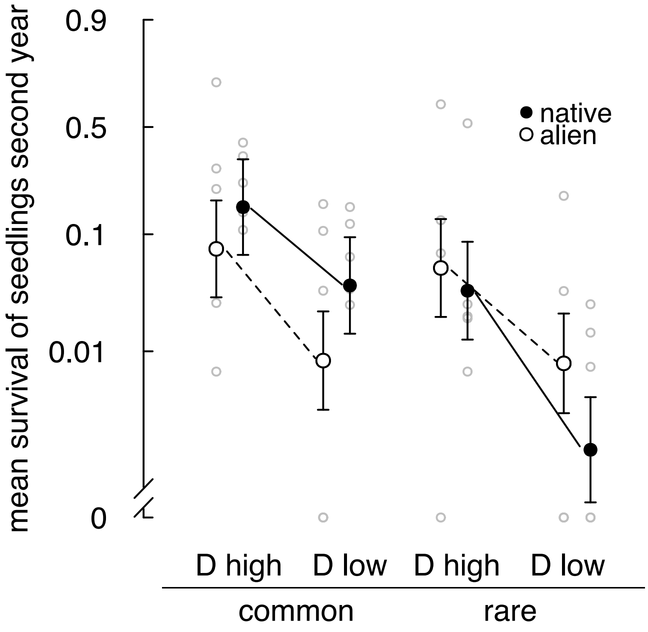


Fig.III.4: Probability of establishment success from seedlings (± SE) of 10 alien and 10 native common or rare species under high and low disturbance treatment in the 2nd growing season. Black dots display means for native species for the respective groups and open white dots display means for alien species. Small grey dots indicate raw data means for each of the species. (Note: y-axis on logit scale)

Discussion

In our multi-factorial field study, we tested whether disturbance, pathogens and herbivores influenced the establishment success from seed and seedlings of common and rare alien and native species. We found that disturbance was the most important driver of establishment success for both alien and native species, and also for both seed and seedling stages. This highlights that biotic resistance by a resident plant community is a major filter for incoming species (Levine, Adler & Yelenik 2004). Apart from the strong effect of disturbance, our results also reveal a minor role for pathogens, as seedlings of alien species showed lower establishment success in undisturbed sites with biocide application. In addition, alien species also showed lower establishment success from seeds when treated with biocide. This indicates that alien species may lose any competitive advantage when the resident community is also released from pathogen pressure (see also Reinhart & Callaway 2004, Reinhart *et al.* 2005). Notably, we did not observe clear effects of aboveground herbivore reduction on species establishment success in our experiment. This suggests that, in contrast with findings of greenhouse-based studies (Engelkes et al. 2008; Morrien, Engelkes & van der Putten 2011) interactions with pathogens may be more important than those with herbivores for establishment success.

The strong effects of disturbance leading to increased establishment as well as higher relative change in plant size can be linked to a reduction of competition (see Appendix III.S2; percentage cover) rather than altered nutrient availability (see Appendix III.S2; NO_2^-/NO_3^- analysis). The role of disturbance as an important driver of establishment success is in line with numerous other studies (Hierro *et al.* 2006; Questad & Foster 2008; Myers & Harms 2009; MacDonald & Kotanen 2010; Maron et al. 2012; Kempel *et al.* 2013). For example, Kempel *et al.* (2013) found that disturbance generally increased establishment success across a set of 93 alien and native plant species, but the importance of disturbance decreased over time relative to other factors (e.g. resistance against herbivores, species origin). Moreover, they found that native species showed a higher establishment success than aliens. In our experiment, native and alien species strongly profited from disturbance, but while native species also tended to show a higher establishment than alien species, a significant difference was only observed for rare aliens (compared to rare natives) under low disturbance, in the seed experiment. This suggests that rare alien species specifically suffer from competition with the intact resident community at initial establishment stages. The positive effect of disturbance on establishment success has also been observed by Radford, Dickinson & Lord

(2010) in a study on *Hieracium lepidulum* in New Zealand. However, Radford, Dickinson & Lord (2010) argue that (low) nutrient levels may be more important for *Hieracium* persistence after initial establishment. These findings point out that disturbance acts as a major factor for plant species establishment and strongly increases the likelihood that incoming species can overcome biotic resistance from a resident community (Lozon and MacIsaac 1997; Levine, Adler & Yelenik 2004). However, while the magnitude of the response to disturbance depended on origin and commonness of the species, overall both native and alien species benefited from disturbance with regard to establishment success as well as growth.

Besides the dominant role of disturbance, we also observed a small effect of biocide application on establishment success. Alien species established significantly worse from seeds when treated with biocide than native species. For seedlings, biocide application led to a similar decrease in establishment success from high to low disturbance for both aliens and natives. However, under the control treatment native species also showed a significant decrease from high to low disturbance, whereas alien species did not. Alien species may have lost their initial advantage of pathogen release relative to the resident plant community when the resident community is also released from pathogens because of biocide application. Consequently, greater competition with the resident community could explain the lower establishment success of alien species under low disturbance. We found a marginally non-significant increase in biomass for the community under biocide treatment (Appendix III.S2), which likely corresponds to a stronger competitive environment. As competitor removal was the likely driver behind the strong effects of disturbance, the slight increase in biomass accumulated by the resident community due to the biocide treatment is in line with the minor role that pathogen removal plays in reducing species establishment success.

Although the biocides used are specific against certain groups of pathogens (i.e oomycetes), it could be that the biocide had side effects on other organisms. We can exclude side effects of the biocide treatment on mycorrhiza (Appendix III.S2), which indicates that differences in establishment success due to biocide were not driven by side effects on an important group of soil mutualists. However, we cannot fully rule out that biocide did not affect abiotic soil properties or other soil organisms. However, we could also exclude an effect of the biocide treatment on nitrogen availability (Appendix III.S2) Notwithstanding this, our results indicate that the effects of disturbance and competition from resident communities on establishment success can also be influenced by pathogens, and that these mediatory effects depend on plant origin, likely *via* release of aliens from pathogens.

Contrary to the first growing season, survival of both alien and native species tended to be slightly higher in closed than open cages. Nevertheless, this herbivore-reduction effect was small and not significant. However, a large proportion of the plants surviving until the second growing season were from the Onagraceae (54 %). When we excluded the Onagraceae from the analysis, the results showed a significantly higher survival for common natives when growing in closed cages and generally a higher survival of common natives compared to rare natives in both open and closed cages (Appendix III.S3). In contrast, we found no significant differences for non-Onagraceae aliens. These findings indicate that the effects of herbivory showed large family-specific differences, with limited influence of the herbivory treatment on the Onagraceae potentially explaining the absence of an overall herbivore-reduction effect across all species. In another field experiment, Engelkes *et al.* (2016) found that herbivory reduced plant biomass and could influence which species dominated in a community, but they did not find that herbivory selectively promoted establishment of alien or native species. Evidence on the role of herbivory in plant species success from field and common garden studies remains equivocal (Blaney & Kotanen 2001;

Agrawal & Kotanen 2003; Dostál *et al.* 2013; Dawson *et al.* 2014; Engelkes *et al.* 2016; Korell *et al.* 2016). The lack of clear herbivore-reduction effects observed in our study corroborates these previous findings.

In summary, our study highlights the importance of assessing multiple potentially interacting factors that can contribute to establishment success of incoming alien and native plant species in existing plant communities. Disturbance had a strong effect on establishment success for both alien and native plant species, highlighting the suppressive effect of intense competition with the resident community for incoming species. Herbivory, in contrast, did not have a clear impact on species establishment success. However, we found evidence that the effects of disturbance can also be mediated by pathogens at both seed and seedling stages of establishment, and depending on species origin. Although the biocide effects in our study were small, alien species still may profit from pathogen release in intact grassland, but this benefit is lost when pathogens are suppressed and the resident community increases in biomass. This, and the overall difference in establishment success between alien and native establishment success supports the recent assertion that plant origin can matter when considering the drivers of alien species establishment and invasion (Buckley & Catford 2016). To conclude, our study shows that disturbance is a major driver for establishment success of incoming species, and interactions with pathogens can, to a lesser degree influence the level of biotic resistance of native communities to alien species.

Acknowledgements

G.M. and W.D. were funded by the Deutsche Forschungs Gemeinschaft (Project DA 1502/1-1). We thank O.Ficht, Sina Glöckner, Timo Scheu and Marisa Braun for horticultural assistance and practical help. Thanks also go to the International Max Planck Research School for Organismal Biology for supporting G.M.

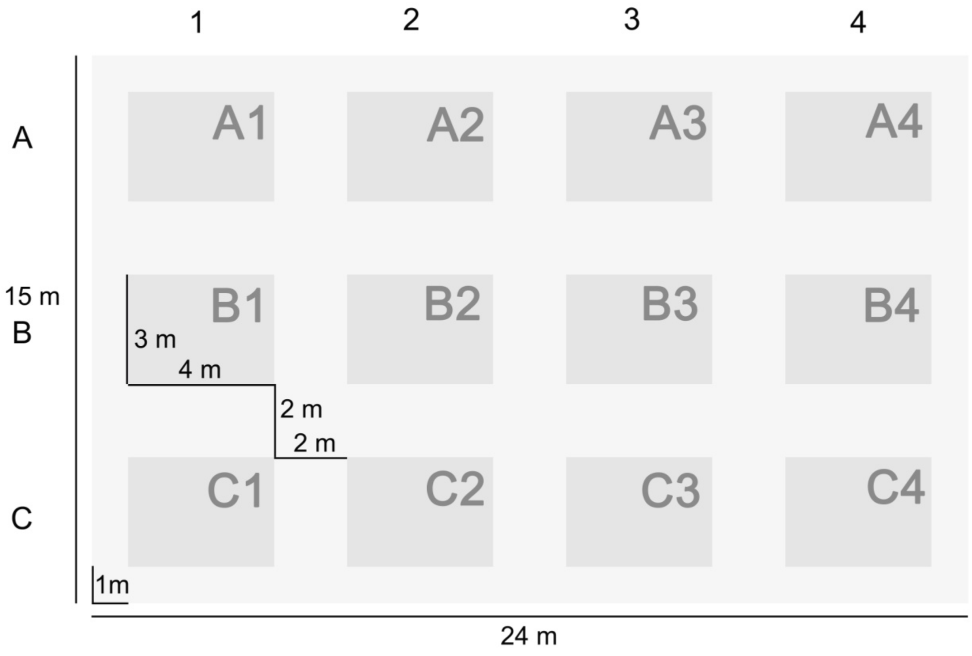
Appendices

Appendix III.S1- Supporting information on experimental setup and model selection

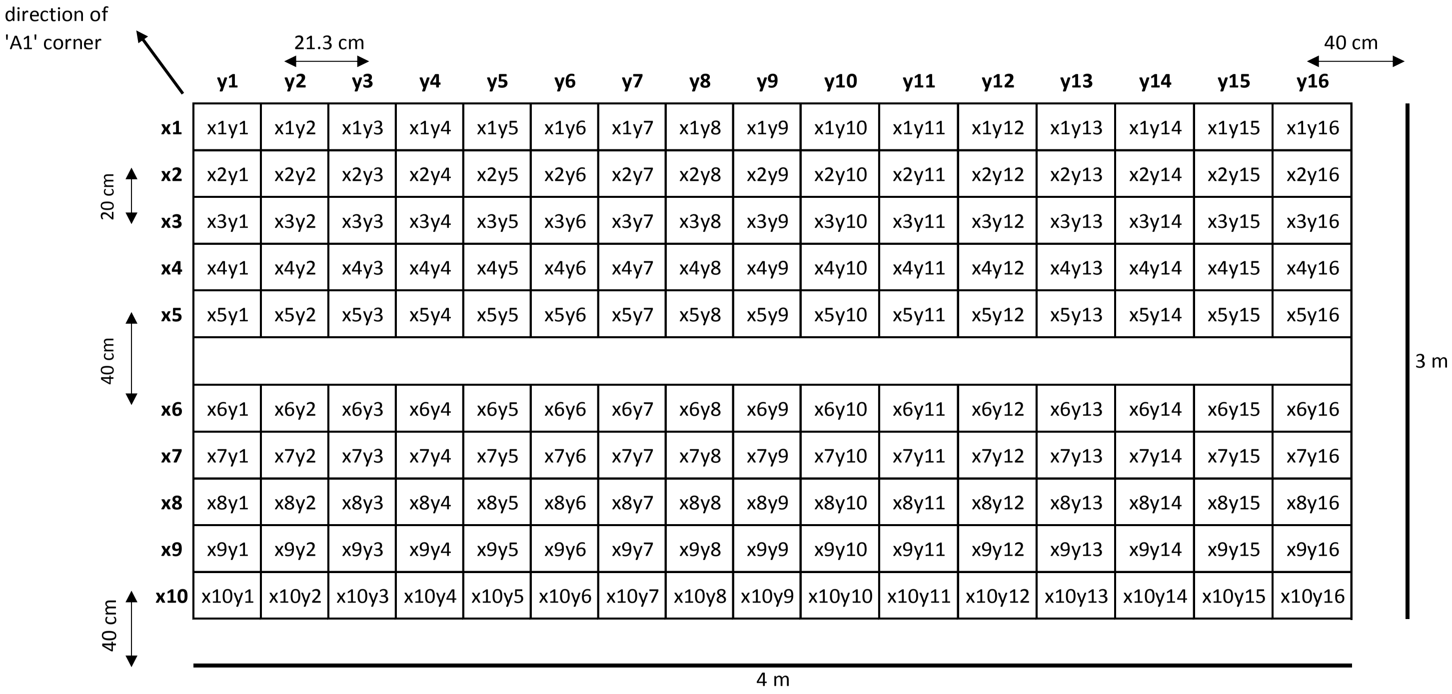
Appendix III.S1 Table A1. Geographic coordinates of the 5 study sites in the vicinity of the University of Konstanz, Germany.

Site	Lat/Long
1	47° 41' 15" N 9° 11' 17" E
2	47° 41' 10" N 9° 11' 24" E
3	47° 41' 4" N 9° 11' 25" E
4	47° 40' 57" N 9° 11' 27" E
5	47° 41' 40" N 9° 11' 31" E

Appendix III.S1 Fig. A1. Schematic figure of the setup for one (out of five) experimental sites. Each of the dark grey rectangles (A1 to C4) represents one of 12 plots (3 × 4 m) per site. Four plots in each site were "seed-experiment" plots and eight plots in each site were "seedling-experiment" plots. Plots were separated by 2 m wide paths.



Appendix III.S1 Fig. A2. Schematic layout of one plot (3x4m). Each x/y position defines one planting position in a plot



	y1	**y2**	**y3**	**y4**	**y5**	**y6**	**y7**	**y8**	**y9**	**y10**	**y11**	**y12**	**y13**	**y14**	**y15**	**y16**
x1	x1y1	x1y2	x1y3	x1y4	x1y5	x1y6	x1y7	x1y8	x1y9	x1y10	x1y11	x1y12	x1y13	x1y14	x1y15	x1y16
x2	x2y1	x2y2	x2y3	x2y4	x2y5	x2y6	x2y7	x2y8	x2y9	x2y10	x2y11	x2y12	x2y13	x2y14	x2y15	x2y16
x3	x3y1	x3y2	x3y3	x3y4	x3y5	x3y6	x3y7	x3y8	x3y9	x3y10	x3y11	x3y12	x3y13	x3y14	x3y15	x3y16
x4	x4y1	x4y2	x4y3	x4y4	x4y5	x4y6	x4y7	x4y8	x4y9	x4y10	x4y11	x4y12	x4y13	x4y14	x4y15	x4y16
x5	x5y1	x5y2	x5y3	x5y4	x5y5	x5y6	x5y7	x5y8	x5y9	x5y10	x5y11	x5y12	x5y13	x5y14	x5y15	x5y16
x6	x6y1	x6y2	x6y3	x6y4	x6y5	x6y6	x6y7	x6y8	x6y9	x6y10	x6y11	x6y12	x6y13	x6y14	x6y15	x6y16
x7	x7y1	x7y2	x7y3	x7y4	x7y5	x7y6	x7y7	x7y8	x7y9	x7y10	x7y11	x7y12	x7y13	x7y14	x7y15	x7y16
x8	x8y1	x8y2	x8y3	x8y4	x8y5	x8y6	x8y7	x8y8	x8y9	x8y10	x8y11	x8y12	x8y13	x8y14	x8y15	x8y16
x9	x9y1	x9y2	x9y3	x9y4	x9y5	x9y6	x9y7	x9y8	x9y9	x9y10	x9y11	x9y12	x9y13	x9y14	x9y15	x9y16
x10	x10y1	x10y2	x10y3	x10y4	x10y5	x10y6	x10y7	x10y8	x10y9	x10y10	x10y11	x10y12	x10y13	x10y14	x10y15	x10y16

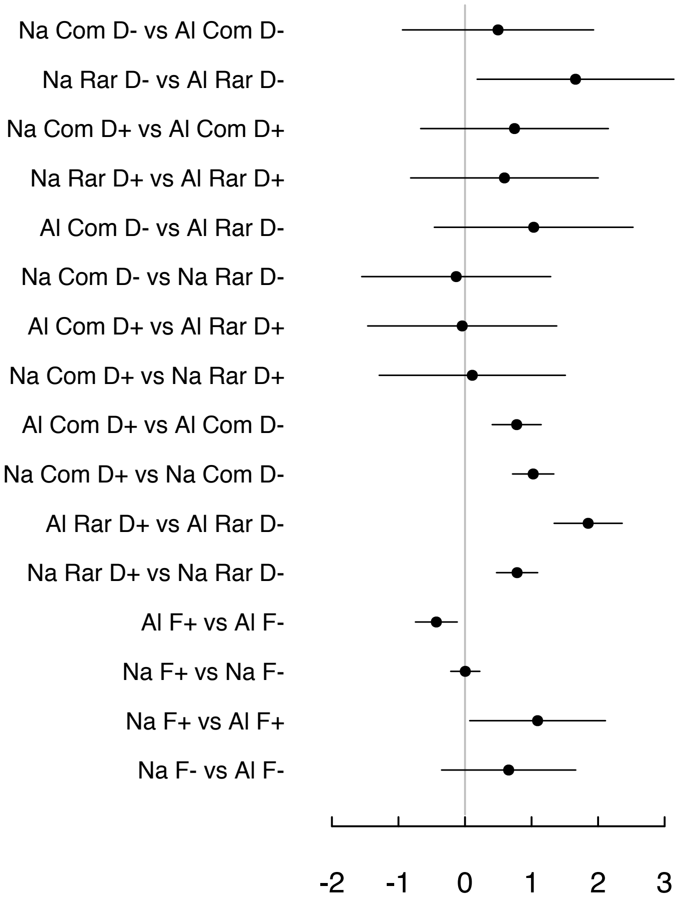
Appendix III.S1 Table A2. Timeline for setup measurements, application of treatments and additional side experiments.

Tasks	Experiment	Date
Setup of sites		
Identification of resident community	both	03.04. - 06.04.2014
mowing to 5cm	both	07.04. - 08.04.2014
tillage	both	09.04. - 10.04.2014
sowing seeds	seed exp.	14.04. - 16.04.2014
building of cages	seedling exp.	01.05. - 20.05.2014
rearing seedlings	seedling exp.	17.03. - 16.04.2014
planting seedlings	seedling exp.	17.04. - 29.04.2014
biocide application		
1st biocide application (Previcur En.)	both	30.04. - 01.05.14
2nd biocide application (Fenomenal)	both	11.06.14
3rd biocide application (Previcur En.)	both	23.07. - 24.07.2014
4th biocide application (Fenomenal)	both	10.09.14
5th biocide application (Previcur En.)	both	22.10. - 23.10.2014
6th biocide application (Fenomenal)	both	25.03.15
7th biocide application (only subplots) (Previcur En.)		14.05. - 15.05.2015
Surveys		
1st survey (seeds)	seed exp.	02.06. - 17.06.2014
2nd survey (seeds - no sufficient data)	seed exp.	12.04. - 14.04.2015
1st survey (seedlings)	seedling exp.	05.05. - 12.05.2014
2nd survey (seedlings)	seedling exp.	28.05. - 12.08.2014
3rd survey (seedlings)	seedling exp.	16.04. - 24.04.2015
Additional measures		
per centage cover	seedling exp.	23.06. - 27.06.2014
soil samples	both	22.07.14
setting up subplots (effect on resident community)	side exp.	24.03.15
harvest of subplots (effect on resident community)	side exp.	17.06.15
beer traps (cage efficiency meassurement)	seedling exp.	21.06. - 22.06.2015
insect collection (cage efficiency meassurement)	seedling exp.	18.06. - 19.06.2015
Plantago root collection (test side effects of biocide)	seedling exp.	18.06. - 21.06.2015

Appendix III.S1 Table A3. Results for each step of the stepwise backward model selection via likelihood-ratio tests for the generalized linear mixed effects model of establishment success of seeds. The main effects that were contained in a significant interaction were not tested, as the interaction was retained in the minimum model.

Term	χ^2	**df**	**p**
4-way-interaction			
Disturbance x Biocide *x* Origin *x* Commonness	0.30	1	0.583
3-way-interactions			
Biocide *x* Origin x Commonness	0.24	1	0.624
Disturbance x Biocide x Commonness	0.56	1	0.454
Disturbance x Biocide x Origin	3.54	1	0.059
Disturbance x Origin x Commonness	24.16	1	**<0.001**
2-way-interactions			
Biocide x Commonness	0.42	1	0.516
Disturbance x Biocide	1.54	1	0.214
Biocide x Origin	6.66	1	**0.009**

Appendix III.S1 Fig A3. Plot with estimates of differences between means and respective 95% confidence intervals for all pairwise comparisons of the terms included in significant interactions in the model for seeds.

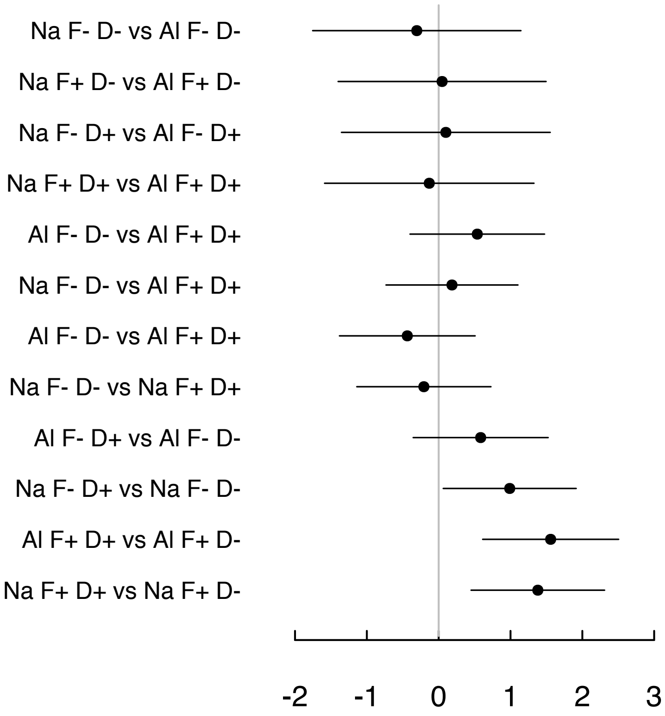


Appendix III.S1 Table A4. Results for each step of the stepwise backward model selection via likelihood-ratio tests for the generalized linear mixed effects model of establishment success of seedlings in the 1st growing season. The main effects that were contained in a significant interaction were not tested, as the interaction was retained in the minimum model.

Term	χ^2	**df**	**p**
5-way-interaction			
Biocide : Disturbance : Herbivory : Commonness : Origin	0	1	0.986
4-way-interaction			
Biocide : Herbivory : Commonness : Origin	0.007	1	0.931
Biocide : Disturbance : Herbivory : Origin	0.064	1	0.800
Biocide : Disturbance : Commonness : Origin	0.107	1	0.743
Disturbance : Herbivory : Commonness : Origin	1.086	1	0.297
Biocide : Disturbance : Herbivory : Commonness	1.485	1	0.223
3-way-interaction			
Biocide : Herbivory : Commonness	0.068	1	0.793
Disturbance : Commonness : Origin	0.225	1	0.635
Disturbance : Herbivory : Commonness	0.63	1	0.427
Biocide : Commonness : Origin	0.892	1	0.345
Biocide : Disturbance : Commonness	1.061	1	0.302
Disturbance : Herbivory : Origin	1.431	1	0.231
Herbivory : Commonness : Origin	1.707	1	0.191
Biocide : Disturbance : Herbivory	2.529	1	0.111
Biocide : Herbivory : Origin	3.001	1	0.083
Biocide : Disturbance : Origin	4.370	1	**0.036**
2-way-interaction			
Herbivory : Commonness	0.007	1	0.933
Herbivory : Origin	0.032	1	0.858
Biocide : Herbivory	0.125	1	0.723
Commonness : Origin	0.185	1	0.667
Biocide : Commonness	0.591	1	0.442

Disturbance : Herbivory	1.196	1	0.274
Disturbance : Commonness	2.895	1	0.088
Main effects			
Commonness	1.153	1	0.282
Height	2.529	1	0.111
Herbivory	3.561	1	0.059
Leaves	78.637	1	**<0.001**

Appendix III.S1 Fig A4. Plot with estimates of differences between means and respective 95% confidence intervals for all pairwise comparisons of the terms included in significant interactions in the model for seedling establishment in the 1st season.



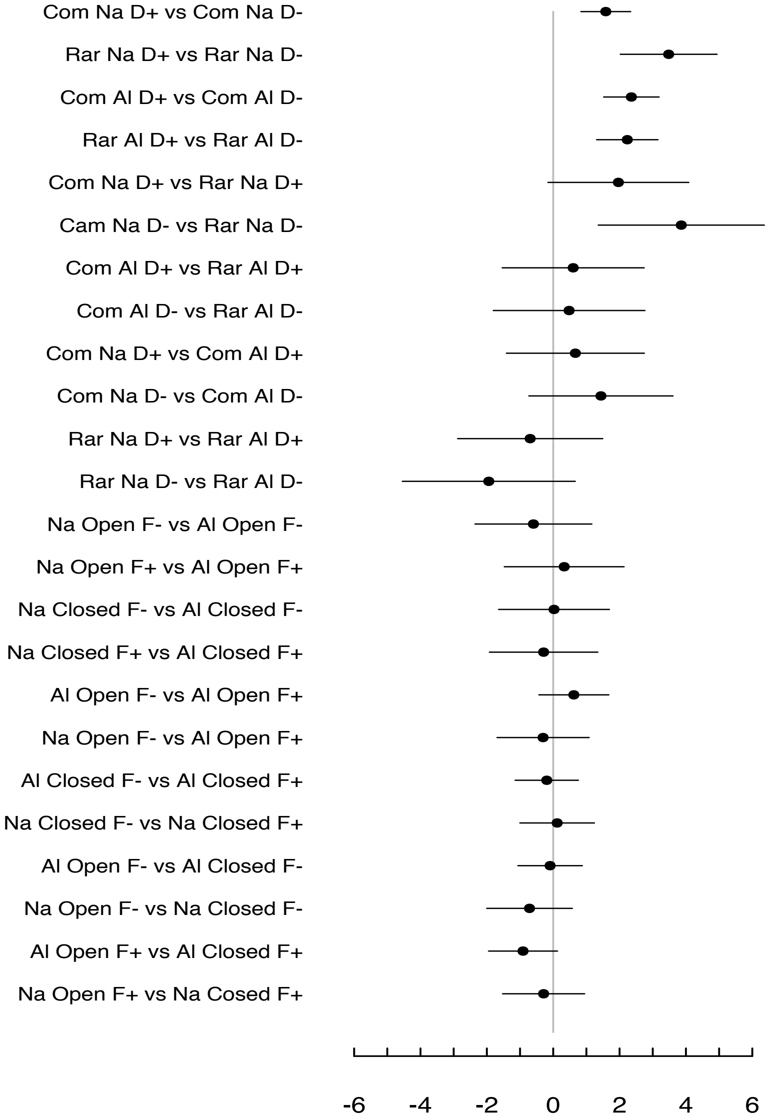
Appendix III.S1 Table A5. Minimum generalized linear mixed effects model explaining probability of seedling establishment success in the 2nd growing season, for 20 alien and native rare and common plant species under high and low disturbance treatment and biocide or water control treatment as well as open and closed cages.

Parameters	**Estimate (Std. error)**	**t-value**	**p-value**
Fixed Effects			
Intercept	-2.266 (1.012)	-2.239	0.025
Biocide (yes)	0.032 (0.333)	0.096	0.923
Disturbance (low)	2.288 (0.287)	7.969	<0.001
Herbivory (open)	-0.279 (0.337)	-0.827	0.408
Commonness (rare)	-0.391 (0.746)	-0.524	0.600
Origin (native)	1.059 (0.739)	-1.432	0.152
Leaves	0.621 (0.095)	6.479	<0.001
Height	0.404 (0.067)	6.015	<0.001
Biocide (yes) : Herbivory (open)	-0.425 (0.479)	-0.887	0.374
Disturbance (low) : Commonness (rare)	0.334 (0.312)	1.07	0.284
Biocide (yes) : Origin (native)	-0.255 (0.256)	-0.996	0.319
Disturbance (low) : Origin (native)	0.682 (0.271)	2.517	0.011
Herbivory (open) : Origin (native)	-0.612 (0.270)	-2.269	0.023
Commonness (rare) : Origin (native)	-1.321 (1.048)	-1.26	0.207
Biocide (yes) : Herbivory (open) : Origin (native)	0.924 (0.383)	2.414	0.157
Disturbance (low) Commonness (rare) : Origin (native)	-1.987 (0.551)	-3.605	<0.001
Random Effects	**Std. deviation**		
Family	1.644		
Species nested in family	1.100		
Site	0.187		
Plot nested in site	0.601		

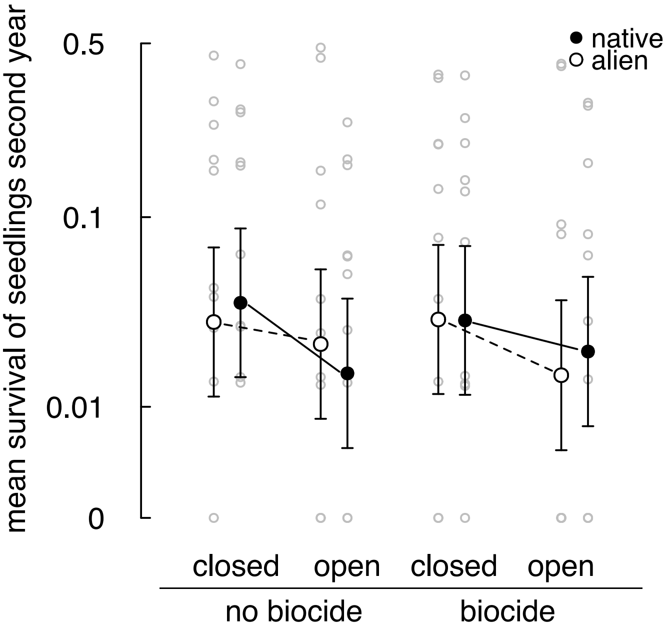
Appendix III.S1 Table A6. Results for each step of the stepwise backward model selection via likelihood-ratio tests for the generalized linear mixed effects model explaining establishment success of seedlings in the 2nd growing season. The main effects that were contained in a significant interaction were not tested, as the interaction was retained in the minimum model.

Term	χ^2	**df**	**p**
5-way-interaction			
Biocide : Disturbance : Herbivory : Commonness : Origin	0.084	1	0.772
4-way-interaction			
Biocide : Disturbance : Herbivory : Commonness	0.340	1	0.559
Biocide : Disturbance : Commonness : Origin	0.450	1	0.502
Biocide : Herbivory : Commonness : Origin	0.627	1	0.428
Disturbance : Herbivory : Commonness : Origin	1.986	1	0.158
Biocide : Disturbance : Herbivory : Origin	3.525	1	0.064
3-way-interaction			
Biocide : Herbivory : Commonness	0.058	1	0.809
Disturbance : Herbivory : Commonness	0.134	1	0.713
Biocide : Disturbance : Origin	0.343	1	0.557
Biocide : Herbivory : Origin	0.515	1	0.473
Biocide : Disturbance : Commonness	1.277	1	0.258
Biocide : Commonness : Origin	1.263	1	0.261
Biocide : Disturbance : Herbivory	1.973	1	0.16
Herbivory : Commonness : Origin	3.262	1	0.07
Biocide : Herbivory : Origin	5.723	1	**0.016**
Disturbance : Commonness : Origin	13.957	1	**<0.001**
2-way-interaction			
Biocide : Commonness	0	1	0.996
Disturbance : Herbivory	0.114	1	0.734
Biocide : Disturbance	1.428	1	0.231
Herbivory : Commonness	3.28	1	0.07
Main effects			
Leaves(log)	43.507	1	**<0.001**
Height	34.916	1	**<0.001**

Appendix III.S1 Fig A5. Plot with estimates of differences between means and respective 95% confidence intervals for all pairwise comparisons of the terms included in significant interactions in the model for seedlings in the 2nd season.



Appendix III.S1 Fig. A6. Probability of establishment success from seedlings (± SE) of 10 alien and 10 native species in open and closed cages under biocide or water control treatment in the 2nd growing season. Black dots display means for native species for the respective groups and open white dots display means for alien species. Small grey dots indicate raw data means for each of the species. (Note: y-axis on logit scale).



Appendix III.S1 Table A7 Minimum generalized linear mixed effects model explaining seedling relative change in plant size of 20 alien and native rare and common plant species under high and low disturbance treatment and biocide or water control treatment as well as open and closed cages.

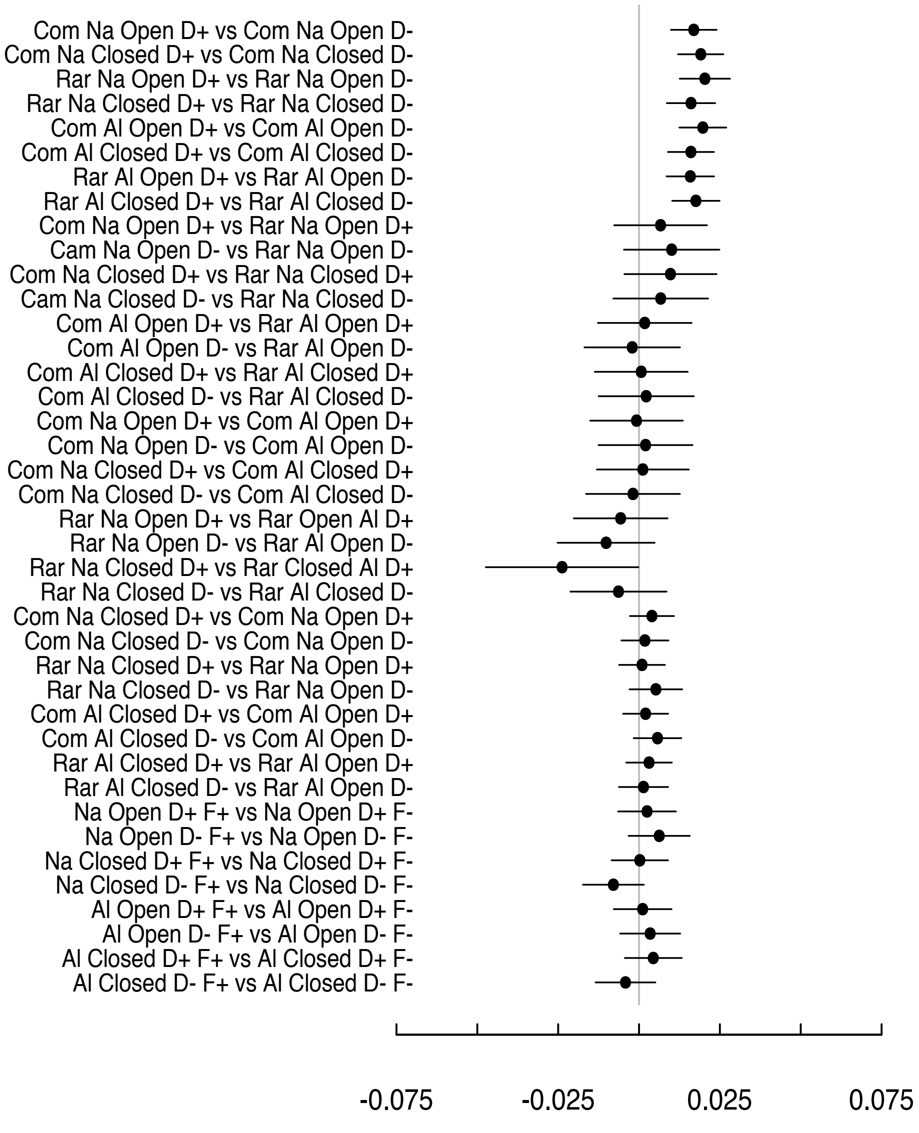
Parameters	**Estimate (Std. error)**	**t-value**
Fixed Effects		
Intercept	0.023 (0.007)	3.167
Biocide (yes)	0.004 (0.002)	1.597
Disturbance (low)	-0.011 (0.002)	-4.002
Herbivory (open)	-0.000 (0.002)	-0.087
Commonness (rare)	-0.000 (0.004)	-0.167
Origin (native)	0.003 (0.004)	0.674
Biocide (yes) :Disturbance (low)	-0.008 (0.003)	-2.232
Biocide (yes) : Herbivory (open)	-0.003 (0.003)	-0.916
Disturbance (low) : Herbivory (open)	-0.009 (0.004)	-2.247
Disturbance (low) : Commonness (rare)	-0.001 (0.002)	-0.718
Herbivory (open) : Commonness (rare)	-0.001 (0.001)	-0.521
Biocide (yes) : Origin (native)	-0.003 (0.001)	-2.551
Disturbance (low) : Origin (native)	-0.003 (0.002)	-1.549
Herbivory (open) : Origin (native)	-0.004 (0.002)	-2.180

Commonness (rare) : Origin (native)	-0.009 (0.006)	-1.424
Biocide (yes) : Disturbance (low) : Herbivory (open)	0.011 (0.005)	2.146
Disturbance (low) : Herbivory (open) : Commonness (rare)	0.005 (0.003)	1.781
Biocide (yes) : Herbivory (open) : Origin (native)	0.005 (0.002)	2.638
Disturbance (low) : Herbivory (open) : Origin (native)	0.005 (0.002)	2.023
Disturbance (low) : Commonness (rare) : Origin (native)	0.004 (0.004)	1.573
Herbivory (open) : Commonness (rare) : Origin (native)	0.004 (0.002)	1.522
Disturbance (low) : Herbivory (open) : Commonness (rare) : Origin (native)	-0.012 (0.004)	-2.757
Random Effects	**Std. deviation**	
Family	<0.001	
Species nested in family	<0.001	
Site	<0.001	

Appendix III.S1 Table A8. Results for each step of the stepwise backward model selection via likelihood-ratio tests for the generalized linear mixed effects model of relative change in plant size between the 1st and 2nd survey. The main effects that were contained in a significant interaction were not tested, as the interaction was retained in the minimum model

Term	χ^2	**df**	**p**
5-way-interaction			
Biocide : Disturbance : Herbivory : Commonness : Origin	0.165	1	0.684
4-way-interaction			
Biocide : Disturbance : Herbivory : Origin	0.085	1	0.77
Biocide : Disturbance : Commonness : Origin	0.123	1	0.725
Biocide : Disturbance : Herbivory : Commonness	0.598	1	0.439
Biocide : Herbivory : Commonness :Origin	2.168	1	0.14
Disturbance : Herbivory : Commonness :Origin	7.619	1	**0.005**
3-way-interaction			
Biocide : Commonness : Origin	0.103	1	0.748
Biocide : Disturbance : Origin	0.225	1	0.634
Biocide : Disturbance : Commonness	0.316	1	0.573
Biocide : Herbivory : Commonness	1.7	1	0.192
Biocide : Disturbance : Herbivory	5.312	1	**0.211**
Biocide : Herbivory : Origin	7.013	1	**0.008**

Appendix III.S1 Fig A7. Plot with estimates of differences between means and respective 95% confidence intervals for all pairwise comparisons of the terms included in significant interactions in the model explaining seedling relative change in plant size.



Appendix III.S2 - Additional experiments to test for the effects of the applied treatments

Disturbance effects on plant cover

To assess the effects of the disturbance treatment, we recorded percentage cover of plants and bare ground in a 20 cm x 20 cm square centered on each target position in the seedling experiment. We analysed percentage of bare ground using a linear mixed model in lme4 (Bates *et al.* 2014). The percentage data were arc sin square root transformed to achieve normality of the data. Disturbance, biocide treatment and herbivory reduction treatment and all respective interactions were included as explanatory variables in the model. Site and plot nested in site were included as random effects, and likelihood-ratio tests were performed to assess significance of the model terms.

The disturbance treatment had a significant effect on percentage bare ground ($\chi^2 = 32.77$, df = 1, $P < 0.001$). High disturbance plots had 34.2% bare ground on average compared to 19.4% in low-disturbance plots. This indicates that the disturbance treatment was effective in regard to removal of resident plants (i.e. competitors for incoming species).

Disturbance effects on nutrient availability

Soil samples were taken in the middle of each plot using a soil corer with 5 cm diameter to a depth of approximately 8 cm. The soil samples were stored at -80° C immediately after collecting until further processing. Leaf and root material was removed from defrosted soil samples, which were then sieved (2-mm mesh). We added 20 g of soil to 80 ml of 0.0125 M $CaCl_2$ (1:4 soil/salt-solution). These suspensions were put for 2 h on an orbital shaker (speed dial 120). Then we left them to settle for 1 minute, poured the suspension through a filter paper (Whatman 595 ½, 70mm filter paper, GE Healthcare) and froze the filtrate until nitrogen-availability analysis. The total nitrate and nitrite concentration (NO_2^- and NO_3^- in µg/g dry soil) of the filtrate was analyzed using a segmented flow auto-analyzer (Technicon® AutoAnalyzer II, Technicon®). Per sample, 4 ml was analyzed.

We analyzed NO_2^-/NO_3^- concentration using a linear mixed model with lme4. Disturbance, biocide treatment and herbivory-reduction treatment, as well as all possible interactions were used as model terms, and site was used as a random effect. We used likelihood ratio tests to assess the significance of the model terms.

None of the model terms had a significant effect on the NO_2^-/NO_3^- concentration of the soil. This indicates that none of our treatments significantly altered the nutrient availability for the plants.

Biocide effects on resident community

To test the effect of the biocides on the resident community, we installed one pair of 30 x 30 cm subplots ("block") on three sides of each site in the 1 m strips around the plots at the beginning of the growing season in the second year (Appendix III.S1 Table A3). We treated one subplot in each pair once with each of the two biocides (same concentrations as the biocide treatment of the experimental plots; 1st application Fenomenal®, 2nd application Previcur Enegy®), whereas the other one received the same amounts of water as a control. We harvested the biomass of the subplots on the

17th of June 2015, dried the samples at 80°C for 48 h and weighed them to assess the performance of the resident community with and without biocide application.

We analysed the total biomass of the subplots treated with and without biocide using a generalized linear mixed model in lme4 (Bates *et al.* 2014). Biomass data was natural-log transformed to achieve normality. Biocide treatment (with/without) was included as a fixed effect. Block nested in site was included as a random effect. A likelihood-ratio test was performed to assess significance of biocide treatment.

Subplots treated with biocide had a slightly larger biomass (mean= 3.737, SE = ±0.086) than the control plots (mean= 3.608, SE = ±0.086), however, this effect was marginally non-significant (χ^2 = 2.76, df = 1, P = 0.095).

Mycorrhiza analysis

To test for any potential side effects of the biocide treatment on mycorrhization of plants we also collected roots of three *Plantago lanceolata* plants (growing in sufficient numbers in all plots – therefore used as a bioassay) in each of the plots in all five sites. We washed the roots and heated them at 80°C in a 10% KOH solution in a water bath until the roots became transparent. After that, the roots were heated again for five minutes in a 5 % vinegar, 5 % ink solution (Parker Quink Black, NWL France Services, Boulogne, France) to stain mycorrhizal fungal structures. We mounted the stained roots on glass slides, and analysed them by counting mycorrhizal fungal structures (vesicles, arbuscules and hyphae) at 50 intersections per sample under a microscope at 100x magnification (Zeiss Axioscope, Carl Zeiss, Jena Germany).

We analysed the number of intersections containing mycorrhiza fungal structures or not, using binomial generalized linear mixed models in the lme4 package. We used separate models for “seed” and “seedling” plots. The model for the “seed plots” contained the fixed factors biocide and disturbance as well as the interaction between both. Site, and plot nested in site were added as random effects. The model for the “seedling plots” was the same, but additionally contained herbivory treatment as a fixed term as well as all possible interactions with it. We used likelihood ratio tests to determine the significance of the model terms.

Plantago lanceolata roots from high disturbance “seed plots” showed a lower rate mycorrhizal colonisation (high disturbance mean= -0.294, SE = ±0.143, low disturbance mean= 0.215, SE = ±0.1014; $\chi^2 = 3.832$, df = 1, P = 0.050). *Plantago lanceolata* roots in the seedling plots did not show any significant differences in mycorrhizal colonisation between treatments.

Effectiveness of herbivore-reduction treatment

To assess the effectiveness of the cages in regard to herbivore reduction, we set up beer traps in the open cages on the 21st of June 2015 (i.e. in the 2nd season), in addition to the beer traps that we had already in the closed cages. We filled all traps with beer (Fürstenbergische Brauerei, Donaueschingen, Germany), and counted the slugs found in the traps of open and closed cages on the following day.

To assess whether there were differences in arthropod abundance in the open and closed cages, we used a vacuum suction device (Type LB37CCM, ECON Handel, Herzebrock-Clarholz, Germany) to collect arthropods. We used the vacuum suction device for five minutes in every plot. We performed the arthropod sampling between the 18th and 19th of June 2015. The collected arthropods were put in a -80°C freezer for five minutes and were then classified according to their mode of feeding into groups of herbivores, omnivores and non-herbivores.

We analysed the number of slugs, the number of herbivorous arthropods and the total number of arthropods per plot using negative binomial generalized linear mixed models in the glmmADMB package (Fournier *et al.* 2012). We included herbivore treatment (open cages/closed cages) as a fixed effect and site as a random effect in the model. As for the other analyses, we used a likelihood-ratio test to determine the significance of the fixed effect.

Compared to the open cages, the closed cages had significantly fewer slugs (open: mean= 3.874, 95% CI = ±0.250, closed: 1.689, SE = ±0.266; $\chi^2 = 91.01$, df = 1, $P < 0.001$), herbivorous arthropods (open: 2.829, SE = ±0.151, closed: 2.230, SE = ±0.159; $\chi^2 = 10.26$, df = 1, $P = 0.001$) and total arthropods (open: 4.326, SE = ±0.193, closed: 3.457, SE = ±0.195; $\chi^2 = 10.26$, df = 1, $P = 0.001$).

Appendix III.S3 – Additional analysis excluding the Onagraceae

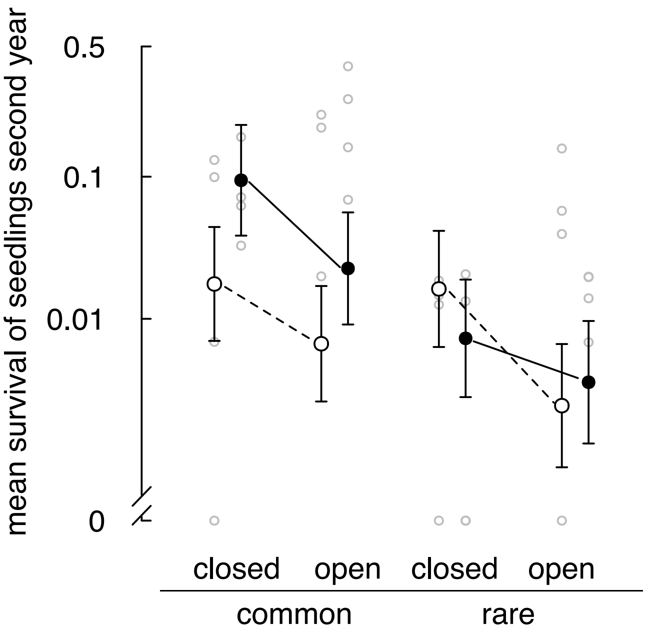
Due to high mortality over the winter and differences in survival between the species, a large proportion of surviving plants were from a single family - the Onagraceae. Thus, to test the robustness of the results, we also analysed the survival in the 2nd growing season excluding the Onagraceae using the same procedure as for the full data set.

The minimum model excluding the Onagraceae contained a significant three-way interaction between herbivory treatment, species commonness and origin (X^2 = 4.606, df = 1, P = 0.031, Appendix III.S3 Table C1). This interaction showed that common native species survived significantly less well in open cages than in closed cages (mean difference = -1.292, SE = ±0293, P <0.001, Appendix III.S3 Fig. C1), while common natives survived significantly more than rare natives in both open (2.494, SE = ±0.852, P =0.038, Appendix III.S3 Fig. C1) and closed cages (3.143, 95% CI = ±0.761, P <0.001, Appendix III.S3 Fig. C1). These differences were not observed for the non-Onagraceae aliens. Furthermore, the minimum model contained the significant main effects of plant height (χ^2 = 31.915, df = 1, P < 0.001, Appendix III.S3 Table C1) and number of leaves (χ^2 = 23.412, df = 1, P < 0.001, Appendix III.S3 Table C1), as well as disturbance (χ^2 = 27.726, df = 1, P < 0.001, Appendix III.S3 Table C1). These findings indicate that larger plants showed a higher survival and that disturbance also increased survival regardless of species origin or commonness (1.640, SE = ±0.316, P <0.001)

Appendix III.S3 Table C1. Minimum generalized linear mixed effects model for probability of establishment success of seedlings in the second growing season of 16 alien and native rare and common plant species (excluding the Onagraceae family) under high and low disturbance treatment and biocide or water control treatment as well as open and closed cages.

Parameters	**Estimate (Std. error)**	**t-value**	**p-value**
Fixed Effects			
Intercept	-3.210 (0.965)	-3.326	<0.001
Disturbance (low)	1.592 (0.260)	-6.123	<0.001
Herbivory (open)	-1.008 (0.318)	-3.168	0.002
Commonness (rare)	-0.083 (0.852)	-0.098	0.921
Origin (native)	1.749 (0.816)	2.142	0.032
Leaves	0.587 (0.122)	4.780	<0.001
Height	0.459 (0.079)	5.796	<0.001
Herbivory (open) : Commonness (rare)	-0.960 (0.478)	-2.007	0.044
Herbivory (open) : Origin (native)	-0.478 (0.308)	-1.554	0.120
Commonness (rare) : Origin (native)	-2.583 (1.207)	-2.140	0.032
Herbivory (open) : Commonness (rare) :Origin (native)	1.707 (0.744)	2.294	0.021
Random Effects	**Std. deviation**		
Family	1.237		
Species nested in family	1.095		
Site	0.245		
Plot nested in site	0.618		

Appendix III.S3 Fig. C1.Probability of establishment success from seedlings (± SE) of 8 alien and 8 native common and rare species in open and closed cages in the 2nd growing season, excluding the Onagraceae family. Black dots display means for native species for the respective groups and open white dots display means for alien species. Small grey dots indicate raw data means for each of the species. (Note: y-axis on logit scale)



General discussion

In recent years substantial progress in understanding the interactions between plants and soil has been made and it has been acknowledged that the belowground part, sometimes referred to as 'black box', is an important driver behind species performance (van der Putten *et al.* 2013). However, due to the complex nature of interactions between plant and soil and the multitude of factors involved we still do not fully understand how general the effect of plant-soil feedback is and, what role it plays for species success, particularly in terms of species invasions and how important it is relative to other factors.

In the three studies included in this thesis I specifically tested for the generality of plant-soil feedback effects across common and rare native and alien plant species, revealing insights on whether success of aliens and natives may be similarly driven by belowground interactions or if alien plant species profit disproportionally from less negative (or even positive) feedbacks. Moreover, the role of plant-soil feedback was tested simultaneously with other factors such as intraspecific competition (Chapter I and Chapter II) or disturbance and herbivores (Chapter III). I could show that alien species do not generally profit from belowground enemy release and that they are similarly or even more strongly negatively, affected by soil microbes than native species (Chapter I and Chapter II). Furthermore, alien and native species showed self-limitation due to intraspecific competition, however, these findings were not linked to species commonness (Chapter I). However, alien species showed higher per capita growth at low intraspecific frequency in the common garden mesocosm study, but these findings were also not related to species commonness (Chapter II). Moreover, effects of plant-soil feedback and competition were not interacting i.e. either ameliorating or intensifying one another (Chapter I and Chapter II). Lastly, I could show that disturbance is of overriding importance for plant species establishment compared to plant-soil interactions and herbivores. Nonetheless, it is important to also consider indirect effects, as experimental release from soil borne enemies for the resident species decreased establishment success of incoming alien species (Chapter III). In summary these findings first point to a major role of generalist pathogens affecting natives as well as alien species at least in our study system Second, effects of intraspecific competition and plant-soil feedback seem to act independently. Third, plant-soil feedback effects play only a minor role compared to disturbance, however, *via* indirect effects they still can be important for species establishment success. Fourth species commonness cannot generally be linked to differences in magnitude of plant-soil feedback or intraspecific competition.

Plant-soil feedback and species success

The literature on the role of plant-soil feedback for species success remains mixed. While there are studies that show that abundant species suffer less from negative plant-soil feedback (Mangan *et al.* 2010; MacDougall Rillig & Klironomos 2011; Liu *et al.* 2015; Xu, Wang & Yu 2015) there are also studies that show no relation of plant-soil feedback to local species abundances (Reinhart 2012) or even the opposite, i.e. that dominant species suffer more strongly form negative plant-soil interactions (van der Putten *et al.* 1993; Maron *et al.* 2016). So, while MacDougall, Rillig & Klironomos (2011) for example found less negative plant–soil feedback for dominant alien species (grown in conspecific soil) in a species rich savannah, Reinhart (2012) could not link abundance to strength of plant-soil feedback effects in grassland. Moreover, in a recent study Maron *et al.* (2016) found that common species showed more negative plant-soil-feedback effects than rare species. Notably, none of the three studies included in this thesis, ranging from greenhouse to field studies showed that plant-soil interactions were linked to species commonness. Given that I used a phylogenetically-informed study (Felsenstein 1985) using large sets of common and rare species of both origins my studies suggest that differences in plant-soil feedback do not generally explain species success. Nevertheless, this does still allow for single species to maintain high abundances due to lower self-limitation by less negative negative plant-soil interactions. Thus, the context-dependency of species responses to plant-soil interactions needs to be considered. This means that the study system may have a strong influence (e.g. soil type, fertility of soil, life form, phylogeny of the studied species) on the strength of plant-soil feedback, likely explaining mixed results in the literature. For example, Bezemer *et al.* (2006) showed in a study comparing sandy loam soil with calcareous soil that plant-soil-feedback effects and the respective soil microbial communities were largely dependent on soil type and host plant species (but see Perkins, Hatfield & Espeland 2016). Furthermore, Larios & Suding (2015) found that plant-soil feedback responses shifted along a nitrogen gradient for a native and an exotic grass species (see also Johnson *et al.* 2003). The role of phylogeny of the studied species in explaining plant-soil feedback responses was recently highlighted by Anacker *et al.* 2014 (but see Merhabi & Tuck 2015). Thus, to shed light on when and under what conditions plant-soil feedback eventually may play a role in explaining species success, it is important to integrate the influence of various other ecological factors that influence species responses to plant-soil interactions (see also section on “the relative role of plant-soil feedback and multiple factors” below)

A crucial point for comparing studies investigating species success is the measure used for species commonness. While I used a regional measure of commonness (i.e. number of grid cells occupied by the species in Germany) based on the assumptions of the abundance-occupancy relationship that widespread species also are locally abundant (Gaston 2003), other studies use local measures of abundance (MacDougall, Rillig & Klironomos 2011; Maron *et al.* 2016). Both approaches have their advantages and disadvantages. A local measure of commonness reflects the local scale at which plant-soil feedback effects act. However, these measures may also largely be influenced by other factors such as microclimate or local soil differences and may particularly include species that are highly specific for the studied system or habitat. While this allows for more accuracy in inferring what drives local abundance in the studied system it limits conclusions on whether plant-soil feedback is the driver behind species success in general and specifically if it may explain commonness or rarity of both alien and native species. Consequently, using a combined approach including data on local abundances with data on regional scale distribution of species, although hard to achieve, could further help improving our understanding on how plant-soil feedback influences species success. In the same vein Catford *at al.* (2016) argue to use four dimensions including local abundance, geographic range, environmental range and spread rate, to improve our abilities to understand what makes a species successful, as none of these dimensions alone clearly separated invasive form non-invasive species.

A particularly notable aspect of my findings is that alien species did not generally profit from soil-borne enemy release. Based on the enemy release hypothesis (Keane & Crawley 2002), it has been suggested that the disproportional success of invasive alien species may be linked to the release from natural enemies in the invaded range. While the majority of studies on enemy release focused on aboveground enemies, I considered the below ground perspective in my studies. However, despite several of the species in my experiments being invasive in Germany, I could not detect that aliens in general or specifically the common aliens were released from belowground enemy attack. In contrast, aliens but not natives were affected by negative plant-soil feedback effects in my greenhouse study (Chapter I). Moreover, also in the common garden mesocosm study (Chapter II) native and alien species similarly profited form fungicide application, which suggests that species of both origins were affected by soil-borne pathogens. This highlights that generalist pathogens may play a substantial role in the outcomes of plant-soil feedback. Furthermore, alien species may also suffer more from novel belowground interactions with pathogens they have not encountered in their home range (Chapter I). Thus, the balance between generalist and specialist pathogens and how virulent they are, or how susceptible the host species is, rather than just presence or absence of species-specific pathogens seems important for explaining when alien species profit from enemy release.

Assessing specific pathogen virulence is also an important yet largely neglected avenue in research on plant-soil interactions. So far there are only few studies that identify and assess the virulence of the active agents behind plant-soil-feedback effects. A remarkable example is the work on the interactions between *Prunus serotina* and the pathogenic oomycetes of the genus *Pythium* (Reinhart *et al.* 2003, 2005, 2010). Reinhart *et al.* (2010) for example showed that *Pythium* species from soil of the native North American range were more detrimental to *Prunus* *serotina* plants than those of the introduced range. While this corroborates the enemy release hypothesis in so far that effects of the soil-born enemies were weaker in the introduced range, it shows that *Prunus serotina* was not generally released from soil-borne enemies. Hence, such studies along with modern molecular tools may provide promising pathways for ecologist to further elucidate when belowground enemy release may be the underlying driver behind species invasions.

Futhermore effects of plant-soil feedback may not always be direct in the effects on host plant performance, but may also be mediated indirectly. If enemy release, or rather a reduced suppression by soil-borne enemies relative to natives occurs in the context of a (native) resident community, this may result in an advantage for alien species. I detected such indirect effects in my field study on species establishment success (Chapter III). Fungicide application reduced establishment success for alien species, by reducing pathogen pressure on the resident community and thereby likely leading to stronger competition with the residents. These effects were not very strong but consistent across seed and seedling stages and influence at least to a limited degree alien plant establishment. Indirect effects can also promote alien success via accumulation of soil pathogens by the alien species and subsequent spillover to native residents. For example, Mangla, Inderjit & Callaway (2008) report on a case where *Chromolaena ordorata* accumulatesd pathogenic *Fusarium* species, which decreased plant performance of co-occurring species (but see Del Fabbro & Prati 2015). Consequently, direct and indirect effects need to be considered for understanding the impact of plant-soil interactions on plant performance or invasive species success.

Taken together, my studies suggest that generalist pathogens and specific virulence rather than solely presence or absence of species-specific pathogens are important aspects for the outcomes of plant-soil feedback. Therefore, it is necessary to identify the active agents and to assess their impact on plant performance to disentangle the complex interactions between potentially many belowground players (pathogenic but also mutualistic). Furthermore, the community of co-occurring species may affect or be themselves affected by plant-soil interactions making it necessary to consider indirect effects.

Competition and plant-soil feedback - their relative roles and other ecological factors

To be able to assess he relative role that plant-soil feedback plays for plant performance, it is necessary to also test its impact in context with other ecological factors. Much knowledge of the effects of plant-soil interactions comes from greenhouse studies that test plant-soil feedback in isolation from other factors. Specifically, it has been suggested that plant-soil feedback and competition should not be seen as separate processes (Casper & Castelli 2007). For example Petermann *et al.* 2008 highlight that negative plant-soil feedback effects were much stronger when plants were grown in competition. To elucidate the relative role that plant-soil feedback plays we tested for both the effects of intraspecific competition and plant-soil feedback (Chapter I and Chapter II).

In the greenhouse study we found that increasing intraspecific competition decreased plant performance regardless of species origin (Chapter I). Interestingly, in the mesocosm study alien species showed significantly higher per capita performance when grow under low sowing frequencies compared to high sowing frequencies, while native species did not show significant difference between sowing frequencies

(Chapter II). The negative effects of intraspecific competition and the effects of plant-soil interactions for both studies were roughly similar in magnitude. This suggests that if plant-soil feedback occurs, it has the potential to play a substantial role for species performance. Moreover, similar to plant-soil feedback, intraspecific competition did not explain species commonness, suggesting that differences in magnitude of self-limitation by intraspecific competition cannot explain species success. Furthermore, I did not find that plant-soil feedback and competition did interact. This suggests that both effects act independently from one another, in contrast for example to the findings of Petermannn *et al.* (2008) and Kardol *et al.* (2007). However, Maron *et al.* (2016) also report, that, opposite to their hypothesis, competition did not interact with plant-soil feedbacks in a study on 10 common and rare savannah plant species. Thus, these conflicting results show that, competitive context has the potential to alter plant-soil-feedback effects, but these effects seem to be species-specific and I could not find in my studies that competition and plant-soil feedback generally interact. However, it has to be stated that I focused on intraspecific competition and my setup manipulated intraspecific plant frequencies, which did not allow disentangling specifically the contribution of interspecific effects of matrix and community species.

Apart from testing plant-soil interactions together with intraspecific competition I also tested the relative role of plant-soil feedback to aboveground enemies and disturbance under realistic field conditions (Chapter III). I did find weak effects of enemy release for alien species, however, they played only a minor role relative to the effect of disturbance, highlighting that biotic resistance is a major factor for establishment success of incoming species in the studied grassland. Moreover, I found that aboveground herbivores did not play a role in explaining differences between species establishment success for the set of 20 common and rare alien and native species. Yet, the effect of herbivores was also dependent on plant family. These findings show that it is important to test for the relative importance of multiple factors in a realistic setup to be able to get a clearer picture of when and under what circumstances specific drivers matter for species success. In my third study I showed that reduction of the competitive environment is much more important than plant-soil interactions, which expands our knowledge about the role of plant-soil interactions relative to other factors beyond findings of simplified greenhouse studies (see also Schittko *et al.* 2016; Heinze *et al.* 2016). Hence, more studies testing under realistic conditions what role that plant-soil feedback plays relative to other factors are needed (van der Putten *et al.* 2013; Dawson & Schrama 2016; van der Putten *et al.* 2016).

Future directions for research

Our understanding of the role of plant soil interactions has immensely grown over the past decades, yet in many aspects it is still in its infancy and there are numerous questions that still need to be answered and many promising pathways for future research. A major step forward would be intensifying efforts to identify the active agents (pathogens, mutualists, decomposers) and figuring out their relative contributions as that may help resolving inconclusive findings in the literature. Particularly modern molecular tools may help to shed more light into the ‘black box’ of soil (Birnbaum *et al.* 2014; Crocker, Karp & Nelson 2015; Dawson & Schrama 2016; Schlaeppi *et al.* 2016). For example, building on studies like Reinhart *et al.* (2010) or Crocker, Karp & Nelson (2015), identifying the responsible pathogens and assessing their virulence can help to shed more light on the specific effect that antagonistic microbes can play. However, the roles of mycorrhizal fungi or bacterial symbionts also needs to be integrated to disentangle the factors that lead to overall net effects of plant-soil interactions (van der Heijden *et al.* 1998; Callaway *et al.* 2011; Liang *et al.* 2015). Moreover, culturing microbial isolates and manipulating the different microbial components of soil, testing them singularly and in combination with others to understand their contribution to net effects of plant-soil interactions will be a challenging but promising approach for further progress (Dawson & Schrama 2016; van der Putten *et al.* 2016).

Furthermore, research on plant-soil feedback needs to adopt more of a dynamic perspective integrating also time and space, as heterogeneity in soil and respective microbial soil communities may be of importance as not 'everything is everywhere' (Brandt *et al.* 2013; Burns & Brand 2014; Mack & Bever 2014; Burns *et al.* 2015, Wubs & Bezemer 2016). This means that the paradigm, that microbes are not influenced by biogeographic patterns and evolutionary processes such as geographical isolation, limited dispersal and natural selection may not hold true and that microbes may also show regional evolutionary trajectories (Rout & Callaway 2012). This aspect is important across multiple spatial scales from differences in local soils to differences across biogeographical scales (i.e. continents) and has potential to influence patterns of plant-soil feedback across these scales. Regarding the temporal aspect, studies have shown that plant-soil feedbacks can change substantially over time (Kardol *et al.* 2006; Diez et al. 2010 but see Speek *et al.* 2015). These temporal changes may be driven by evolutionary changes in both plants as well as microbes (van der Putten *et al.* 2013; Bailey & Schweitzer 2016; Evans *et al*. 2016; van Nuland *et al.* 2016). Thus, incorporating spatiotemporal and evolutionary processes is necessary to foster our understanding of plant-soil interactions. Furthermore, regarding the strong context dependency of plant-soil-feedback effects, a framework combining several different approaches as suggested for invasion ecology by Küffer, Pysek & Richardson (2013), may prove helpful for further progress. Küffer, Pysek & Richardson (2013) propose that using in-depth research on 'model systems' much like 'model organisms' combined with comparisons across such model systems, focused meta-analysis and an aim to identify reoccurring patterns in species-ecosystem interactions may help to better understand, evaluate and predict context-dependent ecological processes – not only in respect to species invasions.

Concluding remarks

This thesis provides one of the few examples of phylogenetically-informed experiments testing the role and generality of plant-soil interactions for common and rare species of both origins relative to other factors in explaining species success. The results do suggest that plant-soil feedback and differences in intraspecific competition generally do not explain species success. And I find only little evidence that alien species are profiting from soil-borne enemy release. Effects of plant-soil feedback often seem rather species specific and context dependent and may not be as prevalent under field conditions compared to under controlled greenhouse conditions. Future studies need to explicitly focus on identifying and evaluating the impact of the driving agents (i.e. microbes), include spatiotemporal and evolutionary aspects of both plants and microbes, and test the roles of plant-soil interactions in concert with other ecological factors under realistic conditions to further advance the understanding of the 'dark side' of plant ecology.

References

Adler, F.R. (2011) The effects of intraspecific density dependence on species richness and species abundance distributions. *Theoretical Ecology,* **4,** 153-162.

Adler, F.R. & Muller-Landau, H.C. (2005) When do localized natural enemies increase species richness? *Ecology Letters,* **8,** 438-447.

Adler, P.B., HilleRisLambers, J. & Levine, J.M. (2007) A niche for neutrality. *Ecology Letters,* **10,** 95-104.

Agrawal, A.A. & Kotanen, P.M. (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters*, **6**, 712-715.

Agrawal, A.A., Kotanen, P.M., Mitchell, C.E., Power, A.G., Godsoe, W. & Klironomos, J. (2005) Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology,* **86,** 2979-2989.

Anacker, B.L., Klironomos, J.N., Maherali, H., Reinhart, K.O. & Strauss, S.Y. (2014) Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. *Ecology Letters,* **17,** 1613-1621.

Andonian, K., Hierro, J.L., Khetsuriani, L., Becerra, P., Janoyan, G., Villarreal, D., Cavieres, L., Fox, L.R. & Callaway, R.M. (2011) Range-Expanding Populations of a Globally Introduced Weed Experience Negative Plant-Soil Feedbacks. *Plos One,* **6,** 8.

Aponte, C., Garcia, L.V. & Maranon, T. (2013) Tree species effects on nutrient cycling and soil biota: A feedback mechanism favouring species coexistence. *Forest Ecology and Management,* **309,** 36-46.

Bachelot, B., Uriarte, M., Thompson, J. & Zimmerman, J.K. (2016) The advantage of the extremes: tree seedlings at intermediate abundance in a tropical forest have the highest richness of above-ground enemies and suffer the most damage. *Journal of Ecology,* **104,** 90-103.

Bagchi, R., Gallery, R.E., Gripenberg, S., Gurr, S.J., Narayan, L., Addis, C.E., Freckleton, R.P. & Lewis, O.T. (2014) Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature,* **506,** 85-88.

Bailey, J.K. & Schweitzer, J.A. (2016) The rise of plant-soil feedback in ecology and evolution. *Functional Ecology,* **30,** 1030-1031.

Bates, D., Maechler, M., Bolker, B. & Walker, S.(2014) lme4: linear mixed-effects model using Eigen and S4. R package ver. 1.1-7. – < http://CRAN.R-project.org/package = lme4 >.

Beckstead, J. & Parker, I.M. (2003) Invasiveness of Ammophila arenaria: Release from soil-borne pathogens? *Ecology,* **84,** 2824-2831.

Bever, J.D. (1994) Feedback between palnts and their soil communities in an old field community. *Ecology,* **75,** 1965-1977.

Bever, J.D. (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist,* **157,** 465-473.

Bever, J.D., Westover, K.M. & Antonovics, J. (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology,* **85,** 561-573.

Bever, J.D., Mangan, S.A. & Alexander, H.M. (2015) Maintenance of Plant Species Diversity by Pathogens. *Annual Review of Ecology, Evolution, and Systematics, Vol 46* (ed. D.J. Futuyma), pp. 305-325. Annual Reviews, Palo Alto.

Bezemer, T.M., Lawson, C.S., Hedlund, K., Edwards, A.R., Brook, A.J., Igual, J.M., Mortimer, S.R. & Van der Putten, W.H. (2006b) Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology,* **94,** 893-904.

Birnbaum, C., Bissett, A., Thrall, P.H. & Leishman, M.R. (2014) Invasive legumes encounter similar soil fungal communities in their non-native and native ranges in Australia. *Soil Biology & Biochemistry,* **76,** 210-217.

Blaney, C.S. & Kotanen, P.M. (2001) Post-dispersal losses to seed predators: an experimental comparison of native and exotic old field plants. *Canadian Journal of Botany-Revue Canadienne De Botanique*, **79**, 284-292.

Blank, R.R. (2010) Intraspecific and interspecific pair-wise seedling competition between exotic annual grasses and native perennials: plant-soil relationships. *Plant and Soil,* **326,** 331-343.

Blumenthal, D.M. (2006) Interactions between resource availability and enemy release in plant invasion. *Ecology Letters*, **9**, 887-895.

Bonanomi, G., Giannino, F. & Mazzoleni, S. (2005) Negative plant-soil feedback and species coexistence. *Oikos,* **111,** 311-321.

Bradley, B.A., Blumenthal, D.M., Wilcove, D.S. & Ziska, L.H. (2010) Predicting plant invasions in an era of global change. *Trends in Ecology & Evolution,* **25,** 310-318.

Brandt, A.J., de Kroon, H., Reynolds, H.L. & Burns, J.H. (2013) Soil heterogeneity generated by plant-soil feedbacks has implications for species recruitment and coexistence. *Journal of Ecology,* **101,** 277-286.

Brinkman, E.P., Van der Putten, W.H., Bakker, E.J. & Verhoeven, K.J.F. (2010) Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology,* **98,** 1063-1073.

Buckley Y.M., & Catford J. (2016) Does the biogeographic origin of species matter? Ecological effects of native and non-native species and the use of origin to guide management. *Journal of Ecology*, **104**, 4-17.

Bundesamt für Naturschutz, FloraWeb. - Available: http://www.floraweb.de

Burns, J.H. & Brandt, A.J. (2014) Heterogeneity in plant-soil feedbacks and resident population dynamics affect mutual invasibility. *Journal of Ecology,* **102,** 1048-1057.

Burns, J.H., Anacker, B.L., Strauss, S.Y. & Burke, D.J. (2015) Soil microbial community variation correlates most strongly with plant species identity, followed by soil chemistry, spatial location and plant genus. *AoB PLANTS,* **7,** 10.

Callaway, R.M., Thelen, G.C., Barth, S., Ramsey, P.W. & Gannon, J.E. (2004) Soil fungi alter interactions between the invader Centaurea maculosa and North American natives. *Ecology,* **85,** 1062-1071.

Callaway, R.M., Bedmar, E.J., Reinhart, K.O., Silvan, C.G. & Klironomos, J. (2011) Effects of soil biota from different ranges on Robinia invasion: acquiring mutualists and escaping pathogens. *Ecology,* **92,** 1027-1035.

Casper, B.B. & Jackson, R.B. (1997) Plant competition underground. *Annual Review of Ecology and Systematics,* **28,** 545-570.

Casper, B.B. & Castelli, J.P. (2007) Evaluating plant-soil feedback together with competition in a serpentine grassland. *Ecology Letters,* **10,** 394-400.

Catford, J. A., Baumgartner, J. B., Vesk, P. A., White, M., Buckley, Y. M. & McCarthy, M. A. (2016), Disentangling the four demographic dimensions of species invasiveness. *Journal of Ecology* doi:10.1111/1365-2745.12627

Chesson, P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics,* **31,** 343-366.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, **7**, 721-733.

Connell, J.H. (1971) On the role of natural enemies in preventing competetive exclusion in some marine animals and rain forest trees. *Dynamics of populations* (ed. P.J. den Boer, Gradwell, G. R.), pp. 298-310. Centre for Agricultural Publishing and Documentation, Wageningen.

Chisholm, R.A. & Muller-Landau, H.C. (2011) A theoretical model linking interspecific variation in density dependence to species abundances. *Theoretical Ecology,* **4,** 241-253.

Chiuffo, M.C., Macdougall, A.S. & Hierro, J.L. (2015) Native and non-native ruderals experience similar plant-soil feedbacks and neighbor effects in a system where they coexist. *Oecologia,* **179,** 843-852.

Chu, C.J. & Adler, P.B. (2015) Large niche differences emerge at the recruitment stage to stabilize grassland coexistence. *Ecological Monographs,* **85,** 373-392.

Collins, A., Hart, E.M. & Molofsky, J. (2010) Differential response to frequency-dependent interactions: an experimental test using genotypes of an invasive grass. *Oecologia,* **164,** 959-969.

Comita, L.S., Muller-Landau, H.C., Aguilar, S. & Hubbell, S.P. (2010) Asymmetric Density Dependence Shapes Species Abundances in a Tropical Tree Community. *Science,* **329,** 330-332.

Crocker, E.V., Karp, M.A. & Nelson, E.B. (2015) Virulence of oomycete pathogens from Phragmites australis-invaded and noninvaded soils to seedlings of wetland plant species. *Ecology and Evolution,* **5,** 2127-2139.

Dawson, W., Fischer, M. & van Kleunen, M. (2011) The maximum relative growth rate of common UK plant species is positively associated with their global invasiveness. *Global Ecology and Biogeography*, **20**, 299-306.

Dawson, W., Fischer, M. & van Kleunen, M. (2012) Common and rare plant species respond differently to fertilisation and competition, whether they are alien or native. *Ecology Letters,* **15,** 873-880.

Dawson, W., Bottini, A., Fischer, M., van Kleunen, M. & Knop, E. (2014) Little evidence for release from herbivores as a driver of plant invasiveness from a multi-species herbivore-removal experiment. *Oikos*, **123**, 1509-1518.

Dawson, W. & Schrama, M. (2016) Identifying the role of soil microbes in plant invasions. *Journal of Ecology*, **104**: 1211–1218. doi:10.1111/1365-2745.12619

de la Pena, E., de Clercq, N., Bonte, D., Roiloa, S., Rodriguez-Echeverria, S. & Freitas, H. (2010) Plant-soil feedback as a mechanism of invasion by Carpobrotus edulis. *Biological Invasions,* **12,** 3637-3648.

Del Fabbro, C. & Prati, D. (2015) Invasive plant species do not create more negative soil conditions for other plants than natives. *Perspectives in Plant Ecology Evolution and Systematics,* **17,** 87-95.

Diez, J.M., Dickie, I., Edwards, G., Hulme, P.E., Sullivan, J.J. & Duncan, R.P. (2010) Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters,* **13,** 803-809.

Dostal, P., Allan, E., Dawson, W., van Kleunen, M., Bartish, I. & Fischer, M. (2013) Enemy damage of exotic plant species is similar to that of natives and increases with productivity. *Journal of Ecology*, **101**, 388-399.

Duralia, T.E. & Reader, R.J. (1993) Does Abundance Reflect Competitive Ability - A Field-Test With 3 Prairie Grasses. *Oikos,* **68,** 82-90.

Elton, C.S. (1958) The ecology of invasions by animals and plants. The University of Chicago Press, Chicago.

Engelkes, T., Morrien, E., Verhoeven, K.J.F., Bezemer, T.M., Biere, A., Harvey, J.A., McIntyre, L.M., Tamis, W.L.M. & van der Putten, W.H. (2008) Successful range-expanding plants experience less above-ground and below-ground enemy impact. *Nature*, **456**, 946-948.

Engelkes, T., Meissner, A., Morrien, E., Kostenko, O., Van der Putten, W. H. & Macel, M. (2016) Herbivory and dominance shifts among exotic and congeneric native plant species during plant community establishment. *Oecologia*, **180**, 507-517.

Eppinga, M.B., Rietkerk, M., Dekker, S.C., De Ruiter, P.C. & Van der Putten, W.H. (2006) Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. *Oikos,* **114,** 168-176.

Evans, J.A., Lankau, R.A., Davis, A.S., Raghu, S. & Landis, D.A. (2016) Soil-mediated eco-evolutionary feedbacks in the invasive plant Alliaria petiolata. *Functional Ecology,* **30,** 1053-1061.

Felsenstein, J. (1985) Phylogenies and the comparative method. American Naturalist, 125, 1-15.

Flory, S.L. & Clay, K. (2013) Pathogen accumulation and long-term dynamics of plant invasions. *Journal of Ecology,* **101,** 607-613.

Fournier D.A., Skaug H.J., Ancheta J., Ianelli J., Magnusson A., Maunder M., Nielsen A. & Sibert J (2012) ADModel Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software*, **27**, 233-249.

Fricke, E.C., Tewksbury, J.J. & Rogers, H.S. (2014) Multiple natural enemies cause distance-dependent mortality at the seed-to-seedling transition. *Ecology Letters,* **17,** 593-598.

Gaston, K., J. (2003) The structure and dynamics of geographic ranges. Oxford University Press, Oxford.

Gaston, K.J. (2011) Common Ecology. *Bioscience,* **61,** 354-362.

Goldberg, D.E. & Barton, A.M. (1992) Patterns And Consequences Of Interspecific Competition In Natural Communities - A Review Of Field Experiments With Plants. *American Naturalist,* **139,** 771-801.

Gripenberg, S., Bagchi, R., Gallery, R.E., Freckleton, R.P., Narayan, L. & Lewis, O.T. (2014) Testing for enemy-mediated density-dependence in the mortality of seedlings: field experiments with five Neotropical tree species. *Oikos,* **123,** 185-193.

Grotkopp, E. & Rejmanek, M. (2007) High seedling relative growth rate and specific leaf area are traits of invasive species: Phylogenetically independent contrasts of woody angiospernis. *American Journal of Botany*, **94**, 526-532.

Gurevitch, J., Morrow, L.L., Wallace, A. & Walsh, J.S. (1992) A Metaanalysis Of Competition In Field Experiments. *American Naturalist,* **140,** 539-572.

Halbritter, A.H., Carroll, G.C., Gusewell, S. & Roy, B.A. (2012) Testing assumptions of the enemy release hypothesis: generalist versus specialist enemies of the grass Brachypodium sylvaticum. *Mycologia,* **104,** 34-44.

Hantsch, L., Bien, S., Radatz, S., Braun, U., Auge, H. & Bruelheide, H. (2014) Tree diversity and the role of non-host neighbour tree species in reducing fungal pathogen infestation. *Journal of Ecology,* **102,** 1673-1687.

Harley J.L., Harley E.L. (1987) A check-list of mycorrhiza in the British Flora. *New Phytologist,* **105**, 1–102.

Harpole, W.S. & Suding, K.N. (2007) Frequency-dependence stabilizes competitive interactions among four annual plants. *Ecology Letters,* **10,** 1164-1169.

Heinze, J., Sitte, M., Schindhelm, A., Wright, J. & Joshi, J. (2016) Plant-soil feedbacks: a comparative study on the relative importance of soil feedbacks in the greenhouse versus the field. *Oecologia,* **181,** 559-569.

Hendrix, F.F. & Campbell, W.A. (1973) Pythiums as plant pathogens. *Annual Review of Phytopathology,* **11,** 77-98.

Hierro, J.L., Villarreal, D., Eren, O., Graham, J.M. & Callaway, R.M. (2006) Disturbance facilitates invasion: The effects are stronger abroad than at home. *The American Naturalist*, **168**, 144-156.

HilleRisLambers, J., Adler, P.B., Harpole, W.S., Levine, J.M. & Mayfield, M.M. (2012) Rethinking Community Assembly through the Lens of Coexistence Theory. *Annual Review of Ecology, Evolution, and Systematics, Vol 43,* **43,** 227-248.

Hirschfeldt, T., Ellner, F., Buschhaus, H., Großmann, M., Büttner, C. (2009) Einfluss von Thiophanat-Methyl und Methyl-Benzimidazol-2yl-Carbamat auf mykotoxinbildende Fusarium spp. *ALVA-Mitteilungen,* **7,** 31-35.

Hodge, A. & Fitter, A.H. (2013) Microbial mediation of plant competition and community structure. *Functional Ecology,* **27,** 865-875.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal* **50**, 346—363.

Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist,* **104,** 501-528.

Jeschke, J.M. & Strayer, D.L. (2008) Are threat status and invasion success two sides of the same coin? *Ecography,* **31,** 124-130.

Johnson, N.C., Rowland, D.L., Corkidi, L., Egerton-Warburton, L.M. & Allen, E.B. (2003) Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology,* **84,** 1895-1908.

Kardol, P., Bezemer, T.M. & van der Putten, W.H. (2006) Temporal variation in plant-soil feedback controls succession. *Ecology Letters,* **9,** 1080-1088.

Kardol, P., Cornips, N.J., van Kempen, M.M.L., Bakx-Schotman, J.M.T. & van der Putten, W.H. (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs,* **77,** 147-162.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution,* **17,** 164-170.

Kempel, A., Chrobock, T., Fischer, M., Rohr, R.P. & van Kleunen, M. (2013) Determinants of plant establishment success in a multispecies introduction experiment with native and alien species. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 12727-12732.

Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature,* **417,** 67-70.

Klotz, S., Kühn, I. & Durka, W. (2002): BIOLFLOR - Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland. - Schriftenreihe für Vegetationskunde 38. Bonn: Bundesamt für Naturschutz.

Korell, L., Stein, C., Hensen, I. Bruelheide, H., Suding, K. N. & Auge, H. (2016) Stronger effect of gastropods than rodents on seedling establishment, irrespective of exotic or native plant species origin. *Oikos,* doi:10.1111/oik.02696

Krausch, H.-D. (2003) *Kaiserkron und Päonien rot... Entdeckung und Einführung unserer Gartenblumen*. Dölling und Galitz Verlag, Munich, Germany.

Kulmatiski, A., Beard, K.H., Stevens, J.R. & Cobbold, S.M. (2008) Plant-soil feedbacks: a meta-analytical review. *Ecology Letters,* **11,** 980-992.

Kunin, W.E. & Gaston, K.J. (1993) The Biology Of Rarity - Patterns, Causes And Consequences. *Trends in Ecology & Evolution,* **8,** 298-301.

Küffer, C., Pysek, P. & Richardson, D.M. (2013) Integrative invasion science: model systems, multi-site studies, focused meta-analysis and invasion syndromes. *New Phytologist,* **200,** 615-633.

LaManna, J.A., Walton, M.L., Turner, B.L. & Myers, J.A. (2016) Negative density dependence is stronger in resource-rich environments and diversifies communities when stronger for common but not rare species. *Ecology Letters,* **19,** 657-667.

Larios, L. & Suding, K.N. (2015) Competition and soil resource environment alter plant - soil feedbacks for native and exotic grasses. *AoB PLANTS,* **7,** 9.

Leger, E.A. & Espeland, E.K. (2010) The shifting balance of facilitation and competition affects the outcome of intra- and interspecific interactions over the life history of California grassland annuals. *Plant Ecology,* **208,** 333-345.

Levine, J.M., Adler, P.B. & Yelenik, S.G. (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters,* **7,** 975-989.

Levine, J.M., Pachepsky, E., Kendall, B.E., Yelenik, S.G. & HilleRisLambers, J. (2006) Plant-soil feedbacks and invasive spread. *Ecology Letters,* **9,** 1005-1014.

Levine, J.M. & HilleRisLambers, J. (2009) The importance of niches for the maintenance of species diversity. *Nature,* **461,** 254-257.

Liang, M.X., Liu, X.B., Etienne, R.S., Huang, F.M., Wang, Y.F. & Yu, S.X. (2015) Arbuscular mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens. *Ecology,* **96,** 562-574.

Liao, C.Z., Peng, R.H., Luo, Y.Q., Zhou, X.H., Wu, X.W., Fang, C.M., Chen, J.K. & Li, B. (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, **177**, 706-714.

Liu, H. & Stiling, P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions,* **8,** 1535-1545.

Liu, Y., Fang, S.Q., Chesson, P. & He, F.L. (2015) The effect of soil-borne pathogens depends on the abundance of host tree species. *Nature Communications,* **6**.

Lortie, C.J. & Turkington, R. (2002) The effect of initial seed density on the structure of a desert annual plant community. *Journal of Ecology,* **90,** 435-445.

Lozon, J.D. & Macisaac, H.J. (1997) Biological invasions: Are they dependent on disturbance? *Environmental Reviews*, **5**, 131-144.

MacDonald, A.A.M. & Kotanen, P.M. (2010) The effects of disturbance and enemy exclusion on performance of an invasive species, common ragweed, in its native range. *Oecologia*, **162**, 977-986.

MacDougall, A.S., Gilbert, B. & Levine, J.M. (2009) Plant invasions and the niche. *Journal of Ecology,* **97,** 609-615.

MacDougall, A.S., Rillig, M.C. & Klironomos, J.N. (2011) Weak conspecific feedbacks and exotic dominance in a species-rich savannah. *Proceedings of the Royal Society B-Biological Sciences,* **278,** 2939-2945.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications,* **10,** 689-710.

Mack, K.M.L. & Bever, J.D. (2014) Coexistence and relative abundance in plant communities are determined by feedbacks when the scale of feedback and dispersal is local. *Journal of Ecology,* **102,** 1195-1201.

Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M.L., Valencia, M.C., Sanchez, E.I. & Bever, J.D. (2010) Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature,* **466,** 752-755.

Mangla, S., Inderjit & Callaway, R.M. (2008) Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *Journal of Ecology,* **96,** 58-67.

Mangla, S., Sheley, R.L., James, J.J. & Radosevich, S.R. (2011) Intra and interspecific competition among invasive and native species during early stages of plant growth. *Plant Ecology,* **212,** 531-542.

Maron, J.L. & Vila, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**, 361-373.

Maron, J.L., Horvitz, C.C. & Williams, J.L. (2010) Using experiments, demography and population models to estimate interaction strength based on transient and asymptotic dynamics. *Journal of Ecology,* **98,** 290-301.

Maron, J.L., Marler, M., Klironomos, J.N. & Cleveland, C.C. (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters,* **14,** 36-41.

Maron, J.L., Pearson, D.E., Potter, T. & Ortega, Y.K. (2012) Seed size and provenance mediate the joint effects of disturbance and seed predation on community assembly. *Journal of Ecology*, **100**, 1492-1500.

Maron, J.L., Waller, L.P., Hahn, M.A., Diaconu, A., Pal, R.W., Muller-Scharer, H., Klironomos, J.N. & Callaway, R.M. (2013) Effects of soil fungi, disturbance and propagule pressure on exotic plant recruitment and establishment at home and abroad. *Journal of Ecology*, **101**, 924-932.

Maron, J.L., Klironomos, J., Waller, L. & Callaway, R.M. (2014) Invasive plants escape from suppressive soil biota at regional scales. *Journal of Ecology,* **102,** 19-27.

Maron, J.L., Laney Smith, A., Ortega, Y.K., Pearson, D.E. & Callaway, R.M. (2016) Negative plant-soil feedbacks increase with plant abundance, and are unchanged by competition. *Ecology,* **97,** 2055-2063.

McCarthy-Neumann, S. & Ibanez, I. (2013) Plant-soil feedback links negative distance dependence and light gradient partitioning during seedling establishment. *Ecology,* **94,** 780-786.

Mehrabi, Z. & Tuck, S.L. (2015) Relatedness is a poor predictor of negative plant-soil feedbacks. *New Phytologist,* **205,** 1071-1075.

Mills, K.E. & Bever, J.D. (1998) Maintenance of diversity within plant communities: Soil pathogens as agents of negative feedback. *Ecology,* **79,** 1595-1601.

Mitchell, C.E. & Power, A.G. (2003) Release of invasive plants from fungal and viral pathogens. *Nature,* **421,** 625-627.

Mitchell, C.E., Agrawal, A.A., Bever, J.D., Gilbert, G.S., Hufbauer, R.A., Klironomos, J.N., Maron, J.L., Morris, W.F., Parker, I.M., Power, A.G., Seabloom, E.W., Torchin, M.E. & Vazquez, D.P. (2006) Biotic interactions and plant invasions. *Ecology Letters,* **9,** 726-740.

Moles, A.T., Flores-Moreno, H., Bonser, S.P., Warton, D.I., Helm, A., Warman, L., Eldridge, D.J., Jurado, E., Hemmings, F.A., Reich, P.B., Cavender-Bares, J., Seabloom, E.W., Mayfield, M.M., Sheil, D., Djietror, J.C., Peri, P.L., Enrico, L., Cabido, M.R., Setterfield, S.A., Lehmann, C.E.R. & Thomson, F.J. (2012) Invasions: the trail behind, the path ahead, and a test of a disturbing idea. *Journal of Ecology*, **100**, 116-127.

Mordecai, E.A. (2011) Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. *Ecological Monographs,* **81,** 429-441.

Morrien, E., Engelkes, T. & van der Putten, W.H. (2011) Additive effects of aboveground polyphagous herbivores and soil feedback in native and range-expanding exotic plants. *Ecology*, **92**, 1344-1352.

Munzbergova, Z. (2005) Determinants of species rarity: Population growth rates of species sharing the same habitat. *American Journal of Botany,* **92,** 1987-1994.

Myers, J.A. & Harms, K.E. (2009) Seed arrival, ecological filters, and plant species richness: a meta-analysis. *Ecology Letters*, **12**, 1250-1260.

Newton, A.C., Fitt, B.D.L., Atkins, S.D., Walters, D.R. & Daniell, T.J. (2010) Pathogenesis, parasitism and mutualism in the trophic space of microbe-plant interactions. *Trends in Microbiology,* **18,** 365-373.

Nijjer, S., Rogers, W.E. & Siemann, E. (2007) Negative plant-soil feedbacks may limit persistence of an invasive tree due to rapid accumulation of soil pathogens. *Proceedings of the Royal Society B-Biological Sciences,* **274,** 2621-2627.

Ortega, Y.K. & Pearson, D.E. (2005) Weak vs. strong invaders of natural plant communities: Assessing invasibility and impact. *Ecological Applications,* **15,** 651-661.

Packer, A. & Clay, K. (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature,* **404,** 278-281.

Parker, J.D., Burkepile, D.E. & Hay, M.E. (2006) Opposing effects of native and exotic herbivores on plant invasions. *Science*, **311**, 1459-1461.

Parker, I.M. & Gilbert, G.S. (2007) When there is no escape: The effects of natural enemies on native, invasive, and noninvasive plants. *Ecology,* **88,** 1210-1224.

Pearson, D.E., Potter, T. & Maron, J.L. (2012) Biotic resistance: exclusion of native rodent consumers releases populations of a weak invader. *Journal of Ecology*, **100**, 1383-1390.

Perkins, L.B., Hatfield, G. & Espeland, E.K. (2016) Invasive grasses consistently create similar plant-soil feedback types in soils collected from geographically distant locations. *Journal of Plant Ecology,* **9,** 180-186.

Petermann, J.S., Fergus, A.J.F., Turnbull, L.A. & Schmid, B. (2008) Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology,* **89,** 2399-2406.

Poorter, H., Buehler, J., van Dusschoten, D., Climent, J. & Postma, J.A. (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology,* **39,** 839-850.

Preston, F.W. (1948) The Commonness, And Rarity, Of Species. *Ecology,* **29,** 254-283.

Questad, E.J. & Foster, B.L. (2008) Coexistence through spatio-temporal heterogeneity and species sorting in grassland plant communities. *Ecology Letters*, **11**, 717-726.

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Radford, I.J., Dickinson, K.J.M. & Lord, J.M. (2010) Does disturbance, competition or resource limitation underlie *Hieracium lepidulum* invasion in New Zealand? Mechanisms of establishment and persistence, and functional differentiation among invasive and native species. *Austral Ecology*, **35**, 282-293.

Reinhart, K.O. (2012) The organization of plant communities: negative plant-soil feedbacks and semiarid grasslands. *Ecology,* **93,** 2377-2385.

Reinhart, K.O., Packer, A., Van der Putten, W.H. & Clay, K. (2003) Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters,* **6,** 1046-1050.

Reinhart, K.O. & Callaway, R.M. (2004) Soil biota facilitate exotic Acer invasions in Europe and North America. *Ecological Applications,* **14,** 1737-1745.

Reinhart, K.O., Royo, A.A., Van der Putten, W.H. & Clay, K. (2005) Soil feedback and pathogen activity in Prunus serotina throughout its native range. *Journal of Ecology,* **93,** 890-898.

Reinhart, K.O. & Callaway, R.M. (2006) Soil biota and invasive plants. *New Phytologist,* **170,** 445-457.

Reinhart, K.O., Tytgat, T., Van der Putten, W.H. & Clay, K. (2010) Virulence of soil-borne pathogens and invasion by Prunus serotina. *New Phytologist,* **186,** 484-495.

Reynolds, H.L., Packer, A., Bever, J.D. & Clay, K. (2003) Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology,* **84,** 2281-2291.

Rillig, M.C. (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters,* **7,** 740-754.

Rillig, M.C. & Mummey, D.L. (2006) Mycorrhizas and soil structure. *New Phytologist,* **171,** 41-53.

Rodriguez-Echeverria, S., Afonso, C., Correia, M., Lorenzo, P. & Roiloa, S.R. (2013) The effect of soil legacy on competition and invasion by Acacia dealbata Link. *Plant Ecology,* **214,** 1139-1146.

Rout, M.E. & Callaway, R.M. (2012) Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that `everything is not everywhere'. *Annals of Botany,* **110,** 213-222.

Royo, A.A., Bates, R. & Lacey, E.P. (2008) Demographic constraints in three populations of Lobelia boykinii: a rare wetland endemic. *Journal of the Torrey Botanical Society,* **135,** 189-199.

Seastedt, T.R. & Pysek, P. (2011) Mechanisms of Plant Invasions of North America and European Grasslands. *Annual Review of Ecology, Evolution, and Systematics, Vol 42* (eds D.J. Futuyma, H.B. Shaffer & D. Simberloff), pp. 133-153. Annual Reviews, Palo Alto.

Schittko, C., Runge, C., Strupp, M., Wolff, S. & Wurst, S. (2016) No evidence that plant–soil feedback effects of native and invasive plant species under glasshouse conditions are reflected in the field. *Journal of Ecology,* **104,** 1243-1249.

Schlaeppi, K., Bender, S.F., Mascher, F., Russo, G., Patrignani, A., Camenzind, T., Hempel, S., Rillig, M.C. & van der Heijden, M.G.A. (2016) High-resolution community profiling of arbuscular mycorrhizal fungi. *New Phytologist*, doi:10.1111/nph.14070

Schnitzer, S.A., Klironomos, J.N., HilleRisLambers, J., Kinkel, L.L., Reich, P.B., Xiao, K., Rillig, M.C., Sikes, B.A., Callaway, R.M., Mangan, S.A., van Nes, E.H. & Scheffer, M. (2011) Soil microbes drive the classic plant diversity-productivity pattern. *Ecology,* **92,** 296-303.

Speek, T.A.A., Schaminee, J.H.J., Stam, J.M., Lotz, L.A.P., Ozinga, W.A. & van der Putten, W.H. (2015) Local dominance of exotic plants declines with residence time: a role for plant-soil feedback? *AoB PLANTS,* **7**.

Suding, K.N., Harpole, W.S., Fukami, T., Kulmatiski, A., MacDougall, A.S., Stein, C. & van der Putten, W.H. (2013) Consequences of plant-soil feedbacks in invasion. *Journal of Ecology,* **101,** 298-308.

Thompson, K., Hodgson, J.G. & Rich, T.C.G. (1995) Native and alien invasive plants: More of the same? *Ecography,* **18,** 390-402.

van de Voorde, T.F.J., van der Putten, W.H. & Bezemer, T.M. (2012) Soil inoculation method determines the strength of plant-soil interactions. *Soil Biology & Biochemistry,* **55,** 1-6.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. & Sanders, I.R. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature,* **396,** 69-72.

van der Putten, W.H. & Peters, B.A.M. (1997) How soil-borne pathogens may affect plant competition. *Ecology,* **78,** 1785-1795.

van der Putten, W.H., Vet, L.E.M., Harvey, J.A. & Wackers, F.L. (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology & Evolution,* **16,** 547-554.

van der Putten, W.H., Klironomos, J.N. & Wardle, D.A. (2007) Microbial ecology of biological invasions. *Isme Journal,* **1,** 28-37.

van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A., Suding, K.N., Van de Voorde, T.F.J. & Wardle, D.A. (2013) Plant-soil feedbacks: the past, the present and future challenges. *Journal of Ecology,* **101,** 265-276.

van der Putten, W.H., Bradford, M.A., Brinkman, E.P., van de Voorde, T.F.J. & Veen, G.F. (2016) Where, when and how plant-soil feedback matters in a changing world. *Functional Ecology,* **30,** 1109-1121.

van Grunsven, R.H.A., van der Putten, W.H., Bezemer, T.M., Tamis, W.L.M., Berendse, F. & Veenendaal, E.M. (2007) Reduced plant-soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology,* **95,** 1050-1057.

van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J.M. & Fischer, M. (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. *Ecology Letters,* **13,** 947-958.

van Kleunen, M., Weber, E. & Fischer, M. (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, **13**, 235-245.

van Kleunen, M., Dawson, W., Bossdorf, O. & Fischer, M. (2014) The more the merrier: Multi-species experiments in ecology. *Basic and Applied Ecology,* **15,** 1-9.

van Nuland, M.E., Wooliver, R.C., Pfennigwerth, A.A., Read, Q.D., Ware, I.M., Mueller, L., Fordyce, J.A., Schweitzer, J.A. & Bailey, J.K. (2016) Plant-soil feedbacks: connecting ecosystem ecology and evolution. *Functional Ecology,* **30,** 1032-1042.

Verhoeven, K.J.F., Biere, A., Harvey, J.A. & van der Putten, W.H. (2009) Plant invaders and their novel natural enemies: who is naive? *Ecology Letters,* **12,** 107-117.

Vestergard, M., Ronn, R. & Ekelund, F. (2015) Above-belowground interactions govern the course and impact of biological invasions. *AoB PLANTS,* **7**.

Vila, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarosik, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y. & Pysek, P. (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, **14**, 702-708.

Wassmuth, B.E., Stoll, P., Tscharntke, T. & Thies, C. (2009) Spatial aggregation facilitates coexistence and diversity of wild plant species in field margins. *Perspectives in Plant Ecology Evolution and Systematics,* **11,** 127-135.

Williamson, M. & Fitter, A. (1996) The varying success of invaders. *Ecology*, **77**, 1661-1666.

Wubs, E.R.J. & Bezemer, T.M. (2016) Effects of spatial plant-soil feedback heterogeneity on plant performance in monocultures. *Journal of Ecology,* **104,** 364-376.

Xu, M., Wang, Y.F. & Yu, S.X. (2015) Conspecific negative density dependence decreases with increasing species abundance. *Ecosphere,* **6,** 11.

Authors contributions

Chapter I

Wayne Dawson designed research with contribution from Mark van Kleunen. Gregor Müller and Wayne Dawson collected the data. Gregor Müller analyzed the data. Gregor Müller wrote the manuscript. All authors revised and contributed to the final version of the manuscript

Chapter II

Wayne Dawson designed the research and Mark van Kleunen and Gregor Müller contributed. Gregor Müller collected the data. Gregor Müller analyzed the data. Gregor Müller wrote the manuscript. All authors revised and contributed to the final version of the manuscript

Chapter III

Wayne Dawson, Mark van Kleunen and Gregor Müller designed the research. Gregor Müller, Lena Horstmeyer and Tilman Rönneburg collected the data. Gregor Müller analyzed the data. Gregor Müller wrote the manuscript. Wayne Dawson, Mark van Kleunen and Gregor Müller revised and contributed to the final version of the manuscript.

Acknowledgements

Completing this thesis would not have been possible without the help of many others – Hiwis, Bachelor and Master students, colleagues, friends and family. I would like to thank all the people for their help and their patience supporting me through ups and downs while I was working on this thesis.

First and foremost I would like to thank Wayne Dawson for his guidance and support. He always had a sympathetic ear for my questions and I could knock on his door anytime to discuss my work, ecology in general or the opportunities and pitfalls of statistics.

Second I would like to thank Mark van Kleunen for his ideas, suggestions, solutions, comments and general guidance. Without that support I might still be in the process of trying to get a first paper published...

Moreover, I would like to thank the whole Ecology group for constructive discussions, atmosphere and multicultural cake and coffee experiences. Thanks also go to the team in the botanical garden for advise and help in horticultural questions.

Also, my gratitude goes to all the Hiwis that helped me with the experiments. While there are too many to name my special thanks go to Sina for helping me in all three projects at all weather conditions and circumstances with a smile.

Furthermore, many thanks go to Tilman and Lena without whom the “famous Hockgraben Experiment“ would not have been manageable in this size and who contributed substantially to its success. Thanks for sharing the time in the field and for the Krapfen we had in between net-cages, grass, forbs, slugs and spiders.

Furthermore, I want to thank the International Max Planck Research School for Organismal Biology for inspiring courses, the good times with my nice PhD colleagues and the shared activities. Moreover, thanks go to Mäggi Hieber-Ruiz for her assistance with administrative things

And last but not least a very special „ thank you“ goes out to my family for their support and love.