The performance of and interactions between multiple co-occurring alien and native plant species

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Summary

This thesis has a particular focus on investigating aspects of species interactions that are relevant in the context of alien species establishment, since in the light of climate change and globalisation, the ever-increasing number of alien species poses severe threats to species, communities and ecosystems. Given this increasing number of alien species, it is crucial to investigate effects among multiple co-occurring alien species, and how these effects can be explained, be it by niche or fitness differences. Competition outcome can further be altered by so-called priority effects, describing effects of an earlier emerging species on a later emerging species in a given environment. This is especially important in the context of alien species which tend to emerge and grow earlier than native species. Besides negative effects among species, also positive interactions such as facilitation play an important role regarding species interactions, even though it is often neglected. Legumes are a plant functional group widely known for facilitative effects on their neighbour species due to their ability to fix atmospheric nitrogen. Additional nitrogen provided by the legume might facilitate the alien species and lead to even more severe negative effects on the native, but might also reduce resource competition and decrease negative effects on native species.

I addressed these various aspects of alien species interactions with three common garden pot experiment. With the first experiment, I focused on identifying mechanisms driving alien species interactions and compared all pairwise combinations of 20 alien annual plant species in Germany. I first tested whether competition or facilitation occurred more often. Secondly, I determined whether individual traits, hierarchical or absolute trait distances, multivariate trait or phylogenetic distance explained alien plant interactions best. Lastly, I assessed whether accounting for trait plasticity explains plant performance better than species-level trait averages. While the magnitude of interspecific competition compared to intraspecific competition was on average larger across the 190 alien species combinations, interspecific facilitation still occurred in 24% of cases. Interactions could be better explained by hierarchical trait distances which reflect competitive ability, compared to phylogenetic and multivariate trait distance (reflecting niche differences). Accounting for trait plasticity did not necessarily explain plant performance better than models using species-level trait averages. Specifically, when growing taller and having a lower specific leaf area than the co-occurring alien neighbour, biomass and seed number of individuals in mixture increased compared to growing alone. These results advance our fundamental understanding of trait-interaction relationships, strengthening recent findings on the importance of competitive hierarchy in shaping interactions and community structure.

In the second experiment I focused on the interactions among five alien-native species pairs. Firstly, I tested whether growing with one or with two alien neighbour species affected native plants stronger. Secondly, I altered the arrival time of the alien or native neighbour by three weeks. Generally, native species performance decreased when surrounded by two alien species compared to only one, although the magnitude of this effect varied depending on species. Species performance greatly decreased when arriving second in the pot, for both native and alien species. In contrast, alien species tended to benefit more from arriving early. Since these effects tended to be species-specific, it requires further studies to achieve generalisations concerning the response of multiple alien and native plants in response to neighbour species and arrival time.

With a third experiment I investigated legume facilitation in more detail. I grew 30 annual Asteraceae species (neophytes and archaeophytes, plus some natives) in communities with or without legume presence. I measured functional traits and fitness in focal Asteraceae, as well as nitrogen characteristics of Asteraceae and two native community phytometer species. I investigated how legume presence affects Asteraceae fitness and how traits and nitrogen concentration relate to Asteraceae fitness. To elucidate whether mechanisms of facilitation in legume presence differ among native phytometer, neophyte and archaeophyte Asteraceae I used the $\delta^{15}N$ natural abundance method. Specific leaf area negatively affected aboveground biomass and seed production, with a stronger effect in legume absence. Nitrogen concentration (approximating nutrient uptake) was positively correlated with biomass, but notably did not increase seed production. My results hint at N facilitation for the native grass phytometer *Festuca rupicola* when growing in legume presence, whereas the forb *Potentilla argentea* and the 27 alien Asteraceae species did not indicate facilitative effects. Direct legume facilitation in native phytometer species was only detected when growing with archaeophytes neighbours, not with neophytes. This hints at varied mechanisms of competition for nitrogen between natives and alien species of different residence time and deepens the understanding of altered facilitative leguminous effects in alien species presence.

I showed how using concepts and theories of community ecology can be applied to examples of alien species interaction especially with the novel aspect of multiple co-occurring alien species. Since the continuously increasing rate of alien species arrival in new habitats makes co-occurrence of alien species more likely, it becomes even more important to examine their combined impact on native species, communities and ecosystems.

Zusammenfassung

Der Fokus dieser Arbeit liegt auf der Untersuchung von Aspekten der Interaktion zwischen Arten, die im Zusammenhang mit der Ansiedlung gebietsfremder Arten von Bedeutung sind, denn angesichts des Klimawandels und der Globalisierung stellt die ständig wachsende Zahl gebietsfremder Arten eine ernsthafte Bedrohung für heimische Arten, Artengemeinschaften und Ökosysteme dar. In Anbetracht der zunehmenden Zahl gebietsfremder Arten, ist es von entscheidender Bedeutung die Auswirkungen des gleichzeitigen Auftretens mehrerer gebietsfremder Arten zu untersuchen und herauszufinden, wie diese Auswirkungen erklärt werden können, sei es durch Nischen- oder Fitnessunterschiede. Das Ergebnis des Wettbewerbs kann auch durch so genannte ‚priority' Effekte beeinflusst werden, die die Auswirkungen einer früher auftretenden Art auf eine später auftretende Art in einer bestimmten Umgebung beschreiben. Dies ist besonders wichtig im Zusammenhang mit gebietsfremden Arten, die dazu neigen, früher zu keimen und zu wachsen als einheimische Arten. Neben negativen Effekten zwischen den Arten spielen auch positive Wechselwirkungen eine wichtige Rolle bei der Interaktion zwischen den Arten, auch wenn dies oft vernachlässigt wird. Leguminosen sind eine Pflanzengruppe, die aufgrund ihrer Fähigkeit, Stickstoff aus der Luft zu fixieren, weithin dafür bekannt ist, dass sie sich positiv auf ihre Nachbararten auswirkt. Zusätzlicher Stickstoff, der von der Leguminose zur Verfügung gestellt wird, könnte die Ansiedlung der fremden Art erleichtern und zu noch schwerwiegenderen negativen Auswirkungen auf die einheimische Art führen, aber andererseits auch die Konkurrenz um Ressourcen verringern und die negativen Auswirkungen auf die einheimische Art reduzieren.

Ich untersuchte diese verschiedenen Aspekte der Interaktionen mit gebietsfremden Arten mit drei Topf-Experimenten. Beim ersten Experiment konzentrierte ich mich auf die Untersuchung der Mechanismen, die die Interaktionen zwischen gebietsfremden Arten bestimmen, und verglich alle paarweisen Kombinationen von 20 gebietsfremden einjährigen Pflanzenarten in Deutschland. Zunächst testete ich, ob eher Konkurrenz oder Unterstützung auftrat. Zweitens ermittelte ich, ob individuelle Merkmale, hierarchische oder absolute Merkmalsdistanzen, multivariate Merkmalsdistanzen oder phylogenetische Distanzen die Interaktionen zwischen gebietsfremden Pflanzen am besten erklären. Schließlich untersuchte ich, ob die Berücksichtigung der Merkmalsplastizität die Leistung der Pflanzen besser erklärt als die Durchschnittswerte der Merkmale auf Artniveau. Während das Ausmaß des interspezifischen Wettbewerbs im Vergleich zum intraspezifischen Wettbewerb im Durchschnitt der 190 Kombinationen gebietsfremder Arten größer war, kam es in 24 % der Fälle zu einer interspezifischen Unterstützung. Interaktionen konnten besser durch hierarchische Merkmalsdistanzen erklärt werden, die die Konkurrenzfähigkeit widerspiegeln, als durch phylogenetische und multivariate Merkmalsdistanzen (die Nischenunterschiede

widerspiegeln). Die Berücksichtigung der Merkmalsplastizität erklärte die Leistung der Pflanzen nicht unbedingt besser als Modelle, die Durchschnittswerte von Merkmalen auf Artniveau verwendeten. Insbesondere wenn die Pflanzen höher wuchsen und eine geringere spezifische Blattfläche aufwiesen als die gemeinsam vorkommenden fremden Nachbarn, nahmen Biomasse und Anzahl der Samen der Individuen in der Mischung im Vergleich zum Einzelanbau zu. Diese Ergebnisse erweitern unser grundlegendes Verständnis der Beziehungen zwischen Merkmalen und Interaktionen und stärken die jüngsten Erkenntnisse über die Bedeutung der Konkurrenzhierarchie bei der Gestaltung von Interaktionen und der Struktur von Gemeinschaften.

Beim zweiten Experiment konzentrierte ich mich auf die Wechselwirkungen zwischen fünf Paaren aus gebietsfremden und einheimischen Arten. Erstens testete ich, ob der Anbau mit einer oder mit zwei gebietsfremden Nachbararten die einheimischen Pflanzen stärker beeinflusst. Zweitens veränderte ich die Ankunftszeit der fremden oder einheimischen Nachbarn um drei Wochen. Im Allgemeinen verringerte sich die Leistung der einheimischen Arten, wenn sie von zwei gebietsfremden Arten umgeben waren, im Vergleich zu nur einer, obwohl das Ausmaß dieses Effekts je nach Art variierte. Sowohl bei den einheimischen als auch bei den gebietsfremden Arten nahm die Leistung stark ab, wenn sie als zweite Art in den Topf kamen. Im Gegensatz dazu profitierten gebietsfremde Arten eher von einer frühen Ankunft im Topf. Da diese Effekte tendenziell artspezifisch waren, sind weitere Studien erforderlich, um Verallgemeinerungen über die Reaktion mehrerer gebietsfremder und einheimischer Pflanzen auf die Nachbararten und die Ankunftszeit zu erhalten.

In einem dritten Experiment untersuchte ich die Unterstützung durch Leguminosen eingehender. Ich pflanzte 30 einjährige Asteraceae-Arten (Neophyten und Archäophyten sowie einige einheimische Arten) in Gemeinschaften mit oder ohne die Anwesenheit von Leguminosen. Ich habe die funktionellen Eigenschaften und die Fitness von Asteraceae sowie die Stickstoffeigenschaften von Asteraceae und zwei einheimischen Phytometer-Arten gemessen. Ich untersuchte, wie sich die Anwesenheit von Leguminosen auf die Fitness von Asteraceae auswirkt und wie Merkmale und Stickstoffkonzentration mit der Fitness von Asteraceae zusammenhängen. Um herauszufinden, ob sich die Mechanismen der Erleichterung der Anwesenheit von Leguminosen bei einheimischen Phytometer-, Neophyten- und Archäophyten-Asteraceae unterscheiden, verwendete ich die δ15N-Methode der natürlichen Abundanz. Die spezifische Blattfläche wirkte sich negativ auf die oberirdische Biomasse und die Samenproduktion aus, wobei der Effekt bei Abwesenheit von Leguminosen stärker war. Die Stickstoffkonzentration (als Näherung für die Nährstoffaufnahme) war positiv mit der Biomasse korreliert, steigerte aber nicht die Samenproduktion. Meine Ergebnisse deuten darauf hin, dass das einheimische Gras *Festuca rupicola* in Anwesenheit von Leguminosen die Stickstoffaufnahme fördert, wohingegen die krautige Pflanze *Potentilla argentea* und die 27 gebietsfremden Asteraceae-Arten keine förderlichen Auswirkungen zeigten. Eine direkte Unterstützung durch Leguminosen wurde bei einheimischen Phytometer-Arten nur festgestellt, wenn sie mit benachbarten Archäophyten wuchsen, nicht aber mit Neophyten. Dies deutet auf unterschiedliche Mechanismen der Konkurrenz um Stickstoff zwischen einheimischen und gebietsfremden Arten mit unterschiedlicher Verweildauer hin und vertieft das Verständnis der veränderten fördernden Wirkung von Leguminosen durch die Anwesenheit gebietsfremder Arten.

Ich habe gezeigt, wie Konzepte und Theorien der Ökologie auf Beispiele für die Interaktion mit gebietsfremden Arten angewandt werden können, insbesondere unter dem neuen Aspekt des gleichzeitigen Auftretens mehrerer gebietsfremder Arten. Da die ständig zunehmende Zahl gebietsfremder Arten in neuen Lebensräumen das gleichzeitige Auftreten von gebietsfremden Arten wahrscheinlicher macht, wird es künftig noch wichtiger, ihre kombinierten Auswirkungen auf einheimische Arten, Gemeinschaften und Ökosysteme zu untersuchen.

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Chapter 1 General Introduction

1.1. Biological invasions

Biological invasions are shaping much of present environments and occur in most parts of the world. Biological invasion has been defined as the process by which organisms land in an environment where they have not been native before but can persist and form stable populations (Ricciardi 2013). By arriving in a novel environment and competing with native species, invasions are known to have various impacts on species, communities and ecosystems (Vilà et al. 2011; Simberloff et al. 2013). Invasions have been shown to affect primary productivity (Vilà et al. 2011), alter soil nutrient cycles (Ehrenfeld 2003) and threaten biodiversity (Vilà et al. 2011). Besides ecological damage, biological invasions also cause major economic costs, as Diagne et al. (2021) predicted annual mean costs of US$ 162.7 billion in 2017.

In the following I will use the definitions suggested by Pyšek et al. (2004); ‘alien plants’ for plants that occur in an area due to transport by humans; ‘native plants’ for indigenous plant species. Alien plants are further split in archaeophytes and neophytes based on residence time. While archaeophytes have been introduced since the beginning of agriculture in the Neolithic from Southern Europe and the Mediterranean Basin; neophytes have only been introduced to Europe after 1492 (Pyšek et al. 2005; Chytrý et al. 2008)

We do not see a saturation of species accumulation yet (Seebens et al. 2017), but an increasing number of biological invasions (Simberloff et al. 2013; van Kleunen et al. 2015). As biological invasions are predicted to increase by more than one third by the year 2050 (Seebens et al. 2021), it is not surprising that we find co-occurring alien invaders more commonly. Amongst the most important reasons for increasing numbers of invasions, is the increasing global transport and trade (Levine and D’Antonio 2003; Hulme 2009; Seebens et al. 2015; Early et al. 2016), newly accessible pools of potentially invasive species (Seebens et al. 2018), the invasion debt (Essl et al. 2011), as well as abiotic effects such as land-use change and altered resource availability which might further facilitate future invasive spread (Bradley et al. 2010).

Biological invasions are an increasingly important research topic, which had begun to raise interest centuries ago but only over the last few decades became a highly investigated topic (Davis 2011). The strong effects of invasive species which likely become more severe in the future, make it inevitable to study causes and effects of species invasion and predict future impact.

Given the severe impacts alien plant invasions have on native communities, it is crucial to investigate biotic interactions to understand and predict invasive species impacts (Mitchell et al. 2006). Several theories attempt to explain why alien species can be more successful than natives in a given environment. Among the most accepted hypotheses is the Enemy Release Hypothesis, explaining the improved performance of alien species in their new environment by the lack of specialist natural enemies from their origin (Keane and Crawley 2002; Wolfe 2002). This can then lead to the Evolution of Increased Competitive Ability (EICA), another theory attempting to explain improved performance of alien species (Blossey and Notzold 1995). Here the main hypothesis is that species can invest more resources into biomass or reproduction as they need to invest less into defence mechanisms (mechanical or secondary metabolites) as they don't face specialist herbivores in the introduced range. Besides investment of excess energy into growth and reproduction, Callaway and Ridenour (2004) proposed the Novel Weapon Hypothesis which explains better performance of alien species due to investment into allelopathic effects or exudates disrupting native plant-soil interactions.

But not only the interactions of alien species with the native community are important to study, given the increase of alien species naturalizing (Seebens et al. 2017), it becomes more important to study the interaction of co-occurring alien species too (Kuebbing et al. 2013). A previous meta-analysis on the limited studies investigating interactions among co-occurring alien species showed, interactions are predominantly negative or neutral, however also positive interactions have occurred (Kuebbing and Nuñez 2014). When investigating species interactions, facilitation has been long neglected. Facilitation and mutualism are an important part of community ecology, recently gaining more attention. Specifically, the positive interaction of alien species, which jointly would have a more than additive effect, has been termed as "invasional meltdown" before (Simberloff and von Holle 1999). These could come in various forms, such as the suppression of a native community that could give way to a new invader, modification of the habitat or also attraction of common pollinators (Richardson et al. 2000). However, despite some findings of facilitative interactions among alien species (Kuebbing et al. 2013), a full 'invasional meltdown' has yet to be scientifically shown (Simberloff 2006).

1.2. Parallels of invasion biology and community ecology

Much research on biological invasions has aimed at describing and predicting successful alien naturalization (van Kleunen et al. 2018). Shea and Chesson (2002) have proposed a framework linking biological invasions to community ecology under the premise that alien species in a novel environment encounter similar challenges as any species that persists in a community. The core criterion for this is to increase population size from a low density. Given this, alien species invasions can be used as model system to investigate processes of community ecology (Shea and Chesson 2002).

Among the most important factors of a successful establishment of an invading species is the availability of resources on which a potential invader depends (Richardson et al. 2000). It is hypothesized that the more unused resources are available, or resource availability fluctuates in a given environment, the higher the invasibility of this environment (Davis et al. 2000). Still invasive species need to have advantages over local species, but since disturbance is assumed to release resources this provides opportunities for invaders (Davis et al. 2000). This can result in niche opportunities, if there is potential provided for an alien to increase its density because resources are available. The more niche opportunities i.e. resource availabilities there are, the more invasible a community is (Shea and Chesson 2002).

To achieve general answers to pending questions, modern community ecology is focusing less on single species or pairwise interactions but employs two main approaches of investigating community assembly. The evolutionary history of co-occurring species reflects modifications to a common ancestor these species share, which are the basis for phenotypic variation. Therefore, one approach to explain community assembly is community phylogenetics (Webb et al. 2002) which is used as a proxy for niche similarity. On the other hand, trait-based inferences on species similarity or dissimilarity (Violle et al. 2007) can be used which describes trade-offs between plant strategies and links ecological processes to functional traits (Lavorel and Garnier 2002). If the functional traits used for trait-based inferences are phylogenetically fixed, both approaches would achieve similar patterns (Webb et al. 2002). Theory predicts opposing outcomes of such approaches. The limiting similarity hypothesis suggests that more similar species would compete stronger since they occupy similar niche space (MacArthur and Levins 1967) and therefore less similar species are more likely to coexist. Opposingly, the environmental filtering hypothesis predicts the occurrence of more similar, or more closely related, species in an environment since their traits would be best adapted to the given conditions (Keddy 1992). Both of these outcomes together in the context of alien species have been framed Darwin’s naturalization conundrum previously (Diez et al. 2008). Since previous empirical studies have found opposing results in regard to similarity or dissimilarity explaining community composition, Mayfield and Levine (2010) argue that competition among species has to be considered since it affects predictions of competition outcome. Therefore, species dissimilarity should be predominant if niche differences (phylogenetic or trait-based differences) are more important, while species similarity would be driven by fitness differences (trait differences related to competitive ability), otherwise competitive exclusion of inferior species is expected (Chesson 2000; Kunstler et al. 2012).

1.3. Functional Traits

Functional traits are commonly used to address questions on individual, community and ecosystem level (Violle et al. 2007). Functional traits, sensu Violle et al. (2007), describe the morphological physiological and phenological characteristics of species impacting various aspects of species performance such as survival and reproduction, growth and resource acquisition and thereby ultimately species fitness (Adler et al. 2014). Since functional traits describe species characteristics and their performance they also affect ecosystem functioning (Pérez-Harguindeguy et al. 2013; Cadotte 2017).

Among the most commonly measured traits are specific leaf area (SLA), height at maturity and seed mass as proposed by Westoby (1998) as LHS scheme. These three traits are believed to describe fundamental trade-offs which shape plant strategies. SLA describes opposing strategies of resource investment. While a fast-grown large leaf area leads to a large photosynthetic capacity, and enable plants to a more flexible response to environmental conditions this can also be costly since the leaves are not well protected and have a high transpiration rate for example. Low SLA characterises more conservative resource use, with rather long-lived leaves that often even contain defensive compounds. Plant height is considered to describe a trade-off between the advantage of receiving more light by being taller than neighbouring plants, and the costs of building the vegetative structure and reflects strategies to cope with disturbance (Pérez-Harguindeguy et al. 2013). Seed mass is a trait describing reproductive and dispersal strategies. While low seed mass usually means a higher number of seeds is produced and thereby more offspring could germinate, the survival rate is higher with bigger seeds. However smaller seeds tend to be dispersed further while larger seeds disperse less far (Westoby 1998; Moles and Westoby 2006).

Besides the LHS scheme a number of other traits is considered relevant to describe species strategies. For example, root: shoot ratio is used to estimate resource allocation. While a larger investment in roots compared to shoots can be advantageous in limiting conditions, in rich conditions a higher aboveground biomass compared to root biomass is advantageous for competition with neighbouring plants (Tilman 1985; Poorter et al. 2012; Pérez-Harguindeguy et al. 2013). Another commonly noted trait is flower phenology, as this trait is independent from general resource strategies and thus it can be used additionally to characterise plant species (Craine et al. 2012).

Although functional traits can nowadays be gathered from databases such as TRY (Kattge et al. 2020), in situ measurements have the advantage of individuals grown in treatments and those measured for functional traits experience same environmental conditions. As previously shown, intraspecific variation can strongly impact competition outcome (Kraft et al. 2014; Conti et al. 2018) and should therefore be considered in the context of using in situ measures of functional traits. For example, by assuring the same seed origin of individuals in treatment and those for measuring functional traits

1.4. Plant-plant interactions

Plants compete for various resources such as light, water, and soil nutrients which leads to negative interaction between neighbouring plants. Interspecific competition has long been considered as the one fundamental process underlying community ecology. Understanding the mechanisms that enable plants to compete for limiting resources can foster better predictions regarding competitive outcomes and the consequences of competition (Aschehoug et al. 2016). Much of past research on species interactions has focused on testing competitive effects but explaining patterns we observe is more complicated than that. Recently facilitation has gained more attention and is considered to be an important factor shaping species coexistence (Brooker et al. 2008; McIntire and Fajardo 2014).

Facilitation can either mean a neutral effect for one of the species and a beneficial effect for the other (commensalism) or both species benefit from the interaction (mutualism) (Callaway 2007). Such effects have often been observed in harsh environments where species depend on facilitative effects, which has been termed stress-gradient hypothesis by Bertness and Callaway (1994). Additionally, facilitative effects of native on alien species have been described in mild environments (McIntire and Fajardo 2014). A commonly known process in the context of facilitation is nitrogen facilitation by legumes. For example, regarding temperate grasslands, nitrogen (N) is among the main limiting resources in this ecosystem (Vitousek and Howarth 1991; LeBauer and Treseder 2008), where legumes are key species. Legumes can fix atmospheric nitrogen, facilitate neighbouring plants (Mulder et al. 2002) and thereby change N dynamics and community productivity (Spehn et al. 2002; Hille Ris Lambers et al. 2004). This becomes especially important in the context of alien species invasion. Since alien species tend to outcompete native species in regards of resource acquisition and impact nutrient cycling and ecosystem services (Ehrenfeld 2010), the facilitative effects of native legumes might only enhance alien species establishment further (Lucero et al. 2019; Cavieres 2021). Ultimately this might promote a so-called ‘invasional meltdown’ (Simberloff and von Holle 1999). However, legume presence might as well decrease negative effects on the native community by improving nitrogen availability and reducing competition for nitrogen (Eisenhauer and Scheu 2008).

1.5. Relevance of priority effects for community assembly

Many factors determine the outcome of community assembly. Besides the already mentioned resource availability and competitive ability of species, also the timing of species emergence can play a crucial role in shaping community composition (Young et al. 2001). An earlier arrival can affect growth and establishment in a community, especially inhibiting performance of subsequent species with similar niche requirements (Young et al. 2001). Such effects of earlier arrival on the fitness of later arriving species have been termed priority effect (von Gillhaussen et al. 2014). Priority effects were shown to play a crucial role in community composition (Martin and Wilsey 2012) even several years later (Weidlich et al. 2018). Priority effects are not restricted to an earlier arrival of seeds in an area but can also be observed if seeds germinate at different times and therefore an earlier emerging individual can pose effects on a later germinating one (Wainwright et al. 2012). In the context of alien species this is a common occurrence as alien species have been shown to often germinate earlier (Chrobock et al. 2011).

1.6. Annual alien plants as study system

Given that my work mainly focused on mechanisms of interaction among alien or between alien and native species, common garden pot experiments provide an ideal design to investigate such questions, since I can (to a large part) regulate distracting biotic and abiotic conditions. In this thesis I focus on annual alien plant species but also use congeneric or co-occurring native plant species for comparisons or as background communities. I chose a large variety of plant families (except for chapter 4, since here I examined species of varying residence time, all belonging to the Asteraceae family) across functional groups to cover a wide range of different species and thereby variation in niche and trait space to draw generalizable conclusions from my studies. All species used occur in central Europe in similar habitats (ruderal and segetal annuals). Since these are prone to human disturbance, they are commonly invaded by alien species (Chytrý et al. 2008). Given the common occurrence of aliens, this makes them an ideal study system to investigate competitive and facilitative effects of co-occurring plant invaders. However alien plants still interact with native species and communities too, I therefore investigate alien-native interactions as well. Using only annual species reduced bias due to different life forms and gave me the possibility to examine the individual’s full life cycle within one year. Therefore, I could measure total reproductive output of the species as performance measure, which is more closely linked to intrinsic growth rates than biomass for instance, and thereby improves predictions of population dynamics (Laughlin et al. 2020).

1.7. Thesis objectives and outline

In this dissertation I investigate the performance of and interactions among multiple co- occurring alien plant invaders and native species. I studied this by setting up common garden pot experiments at the field station of the University of Hohenheim. Firstly, in Chapter 2 - The stronger, the better - trait hierarchy is driving alien species interaction - I investigated pairwise interactions of 20 alien plant species to elucidate the underlying driver of interaction outcomes and quantify competitive and facilitative interactions. Therefore, I compared models using functional traits, multi-trait- or phylogenetic distance as well as trait hierarchies to explain species interactions. In Chapter 3 - Native and alien species suffer from late arrival, while negative effects of multiple alien species on natives vary - I studied the priority effect and whether alien and native species differ in response to arrive earlier or later than a neighbouring species. At the same time, I investigated whether the occurrence of more than one alien neighbour affects native species stronger than having only one neighbour species. In Chapter 4 - Legume effects in a native community invaded by alien Asteraceae in a multi-species comparison - I aimed to disentangle the relative importance of direct and indirect legume-facilitation for alien species. Further I studied the question how presence of legumes (i.e. not being limited by nitrogen) influences the relationship between traits and species performance. Finally, in Chapter 5 I discuss the findings of the previous chapters and emphasise the importance of investigating co-occurring alien species to improve management implications and predictions of their impact on native communities.

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Chapter 2 The stronger, the better – trait hierarchy is driving alien species interaction

2.1. Abstract

Multiple invaders commonly co-occur in native ecosystems and in some cases have been shown to facilitate each other thus exacerbating impacts on native species, while in other cases one invader may reduce the impact of another due to competition. We therefore aimed at identifying mechanisms driving alien species interactions. We conducted a common garden experiment investigating all pairwise combinations of 20 alien annual plant species in Germany. We first tested whether competition or facilitation occurred more often. Secondly, we determined whether individual traits, hierarchical or absolute trait distances, multivariate trait or phylogenetic distance explained alien plant interactions best. Thirdly, we assessed whether accounting for trait plasticity explains plant performance better than species-level trait averages. While the magnitude of interspecific competition compared to intraspecific competition was on average larger across the 190 alien species combinations, interspecific facilitation still occurred in 24% of cases. Interactions could be better explained by hierarchical trait distances which reflect competitive ability, compared to phylogenetic and multivariate trait distance (reflecting niche differences). This finding supports criticisms about the applicability of testing limiting similarity versus environmental filtering and the community phylogenetic approach. Specifically, when growing taller and having a lower specific leaf area than the co-occurring alien neighbour, biomass and seed number of individuals in mixture increased compared to growing alone. Effects of seed mass, root:shoot ratio and flowering time depended on the performance measure. In contrast to recent suggestions, accounting for trait plasticity did not necessarily explain plant performance better than models using species-level trait averages. These results advance our fundamental understanding of trait-interaction relationships, strengthening recent findings on the importance of competitive hierarchy in shaping interactions and community structure. In particular, applying these concepts to the novel case of alien-alien interactions, is crucial given the continuing accumulation of alien species around the globe.

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2.2. Introduction

Biological invasions have various impacts on ecosystems and communities, posing major threats to biodiversity (Vilà et al. 2011) and are still increasing in number (Seebens et al. 2017). This increase is not only due to well-known reasons such as the invasion debt (Essl et al. 2011) or an increase of global trade and transport (Hulme 2009), but also due to newly accessible source pools (Seebens et al. 2018). Facing increasing accumulation of invasive species, it becomes apparent that more attention must be paid to interactions among invasive species which are especially important in terms of management decisions (Kuebbing et al. 2013). Whether species facilitate each other (which may lead to accelerated impacts, termed as 'invasional meltdown' by Simberloff and von Holle (1999)), interfere with each other or have a neutral interaction has rarely been studied. At least until recently, most studies focused on single invasive species: as the review of Kuebbing et al. (2013) showed, out of 153 studies less than 6% investigated interactions of multiple co-occurring plant invaders. These studies usually focus on few species and their interactions, whereas to identify underlying drivers of species interactions multi-species approaches should be employed, which are suitable for making generalisations across species on mechanisms of species coexistence (van Kleunen et al. 2014).

To achieve such generalisations, functional traits may be used to explain variation in species interaction. Indeed, combining functional trait-based approaches and demographic approaches is necessary to answer pending questions on trait variation (Salguero-Gómez et al. 2018). Trait-based approaches are also a powerful tool explaining community assembly, whereby functional traits influencing plant performance are employed to assess niche differences and species interactions (Violle et al. 2007). Niche differences, which may be reflected by differing trait combinations, enable species to coexist due to, for example, different demands in resource acquisition. Trait-based approaches were often employed in the context of native-alien species interactions (Ordonez et al. 2010), but rarely have studies looked at alien-alien interactions (but see Sheppard et al. 2018). There are two opposite theories predicting the outcome of community assembly in such approaches. The limiting similarity hypothesis suggests that individuals compete more strongly the more similar they are due to occupying similar niche space and competing for resources (MacArthur and Levins 1967), therefore more dissimilar species are more likely to coexist. Assuming the relevant functional traits are fixed in the phylogeny, the same patterns are expected when analysing phylogenetic relatedness as a proxy of niche similarity, which is the approach taken in phylogenetic community ecology (Webb et al. 2002). Conversely, the environmental filtering hypothesis predicts the occurrence of more similar (or related) species in a given environment, as their traits would be best adapted to certain conditions (Keddy 1992). However, Mayfield and Levine (2010) outline that the phylogenetic approach is not a good predictor of interaction outcome as empirical evidence of many studies have not uniformly supported theory. They argue that focusing on trait-based niche description is neglecting competition between dissimilar species and therefore is also often erroneous in predicting interaction patterns. Thus, if niche differences are important, species dissimilarity (phylogenetic or trait-based) is promoted, while fitness differences (hierarchical trait differences related to competitive ability) would promote species similarity in a community (Chesson 2000, Kunstler et al. 2012).

Considering traits related to competitive ability, the plastic response of individuals to biotic or abiotic stress is a common driver of community assembly outcome (Violle et al. 2012) and is an important predictor of biological invasions (Richards et al. 2006). This would imply that to improve predictions of interaction outcome, not only species-level trait averages growing under ideal conditions without competition should be measured, but also traits of individuals growing under competition to account for competition-induced trait plasticity (Richards et al. 2006).

Our study aims to evaluate the previously mentioned hypotheses on trait-interaction relationships to identify which mechanisms determine alien plant species interactions. To this end, we performed a common garden experiment, investigating all inter- and intraspecific pairs of 20 alien plant species (giving 190 interspecific combinations) and additionally growing alone. To explain species interactions we considered five different models to investigate plant performance: a) individual functional traits of each plant individual (targets) to assess performance independent of neighbour traits; b) individual trait hierarchy (trait distances between target and neighbour) as recent studies showed that fitness differences are correlated with competitively superior traits; c) absolute trait distances to test the possibility that trait differences regardless of the direction are relevant (in which case the individual trait hierarchy model would show a quadratic relationship); d) multivariate trait distance, as a measure to describe overall niche differences; and e) phylogenetic distance as another proxy for niche differences. In this study we aimed at answering 1) whether alien species are more likely to compete or facilitate each other when growing in interspecific pairs, as well as assessing whether inter- or intraspecific competition is stronger; 2) which of the above mentioned models explains alien plant interactions best, and consequently how the relative performance of aliens relates to phylogenetic or trait similarity and hierarchy; and 3) whether accounting for trait plasticity explains plant performance better compared to only employing species-level trait averages.

2.3. Methods

Study system

We selected 20 annual plant species from seven different plant families across three functional groups (2 legumes, 4 grasses, 14 forbs). The species occur in ruderal plant communities and were selected as they cover a wide phylogenetic range (phylogenetic tree in the Supplementary material Ch 2, Appendix 1, Figure A1). The study comprises only annual species to reduce bias of different plant life forms and as it enables the measurement of an individual's fitness within one experimental season. We obtained seeds of most species from botanical gardens, the remaining from seed suppliers or monocultures of previous common garden experiments (Table A1). All study species are considered established neophytes in Germany except for *Amaranthus tricolor, Bidens pilosa* and *Cosmos bipinnatus,* which are casual neophytes (Table A1). On the scale of ca. 11 km x 11 km, species range size in Germany ranges from less than 1% to 47%, suggesting that some species are more likely to co-occur than others. Minimum residence time (years since first record in Germany) varied from 38 to 434 years (Table A1).

Experimental set-up

We conducted the experiment during the growing season 2018 at a field station of the University of Hohenheim (48°42'45.2"N, 9°11'23.6"E) in Stuttgart, Germany (400 m a.s.l.; mean annual temperature 2018: 11.15 °C; annual precipitation 2018: 649.7 mm). In this common garden pot experiment, plants were growing either alone or with one neighbour plant. Specifically, all species were planted alone (control) and in all pairwise combinations (20 intraspecific [monoculture], 190 interspecific [mixture]) in a completely randomized design using four replicates for the pairwise pots. Overall, we used 926 pots with a volume of 10 L (28 cm diameter, 21 cm height) filled with field soil (total soil carbon content 1.8 %; total soil nitrogen content 0.075 %; 212 mg potassium (K2O)/kg, 347 mg phosphate (P2O5)/kg; soil texture of 9.8 % clay, 70.7 % sand and 19.5 % silt) and placed on concrete tiles to improve drainage. Seeds of the study species were sown in trays in a greenhouse on 20 April 2018. Five weeks later (22-30 May 2018) seedlings were transplanted into the experimental pots. Individuals in pairwise treatments were placed 5 cm apart, single individuals were placed in the centre of the pot. Due to low germination rates, *Amaranthus blitoides* and *Atriplex sagittata* could only be replicated three times and *Iva xanthiifolia* two times, resulting in a total of 698 mixture pots. Control individuals were planted in eight replicates (respectively six and four) to obtain more accurate trait measurements (see below) adding up to a total of 926 pots. Weeds emerging from the seedbank in the pots were removed before transplanting seedlings and continuously throughout the experiment. All pots were watered sufficiently by a drip watering system. Initial height was measured (longest leaf for species growing as rosettes, stem height for all others) after planting, to correct for initial differences that are not a result of varying neighbour treatment. Individuals which died from transplanting were replaced in the first two weeks of the experiment. We harvested all plants between 15 October and 7 November 2018 (21 weeks after the start of the experiment).

Trait selection and data collection

We measured the traits that are considered to be 'fundamental trade-offs controlling plant strategies' according to Westoby (1998): specific leaf area (SLA), plant height and seed mass. Additionally, we measured flowering onset and root:shoot ratio. Relevant traits for resource acquisition and allocation were chosen and trait measurements followed standard protocols (for further information see Supplementary material Ch 2, Appendix 2). Each of these traits was measured on each individual plant except for root:shoot ratio, which, due to its labour intensiveness, was only measured for control pots. For the remaining traits we thus estimated both species-level trait averages in ideal conditions, that is on the individuals growing alone as well as traits at individual-level accounting for competition-induced plasticity in the various pairwise treatments. Additionally, we classified our species according to plant functional group (forb, grass, legume), which encompasses several traits and can thereby also incorporate traits which are more difficult to measure (Cornelissen et al. 2001). We note that while seed mass was higher for the legumes in our study, the other four quantitative traits greatly overlap across the three functional groups (Appendix 2, Figure A2). We thus consider it likely that functional group adds other dimensions of plant ecological strategies and follow the methods of other studies that included both quantitative and categorical traits in analyses of trait effects (e.g. Carboni et al. 2016). As niche differences of species might be better represented in multi-dimensional space (Kraft et al. 2015), we also calculated multivariate trait distance from the species-level trait averages and plant functional group of control individuals, as well as calculating phylogenetic distances between species pairs (for details see Appendix 2).

To assess plant performance, we considered aboveground biomass as a proxy for competitive ability and total seed number as measure of fitness for such annual species. For biomass, we cut plants at ground level and dried them at 70 °C for 72 h. As seeds ripened during the season, a subset of ten intact flower heads was collected per plant. All seeds of the subsets were counted and weighed. At harvest we counted the total number of flower heads with ripe seeds to extrapolate the counted subset to total seed number. For species releasing whole flower heads which are not countable anymore afterwards (*Diplotaxis muralis, Eragrostis minor, Hirschfeldia incana, Medicago polymorpha* and *Vicia villosa*), all flower heads were collected throughout the season as they ripened (at least two times per week).

Of initially 926 pots with 1700 plants, we removed 59 pots from the dataset due to mortality, resulting in 1582 observations (1288 interspecific, 142 intraspecific, 152 single; for 32 of these individuals (26, 3 and 3, respectively) we could not assess biomass as it was ripped off in a storm just before harvest, but this did not affect neighbouring plants). For the seed number analysis *Berteroa incana* was removed as it did not produce seeds during the experimental period, resulting in 1438 observations (1160 interspecific, 134 intraspecific, 144 single; for 54 of these individuals (43, 7 and 4, respectively) we could not assess seed number because they were released before we could collect them).

Statistical analysis

To address aim 1) of finding out whether competition (interspecific versus intraspecific) or facilitation is more common, we first tested for general differences among treatments: single plants (control), intra- and interspecific pairs (split into respective neighbour plant functional group). We examined log-transformed aboveground biomass and log-transformed seed number+1 for each species separately with ANOVA using R version 3.5.3 (R Core Team 2019). For pots with intraspecific pairs we used the mean performance of both individuals to account for non-independence within the pot.

For each performance measure (i.e. biomass and seed number) relative performance growing in interspecific pairs was calculated as the log response ratio (lnRR, which is the log(performance in mixture/performance in control, Weigelt and Jolliffe 2003) separately for each individual. As a measure for performance in the control, we used the mean of all control individuals of the respective species. These log response ratios (in the following referred to as lnRR_inter/control) represent the relative ability to tolerate a heterospecific individual compared to growing alone. Positive values indicate facilitation and negative values indicate competition. Analogously we calculated the relative performance in interspecific pairs compared to intraspecific pairs (i.e. log(performance in mixture/mean performance in monoculture; in the following referred to as lnRR_inter/intra), here, positive values indicate higher intraspecific competition and negative values indicate higher interspecific competition as compared to each other.

To address aim 2) of exploring whether species interaction is best explained by phylogenetic, multivariate trait based or hierarchical trait models; we built five models for each log response ratio, to investigate the response to a) a target individual’s own traits (log SLA, log maximum height, log seed mass, log root:shoot ratio, flowering onset, plant functional group; in the following called target trait model); b) trait hierarchies between target and neighbour, which is the (except for flowering onset) logarithmized distance of each species-level trait average between the two species (target – neighbour) of a respective pot, plus the plant functional group of the neighbour (trait hierarchy model); c) absolute trait distances (the absolute values of the trait hierarchy model) and neighbour plant functional group (absolute trait distance model); d) multivariate trait distance between the two species in the pot (multivariate trait distance model); e) and phylogenetic distance (phylogenetic distance model). When calculating log response ratios, we considered all individuals as target individuals, while accounting for sources of non-independence in the random effects. We added log-transformed initial height as covariate to all models to account for potential differences in starting conditions. All numeric explanatory variables were scaled to a mean of zero and standard deviation of one.

To address aim 3) of examining whether models accounting for trait plasticity explain plant performance better that only considering species-level trait averages, we compared the three models using separate traits, with the respective individual-level trait based model for lnRR biomass and lnRR seed number. We ran the species-level trait average model again on a reduced dataset comprising only individuals with data for each individual-level trait. As root:shoot ratio could only be assessed for individuals growing alone, these models only consider SLA, height, seed mass, and flowering onset (plus neighbour functional group).

For all models analysing the log response ratios of each target individual, we used the Markov chain Monte Carlo method in the MCMCglmm package (Hadfield 2010) in R version 3.5.3 (R Core Team 2019) to account for phylogenetic correlation between species. We calculated the correlation as the inverse phylogenetic covariance, using the function inverse in the MCMCglmm package. Besides phylogenetic correlation, target species identity was included as a random effect, as well as unique combination of species to account for lower differences between same combinations than between different combinations. To account for non-independence of individuals in the same pot, pot identity nested in combination was further included as a random effect. We defined priors for each random effect corresponding to Gaussian inverse-Gamma distribution with a variance of one and shape and scale parameters set to 0.01 (Hadfield 2010). We ran the models with 1,000,000 iterations, using a burn-in phase of 100,000 and a thinning interval of 200, resulting in 4,500 observations. We assumed convergence after visually checking trace plots of all models. To estimate the phylogenetic signal we used Pagel's lambda (Freckleton et al. 2002). We considered fixed effects as relevant for plant performance if their respective 95% credible interval of the effect size did not overlap zero. The variance explained by each model (R^2) was calculated following Nakagawa and Schielzeth (2013). Since the R^2 value only takes into account posteriori model output, we relied on the Deviance Information Criterion (DIC) to compare model fit, a measure which is commonly employed for model selection in Bayesian statistics (Spiegelhalter et al. 2002).

2.4. Results

Alien species competition versus facilitation

We found species-specific variation concerning whether alien species benefitted or suffered from growing in mixture compared to growing alone or with conspecifics (Figure 2.1). Legumes (*M. polymorpha* and *V. villosa*) and three other species we observed to be more dominant in the pots (*B. pilosa*, *C. bipinnatus*, *Panicum capillare,* i.e. those with highest biomass production) grew equally good or better in interspecific pairs compared to growing alone. Although performance generally varied more within treatment, this trend was also found for seed number in the same species except for *C. bipinnatus* (Appendix, Supplementary material Ch 2, Figure A3).

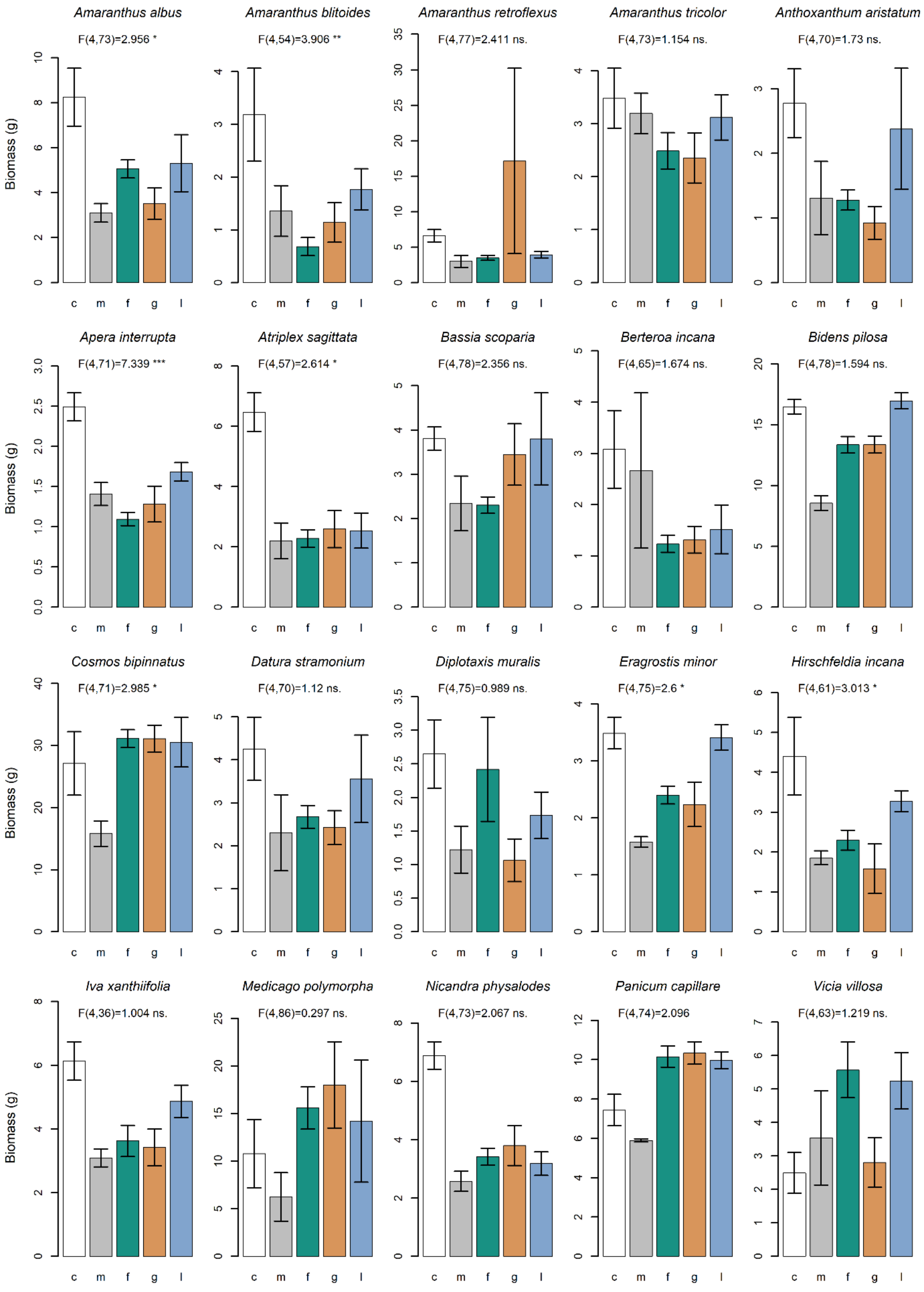

***Figure 2.1** Mean biomass production in g (± 1 standard error) per species depending on competition treatment. White bars represent the control (plants growing alone in the pot, 'c'); grey bars the monoculture treatment (plants grow with a conspecific individual, 'm'); coloured bars represent the mixture treatment with the neighbour being a forb (green, 'f'), a grass (orange, 'g') or a legume (blue, 'l'). Significance of competition treatment effect on log-transformed biomass was tested using ANOVA. F-statistics and significance of p-values (indicated by asterisk; *=p<0.05; **=p<0.01; ***=p<0.001; ns. indicates no significance) are displayed.*

Comparing biomass production over all interspecific pairs, we found competition more often than facilitation (958 vs. 304 cases). Thus, interspecific facilitation occurred in 24% of all observations and was more common among plants growing with a legume than with a grass or forb (31% vs. 23% of cases). Intraspecific facilitation, on the other hand, was rare (ca. 10%, i.e. 14 vs. 128 observations). Comparing interspecific competition with intraspecific competition, higher intraspecific competition was more common than higher interspecific competition (730 vs. 532 cases). Nevertheless, although instances of higher interspecific competition were less common, interspecific competition was on average stronger as the mean lnRR_inter/intra across all species is negative for all response values. Mean (± 1 SE) values for the four log response ratios considered were: -0.75± 0.033 (n=1262) for lnRR_inter/control biomass; -1.24± 0.051 (n=1117) for lnRR_inter/control total seeds; -0.15± 0.033 (n=1262) for lnRR_inter/intra biomass; and -0.45± 0.051 (n=1117) for lnRR_inter/intra total seeds.

Niche differences (phylogenetic or multivariate) versus functional trait hierarchies

While the target trait model and trait hierarchy model explained more variance in the data than the other models, we found the best model fit using DIC with the hierarchical trait model for all response variables (Table 2.1). The phylogenetic signal Pagel's lambda differed between models, being highest for the target trait models. For all models the random effects phylogeny, target species identity and species combination explain more variation than pot identity. For the target trait model species combination explains more variation than for the trait hierarchy model which already includes information on species combination in fixed effects (Supplementary material Appendix 4, Table A2). Concerning the fixed effects of this target trait model, maximum height was the only significant trait, with a strong positive effect on relative biomass, while none of the measured focal traits were significant for seed number (Figure A4).

Table 2.1 *Model overview of the five different models for each response variable (log response ratios of individuals in interspecific competition compared to control individuals or compared to individuals in intraspecific competition, for biomass and seed number); showing the results of the target trait model; trait hierarchy model; absolute trait distance model; multivariate trait distance model; phylogenetic distance model. Marginal R^2 and conditional R^2 are reported as well as the delta DIC (Deviance Information Criterion) value (difference between the respective model and the model with lowest DIC for each response variable). The phylogenetic signal was calculated as Pagel's lambda.*

Response variable (sample size)	**Model**	**R^2 marginal (95% credible interval)**	**R^2 conditional (95% credible interval)**	**ΔDIC**	**Mean phylogenetic signal (95% credible interval)**
Facilitation vs. Competition – *lnRR_inter/control biomass (N=1262)*	Target trait	0.22 (0.077 - 0.362)	0.64 (0.484 - 0.818)	17.2	0.23 (<0.001 - 0.586)
	Trait hierarchy	0.20 (0.136 - 0.263)	0.48 (0.353 - 0.608)	0	0.17 (0.002 - 0.424)
	Absolute trait distance	0.05 (0.023 - 0.086)	0.54 (0.368 - 0.703)	18	0.17 (0.001 - 0.528)
	Multivariate trait distance	0.03 (0.009 - 0.061)	0.54 (0.397 - 0.711)	20.1	0.16 (0.001 - 0.511)
	Phylogenetic distance	0.03 (0.005 - 0.048)	0.53 (0.392 - 0.709)	21.3	0.16 (0.001 - 0.515)
Facilitation vs. Competition – *lnRR_inter/control seed number (N=1117)*	Target trait	0.17 (0.038 - 0.344)	0.46 (0.328 - 0.790)	60.6	0.18 (<0.001 - 0.598)
	Trait hierarchy	0.12 (0.076- 0.172)	0.47 (0.342 - 0.624)	0	0.07 (<0.001 - 0.264)
	Absolute trait distance	0.04 (0.016 - 0.064)	0.36 (0.238 - 0.492)	52.7	0.04 (<0.001 - 0.158)
	Multivariate trait distance	0.02 (<0.001 - 0.036)	0.36 (0.229 - 0.495)	57.7	0.04 (<0.001 - 0.141)
	Phylogenetic distance	0.01 (<0.001 - 0.032)	0.35 (0.233 - 0.499)	59.6	0.04 (<0.001 - 0.149)
Inter- vs. Intraspecific competition – *lnRR_inter/intra biomass (N=1262)*	Target trait	0.22 (0.088 - 0.372)	0.62 (0.463 - 0.803)	17.6	0.24 (0.002 - 0.547)
	Trait hierarchy	0.19 (0.140 - 0.260)	0.44 (0.325 - 0.573)	0	0.11 (0.002 - 0.348)
	Absolute trait distance	0.06 (0.025 - 0.086)	0.51 (0.371 - 0.667)	17.4	0.13 (0.001 - 0.454)
	Multivariate trait distance	0.03 (0.007 - 0.058)	0.52 (0.363 - 0.669)	20	0.14 (0.001 - 0.462)
	Phylogenetic distance	0.02 (0.004 - 0.046)	0.51 (0.364 - 0.665)	21.4	0.13 (0.001 - 0.438)
Inter- vs. Intraspecific competition – *lnRR_inter/intra seed number (N=1117)*	Target trait	0.15 (0.030 - 0.291)	0.52 (0.319 - 0.776)	61.2	0.11 (<0.001 - 0.490)
	Trait hierarchy	0.12 (0.076 - 0.170)	0.49 (0.348 - 0.631)	0	0.07 (<0.001 - 0.267)
	Absolute trait distance	0.04 (0.016 - 0.065)	0.35 (0.231 - 0.486)	52.2	0.03 (<0.001 - 0.148)
	Multivariate trait distance	0.02 (<0.001 - 0.034)	0.35 (0.233 - 0.494)	57.3	0.03 (<0.001 - 0.128)
	Phylogenetic distance	0.01 (<0.001 - 0.029)	0.35 (0.225 - 0.493)	59.1	0.04 (<0.001 - 0.130)

For the best model (based on DIC, trait hierarchy model), maximum height and seed mass had a strong positive effect on relative biomass, meaning growing taller and growing from heavier seeds than the neighbour, increases biomass in mixture compared to growing alone (Figure 2.2a, 2.3a-b). Root:shoot ratio had a positive effect as well, meaning species which are potentially better belowground competitors have a competitive advantage over the neighbour (Figure 2.3c). SLA had a negative effect, meaning species with lower SLA than their neighbour increased relative performance in mixture compared to growing alone (Figure 2.3d). Flowering onset had no effect on relative biomass, whereas the functional group of the neighbour plant had a strong effect. Growing next to a legume increased relative performance, whereas growing next to a grass decreased performance compared to growing with a forb (Figure 2.2a).

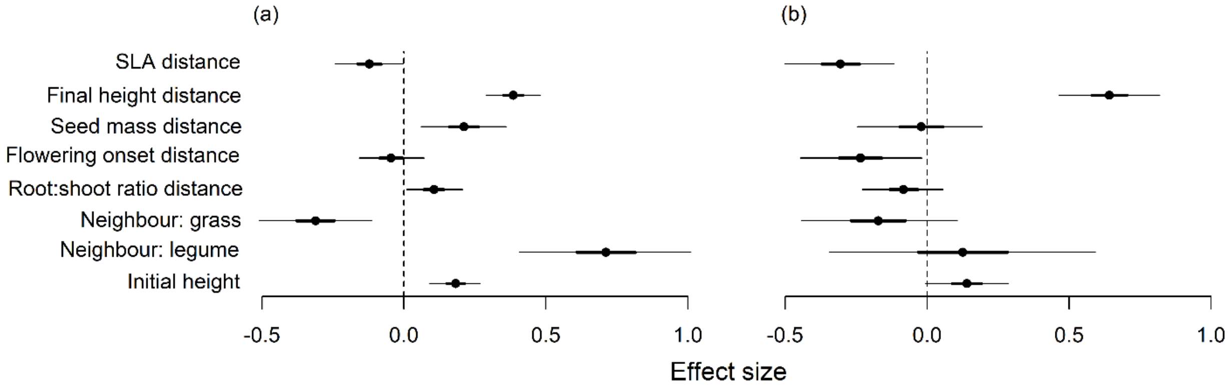

Figure 2.2 *Results of the trait hierarchy model to explain relative performance in interspecific competition compared to growing alone (lnRR_inter/control): effects of specific leaf area (SLA) distance, final height distance, seed mass distance, flowering onset distance, root:shoot ratio distance and the effect of having a grass or a legume as neighbour (relative to a forb) and initial height of the individual on log response ratio biomass (a) and log response ratio seed number (b). All numerical traits are log-transformed and scaled to a mean of 0 and standard deviation of 1 (except for flowering onset which is only scaled). Positive effects indicate that a higher value of the respective trait leads to more biomass (respectively seeds) in interspecific competition (compared to control single individuals). Effects are considered relevant if the 95% credible interval does not overlap zero. Dots show the posterior mean, wide lines indicating one standard deviation (68% credible interval), while narrow lines indicate the 95% credible interval.*

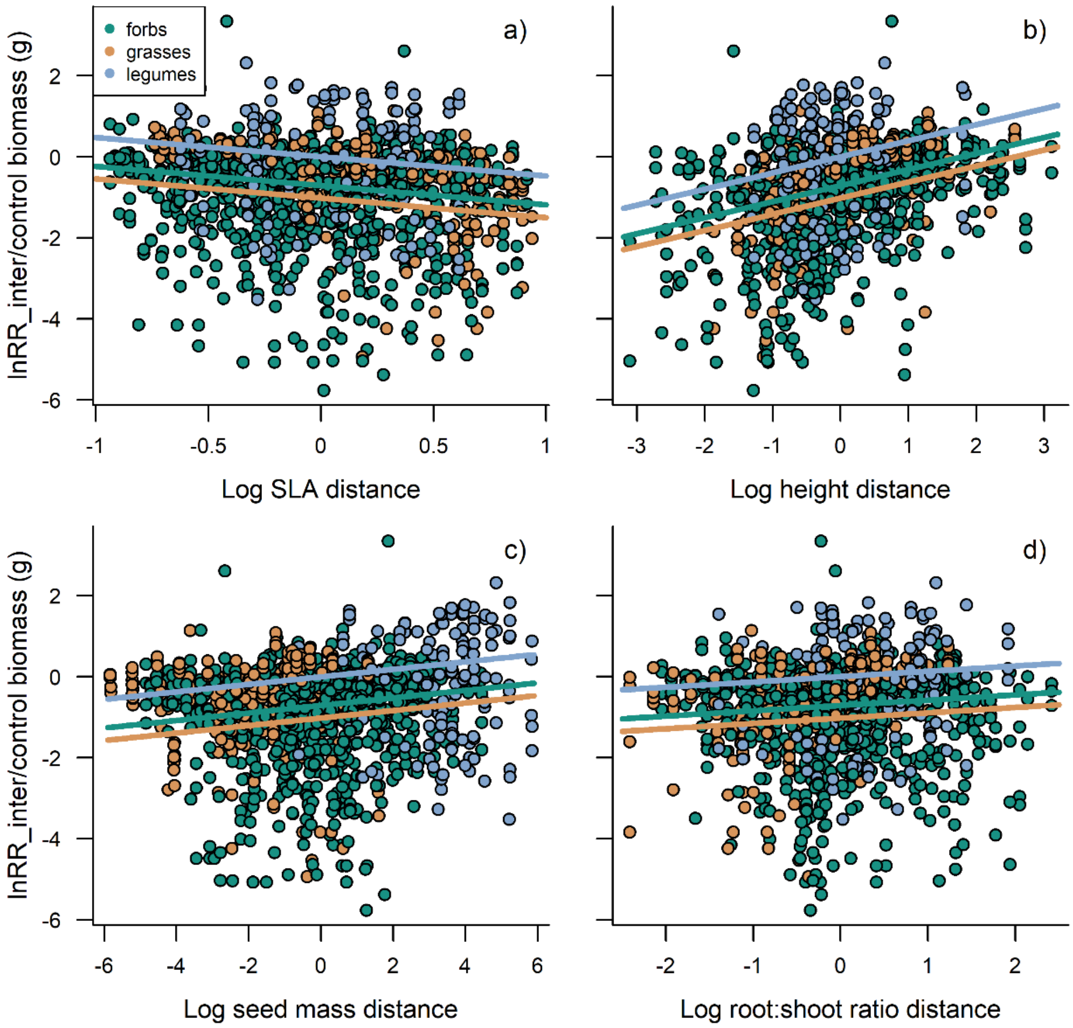

Figure 2.3 *Predictions of trait effects on the log response ratio biomass of alien plants growing in interspecific competition compared to growing alone (lnRR_inter/control). Displayed are the predicted effects of the four relevant traits from the trait hierarchy model: specific leaf area (SLA) distance (a), maximum height distance (b), seed mass distance (c) and root:shoot ratio distance (d) (all log-transformed and scaled to a mean of 0 and standard deviation of 1; predictions for each trait effect shown with the other numeric traits fixed at their mean). Different colours depict the different functional groups: forbs (green), grasses (orange) and legumes (blue). Positive effects indicate that a higher value of the respective trait of the target individual compared to its neighbour leads to more biomass in interspecific competition (compared to control single individuals).*

For seed number the effects of height, SLA and having a grass as neighbour pointed in similar directions (Figure 2.2b, 2.4a-b). In contrast to lnRR biomass, for lnRR seed number flowering onset had a negative effect, meaning the relative number of produced seeds increased when flowering earlier than the neighbour plant (Figure 2.4c). Seed mass, root:shoot ratio and having a leguminous neighbour did not have an effect on lnRR total seed number (Figure 2.2b).

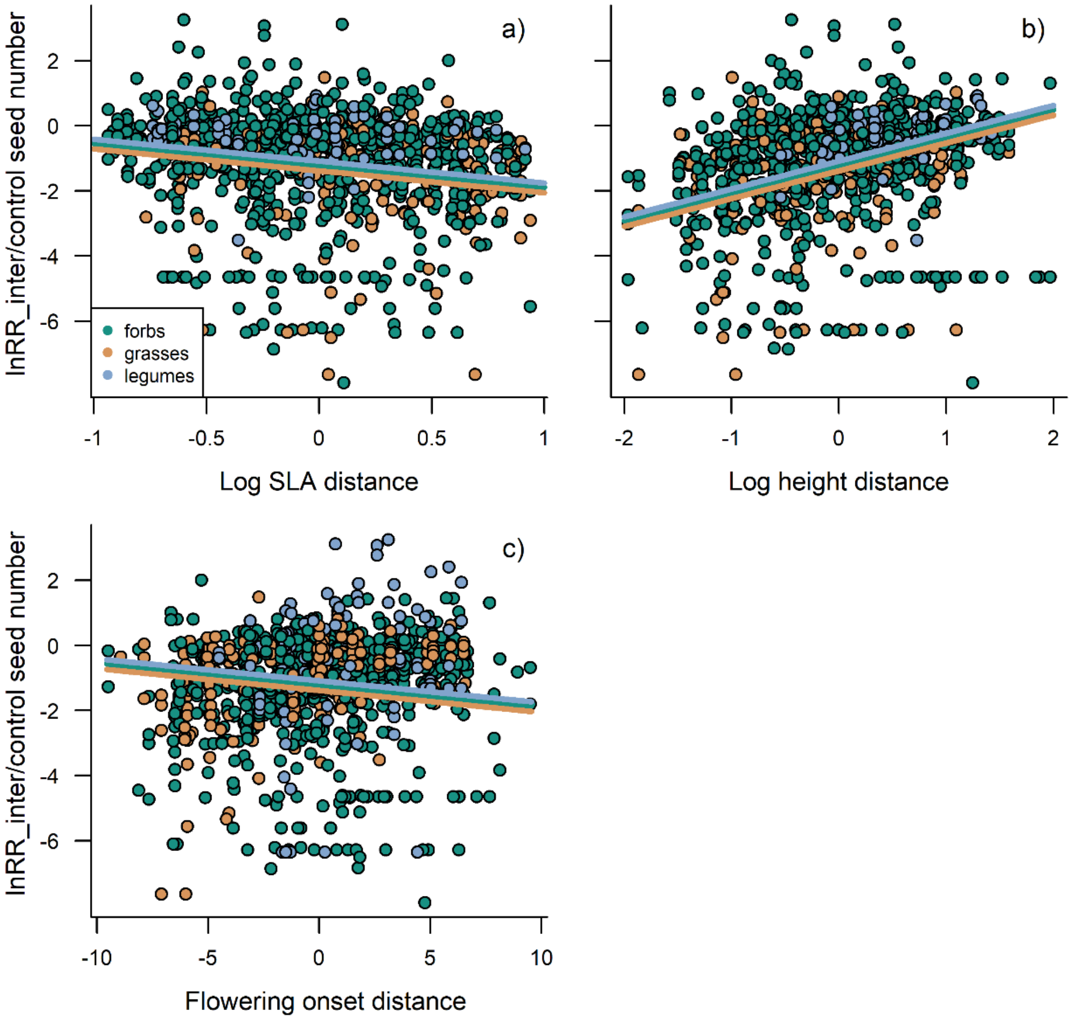

Figure 2.4 *Predictions of trait effects on the log response ratio seed number of alien plants growing in interspecific competition compared to growing alone (lnRR_inter/control). Displayed are the three relevant traits from the trait hierarchy model: specific leaf area (SLA) distance (a), maximum height distance (b) and flowering onset distance (c) (all log-transformed and scaled to a mean of 0 and standard deviation of 1, except for flowering onset which is only scaled; predictions for each trait effect shown with the other numeric traits fixed at their mean). Different colours depict the different functional groups: forbs (green) grasses (orange) and legumes (blue). Positive effects indicate that a higher value of the respective trait of the target individual leads to higher seed number in interspecific competition (compared to control single individuals).*

For the log response ratios comparing performance in mixture to intraspecific pairs (lnRR_inter/intra), results were qualitatively similar for all traits for relative biomass and seed number measures respectively (except that the effect of root:shoot ratio on relative biomass showed only a positive trend but was not significant) (Supplementary material Ch 2, Appendix 4, Figure A5-7).

Individual-level traits versus species-level trait averages

We compared our models using separate traits with models using trait measures at an individual-level (accounting for competition-induced plasticity) instead of traits measured as species-level trait averages (for comparison only SLA, height, seed mass and flowering onset were included as for root:shoot ratios only control individuals could be assessed) (Table 2.2; Figures for the two best models in Supplementary material Appendix 5, Figure A8, A9).

Table 2.2 *Model overview comparing the species-level trait average model; with the corresponding individual-level trait model for the models testing the effects of target traits, trait hierarchy, and absolute trait distance on the log response ratios of individuals in interspecific competition compared to control individuals (lnRR_inter/control), for biomass and seed number; Marginal R^2 and conditional R^2 are reported as well as the DIC (Deviance Information Criterion) value. The phylogenetic signal was calculated as Pagel's lambda.*

Response variable (sample size)	**Model**		**R^2 marginal (95% credible interval)**	**R^2 conditional (95% credible interval)**	**DIC**	**Mean phylogenetic signal (95% credible interval)**
Facilitation vs. Competition lnRR_inter/control biomass (N=571)	Target trait	Average traits	0.14 (0.029 - 0.264)	0.43 (0.221 - 0.665)	448.38	0.17 (0.002 - 0.461)
		Individual traits	0.35 (0.152 - 0.535)	0.95 (0.912 - 0.988)	0	0.42 (0.001 - 0.962)
	Trait hierarchy	Average traits	0.12 (0.060 - 0.180)	0.39 (0.243 - 0.552)	423.03	0.17 (0.003 - 0.403)
		Individual traits	0.08 (0.038 - 0.134)	0.35 (0.209 - 0.524)	428.02	0.14 (0.002 - 0.390)
	Absolute trait distance	Average traits	0.04 (0.012 - 0.079)	0.35 (0.186 - 0.527)	449.70	0.12 (0.002 - 0.366)
		Individual traits	0.08 (0.039 - 0.133)	0.35 (0.204 - 0.515)	428.03	0.14 (0.002 - 0.380)
Facilitation vs. Competition lnRR_inter/control seed number (N=565)	Target trait	Average traits	0.20 (0.063 - 0.351)	0.54 (0.330 - 0.768)	266.54	0.19 (0.001 - 0.572)
		Individual traits	0.46 (0.319 - 0.581)	0.80 (0.713 - 0.892)	0	0.14 (<0.001 - 0.607)
	Trait hierarchy	Average traits	0.15 (0.079 - 0.227)	0.50 (0.348 - 0.671)	222.28	0.23 (0.002 - 0.535)
		Individual traits	0.05 (0.015 - 0.081)	0.46 (0.276 - 0.675)	257.18	0.16 (0.001 - 0.534)
	Absolute trait distance	Average traits	0.04 (0.010 - 0.066)	0.48 (0.288 - 0.687)	263.15	0.17 (0.002 - 0.540)
		Individual traits	0.05 (0.015 - 0.080)	0.46 (0.272 - 0.670)	257.26	0.16 (0.001 - 0.522)

For both, lnRR_inter/control biomass and lnRR_inter/control seed number the datasets were reduced, excluding all individuals where one of the measures was missing (Table 2.2). While the trait hierarchy model is still the best model for this reduced dataset when using species-level trait averages (but with weaker effect sizes for some traits), the target trait model has the lowest DIC when comparing individual-level traits. Furthermore, when considering only traits of the target plant relative performance (both biomass and seed number) were explained better when using individual-level traits, whereas when considering trait hierarchies, species-level trait averages explained performance better (Table 2.2). Height was by far the most important trait influencing species performance for the individual-level model, showing a strong positive effect for lnRR biomass as well as lnRR seed number (Figure A9).

2.5. Discussion

Although individual species varied in their response to neighbouring species, overall competition was more commonly found than facilitation in terms of biomass production. Higher intraspecific competition was slightly more common, though on average weaker, than higher interspecific competition. Hierarchical trait differences between target and neighbour explained interactions between alien species best (even though the target trait model also explained considerable variation in relative performance). Specifically, relative performance advantages (i.e. seed number and aboveground biomass) were greater for species having lower SLA values, growing taller, producing larger seeds, investing into roots compared to shoots, and flowering earlier than the respective neighbour. Notably, using traits measured at individual-level, thereby accounting for plasticity, did not explain relative performance better than using species-level trait averages when looking at species differences (trait hierarchy model). However, for models focusing only on target traits, individual-level traits explained species performance better. Specifically, relative aboveground biomass and seed production was larger, the taller the species grew.

Alien species competition versus facilitation

When addressing aim 1), that is comparing individuals growing in mixture vs. growing alone, we find competition to be more common than facilitation (76% of the cases). Comparisons to previous studies are difficult, as few studies to date have examined interactions among invasive species (Kuebbing et al. 2013) and even less studies have used a multi-species approach to empirically test pairwise interactions among alien species (but see Sheppard 2019). Nevertheless, so far mostly evidence of competition was found for alien plants but also other taxa (Kuebbing and Nuñez 2014, Jackson 2015), which is in line with our results. These predominantly negative interactions among several alien species rather contradict the proposed ‘invasional meltdown’ hypothesis (Simberloff and von Holle 1999). Yet in 24% of the interspecific and 10% of the intraspecific instances we observed facilitation. This was more often - but not exclusively - the case for growing with a legume compared to growing with a forb or a grass species, which is in line with the positive effect of legume neighbours also found in our models. To date very few studies have found facilitating effects among invasive plants (but see Cushman et al. 2011) whereas more commonly it has been demonstrated that native species can facilitate aliens (Abella and Chiquoine 2019, Lucero et al. 2019). Our results thus highlight that attention has to be raised to facilitative interactions among aliens, especially since they are not limited to benefitting from legumes and occur also in casual neophytes. Such positive interactions may thus be highly relevant for invasion management (Kuebbing et al. 2013).

When comparing species growing in mixture with growing in monoculture, we found higher intraspecific competition to be slightly more common (58% of the cases) than higher interspecific competition. However, interspecific competition was on average stronger than intraspecific competition over all species (as also found by Sheppard (2019)). Although a recent meta-analysis showed higher intraspecific competition among plants generally to be more common than higher interspecific competition (Adler et al. 2018), another meta-analysis showed differences for native and alien species in intensity of inter- and intraspecific competition (Golivets and Wallin 2018). They found that while native species suffer more from interspecific competition, regardless of whether competing with alien or native species, alien species equally often suffer from intra- and interspecific competition. Past reviews (Goldberg and Barton 1992 and cited therein) found mainly contradicting results of empirically testing the strength of inter- vs. intraspecific competition. Other mechanisms such as competitive hierarchy may be more important for shaping species coexistence rather than niche differentiation. Considering niche theory one would expect higher intraspecific competition whereas under the importance of fitness differences, interspecific competition may be higher depending on the species pair. This can explain the fact that species greatly differ in individual responses to intra- and interspecific competition in our experiment. Indeed, Stoll and Prati (2001) found that less competitive plants perform better growing with conspecifics whereas more competitive plants tend to perform better growing with heterospecific neighbours (also found in our experiment e.g. for *B. pillosa, C. bipinnatus, M. polymorpha, P. capillare*, or *V. villosa* which were the species reaching highest absolute biomass, notably the first two are considered to be casual neophytes in Germany).

Relative performance in competition with other aliens is not related to phylogenetic or functional similarity

Considering aim 2), the results generally showed that hierarchical traits related to competitive ability predict alien species interactions best, explaining 12-20% of the variance in relative performance. Additionally, the actual traits of the target individuals, irrespective of their neighbour, also explained considerable variance. Conversely, phylogenetic distance or overall trait distance only explained 1-3% of variance and thus cannot generally explain relative performance of aliens. While the 'limiting similarity' hypothesis states that species with more similar traits would compete more strongly and that more dissimilar species are more likely to coexist (MacArthur and Levins 1967), several authors questioned this hypothesis and pointed out the importance of fitness differences (described by 'trait hierarchy') rather than niche differences (described by multivariate trait distance or phylogenetic distance) (Chesson 2000, Mayfield and Levine 2010). Indeed, our results also support the argument that fitness differences may be more important in explaining alien species interactions than niche differences. Most recent studies found that phylogenetic distance is not a good predictor of species interactions (Kunstler et al. 2012, Golivets and Wallin 2018), whereas a trait-based approach may be more relevant. For instance, Feng et al. (2019) introduced alien species into native communities of different diversity levels and found that the effect of multivariate trait distance was stronger than phylogenetic distance. Few studies compared multivariate trait distance approaches with trait hierarchy, but Funk and Wolf (2016) studied competition between 47 native species and an alien invader and found that species interaction could only be explained by trait hierarchy, not by a multivariate trait distance approach nor by phylogenetic relatedness. Kraft et al. (2014) found trait hierarchies to best explain interactions of eight studied native species in pairwise competition and Kunstler et al. (2012) compared the effects on competitive response between trait hierarchy and phylogenetic or functional similarity for 22 native tree species and found that only trait hierarchy could explain species interaction. A recent study by Carmona et al. (2019) with a set-up of pairwise competition involving six native species found more support for trait hierarchies determining species competition than for absolute trait distances. The importance of considering hierarchical trait differences has thus recently gained more attention and for the specific case of alien-alien interactions is in line with previous findings of Sheppard (2019).

Which traits determine alien species interactions?

From the five quantitative traits we considered, plant height had the strongest effect; consistently with previous studies, taller individuals had a competitive advantage over their neighbours (Kraft et al. 2015) which reflects an advantage in competition for light (Westoby 1998). When considering only target traits irrespective of the neighbour traits, height was in fact the only significant trait (and only affecting biomass production). Having lower SLA values compared to the neighbour resulted in relatively more aboveground biomass and more seeds and therefore in higher fitness, consistent with previous studies that showed higher competitive ability for individuals with low SLA (Kraft et al. 2015, Conti et al. 2018). High initial seed mass had a positive effect on biomass, because seedlings from heavier seeds tend to have more resources and are more robust facing environmental stress (Westoby 1998). However, we note that seed mass was correlated with plant functional group (see Supplementary material Ch 2, Figure A2); and therefore has to be carefully interpreted. Because root:shoot ratio is a labour intensive measure empirical results are scarce compared to the previously discussed traits. Competition for nutrients may be more relevant than for light in our experimental set up with only two individuals per pot. We found higher root investment to result in higher biomass production, while it did not benefit seed production but rather tended to reduce it. This hints at a trade-off between resource investment in competitive ability (hence biomass) and reproduction (seed number). Our results revealed a negative effect of flowering onset on seed number, meaning the earlier a plant flowers, compared to its neighbour, the more seeds it will produce relative to growing alone. Similarly a meta-analysis showed higher fitness for earlier flowering plants (Munguía-Rosas et al. 2011) which have a higher likelihood to finish seed set within the season.

Besides the quantitative traits we investigated, neighbour functional group played an important role. Having a legume as a neighbour is beneficial for biomass production, but has no effect on seed number. The facilitative effect of legumes due to their symbiosis with nitrogen fixing bacteria (Tate 1995) is well-known, experimental studies showed that the presence of legumes increases productivity (Temperton et al. 2007). However, given that the effect on seed number was not relevant in our study, positive fitness consequences may actually be low. Contrastingly growing next to a grass has a negative effect on biomass and seed number, as also found by Bloor et al. (2008), potentially due to altered soil microbiome or nutrient depletion by a dominant grass neighbour. However, we note that given the unbalanced number of species in each functional group in our study (and particularly the low number of legumes), our results may also reflect species-specific effects.

Generally, the same traits determined species interactions for the comparisons between growing in intraspecific and interspecific pairs. Although all traits point in the same directions the effects are less strong than for comparing individuals in competition with individuals growing alone. Comparing our results to similar studies is difficult as few studies so far have investigated alien species interactions (Kuebbing et al. 2013). While our results on the importance of trait hierarchies and the particular traits relevant for alien interactions match recent findings on native-native or native-alien interactions, certain differences between alien species interactions and native species interactions are nevertheless likely. Alien species are not a random subsample but biased due to species with certain characteristics being more commonly introduced by humans (Maurel et al. 2016). Invasive species in particular are often highly competitive and more dominant in various aspects of performance than native species (van Kleunen et al. 2010). Thus, as aliens represent different trait variation compared to native species, they may be adapted to different abiotic conditions in their native range, and as most alien species did not coexist before introduction to the shared new range, the traits shaping species coexistence may differ for alien-alien interactions. As alien species can have severe impacts when invading new areas, we emphasise the importance of further investigating alien interactions, especially in the light of the high proportion of facilitative interactions found in our study. In fact, we here greatly extend previous work on alien species interactions by Sheppard (2019) who used a similar but smaller set of species and traits (as well as a shorter experimental duration, resulting in less data on seed production as the most important fitness measure for annual species). Importantly, while our results are generally in line with what was previously found by Sheppard (2019) (who however did not compare interspecific competition to growing alone), in the present study we can now distinguish between facilitation and competition, and add insight into the relevance of competition-induced trait plasticity, as unlike Sheppard (2019) we measured traits for every single individual (see section below).

Individual-level traits versus species-level trait averages

Regarding aim 3), using traits measured at an individual-level instead of species-level did not improve the fit of our trait hierarchy model. This result contradicts the general idea that a high degree of intraspecific variation (genetically or plastic) which can be explained with individual-based traits, captures niche differences better than by species-level trait averages (Kraft et al. 2014). For example Kraft et al. (2014) found individual-level trait hierarchies to explain competition among species in vernal pool habitats better than species-level trait averages. Conti et al. (2018) also underlined the importance of investigating traits at an individual-level, as they found that individuals could respond to competition by trait shifts, which increased their competitive ability and resulted in a positive feedback. Similarly, Carmona et al. (2019) concluded, individual trait expression can ameliorate the effect of lower trait hierarchical position. Bennett et al. (2016) found competition to induce changes in expressed traits and concluded that the environment where the sampled plant grows can affect the outcome of trait patterns. Such trait-environment effects may indeed be one reason that the species-level trait averages in our study performed well: while studies often use traits from databases (Kunstler et al. 2012, Carboni et al. 2015), which can come from various environments and populations, our species-level trait averages were grown and measured in the same abiotic conditions from the same populations as individuals in competition. The extent of intraspecific trait variation in our study depended on species, trait and competition treatment (Supplementary material Ch 2, Appendix 5, Figure A11-A14), with height showing the greatest variation within species, although notably for the grasses height hardly differed between competition treatments (Figure A12). The finding that for the target trait model the individual-level model performed better than the species-level trait average model is thus hardly surprising, given that this result appears to be highly driven by the effect of height, which generally is strongly correlated with biomass. Such circularity between explanatory and response variable, combined with the fact that it is often unrealistic to measure traits on each individual, may present arguments against using individual-level traits.

Conclusion

This study showed for a range of alien species in Germany that competition is the predominant type of interaction in pairwise settings, although in a quarter of cases aliens experienced facilitation by other aliens, which may have important management implications. We also showed that alien interactions were better explained by hierarchical traits related to competitive ability rather than by multivariate trait distance or phylogenetic relatedness, supporting recent criticism about the applicability of testing limiting similarity versus environmental filtering and the community phylogenetic approach. Contrasting recent suggestions, traits measured at individual-level did not necessarily explain species interaction better. With the continuing accumulation of alien species around the globe, such a better understanding of interactions among invaders is urgently needed. As a next step, interactions among co-occurring alien plants should be studied in a community context, thereby accounting for more direct and indirect biotic interactions in a more natural environment. Furthermore, as it is unlikely that several alien species arrive at the exact same moment in a certain location, studies should also investigate priority effects among alien species and not only between native and alien species.

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Chapter 3 Native and alien species suffer from late arrival, while negative effects of multiple alien species on natives vary

3.1. Abstract

Ongoing globalisation and climate change are causing plant species to invade new habitats and thereby alter biodiversity and ecosystem functioning. Since numbers of plant invasions continue to increase globally, it is crucial to investigate the effects of multiple co-occurring alien species on native communities. Furthermore, priority effects due to the earlier emergence of certain species affecting fitness of later arriving species can shape community structure and affect native species performance. We investigate in a common garden pot experiment the interactions among five alien-native species pairs. First we focus on the effect of growing with either one or two alien neighbour species on a native plant, second we alter the arrival time of the alien or native neighbour by three weeks. Generally, native species performance decreased when surrounded by two alien species compared to only one, although the magnitude of this effect varied depending on species, with one species even performing better with alien neighbours than in monoculture. Species performance greatly decreased when arriving second in the pot, for both native and alien species. In contrast, alien species tended to benefit more from arriving early. Given that we studied annual ruderal species, their potentially lower competitive ability might explain why we detected negative effects of late arrival. We highlight the need to further elucidate underlying mechanisms of small-scale invasion dynamics to achieve generalisations concerning the response of multiple alien and native plants given their species-specific differences in response to neighbour species and arrival time.

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3.2. Introduction

Invasive species pose a major threat to biodiversity and ecosystem functioning (Pyšek et al. 2020). Global analyses show an increase in the number of invasive species (Seebens et al. 2017, 2018), indicating that the negative impacts of invasive species already observed might become even more severe in the future. Increasing numbers of alien species also raises attention to the importance of examining interactions of co-occurring alien species and how they may affect natives, which was until recently an often neglected aspect of invasion studies (Kuebbing et al. 2013).

Invasive species are often found to be better competitors than native species (Vila and Weiner 2004) as the traits of invaders tend to lead to higher fitness than natives (Ordonez et al. 2010; van Kleunen et al. 2010). In comparisons to single alien-native pairs, the effect of more than one alien species being present has received far less attention. When multiple aliens are present, the following mechanisms might lead to stronger negative effects on a co-occurring native species: asymmetric competition meaning that the alien species compete less with each other than with the native species (Kuebbing and Nuñez 2016), or even facilitation among alien species (Flory and Bauer 2014) leading to higher negative impacts on the native species. The outcome of such an effect was termed 'invasional meltdown' by Simberloff and von Holle (1999).

Besides more favorable trait values related to superior competitive ability explaining alien species advantage over natives, earlier emergence (as commonly observed for alien species) may affect growth, development and reproduction of later arriving native plants, termed priority effects (Young et al. 2001). According to von Gillhaussen et al. (2014), priority effects occur if a species arriving first affects the fitness of a later arriving species. Such effects can strongly influence plant community structure and functioning, even after multiple seasons (Weidlich et al. 2018). The effect can be caused by a direct competitive advantage during the growing period or indirectly, either by reducing resource availability or by residues in the soil such as allelochemicals that interfere with the subsequently arriving species, so called soil legacies (Grman and Suding 2010). Priority effects whereby a later arriving species is affected by the earlier arriving one can not only occur if one species arrives later in a certain site than the other species, but also if all seeds are present already but species germinate at different times (Wainwright et al. 2012). Alien species tend to germinate earlier and more successfully than native species (Chrobock et al. 2011; Wainwright and Cleland 2013) as well as starting to grow earlier (Wilsey et al. 2011). Consequently, a prior establishment of alien species has been found to negatively impact native species (Dickson et al. 2012; Delory et al. 2019a). In contrast, it is possible that late-arriving alien species may actually suffer less from the earlier arrival of natives (Stuble and

Souza 2016), as alien species tend to have higher competitive ability and growth rates (Levine et al. 2003; van Kleunen et al. 2010). Furthermore, arriving late may even result in a positive priority effect, depending on whether the species is alien or native and to which functional group it belongs (Delory et al. 2019b). For instance, legume species fix nitrogen and enrich soil (Temperton et al. 2007), which may benefit subsequently arriving species.

In this study, we thus aim to find out how these two important aspects of invasion success, the effects of co-occurring aliens and priority effects, affect natives. To this end, we conducted a common garden pot experiment using five alien and five native species of different families. In the first part of the experiment, we investigate the effect of two alien species relative to only one alien species (neighbour experiment) on the growth of five different native species. We expect higher performance of native plants when having one alien neighbour species compared to having two alien neighbour species, as possible synergistic negative effects are not present. In the second part of our experiment, we manipulate the order of arrival of alien and native species to investigate potential priority effects among different alien-native species pairs (priority experiment). We hypothesize that species generally benefit from arriving early but suffer from arriving late (compared to arriving simultaneously). However, we expect the advantage of arriving first compared to simultaneously to be stronger for alien species which often have ruderal strategies and germinate and grow fast. This might either be due to their often stronger competitive ability helping them to compensate for the disadvantage, or because the priority effects of the native species arriving first are weaker.

3.3. Methods

Study Species

Our study included ten annual plant species consisting of five confamilial alien-native species pairs (Table 3.1). We chose the specific plant families to include three different functional groups (forbs: Amaranthaceae, Asteraceae, and Solanaceae; grasses: Poaceae; and legumes: Fabaceae) to cover a wide range of different species, but also these families are common in central Europe and comprise many alien and invasive species. For the alien-native species pairs, we specifically chose natives that are common. While all five native species are widespread in Germany (52 - 98% of area occupied in Germany), all alien species are known to be established neophytes (Jäger 2017) but some of them are not (yet) widespread (5 – 70% of area occupied in Germany, Table 3.1). As the German floristic literature (Footnote Table 3.1) is inconsistent in categorising the species in native or archaeophytes, we also considered archaeophytes (*Solanum nigrum, Setaria pumila, Chenopodium album*) as native species, given that their long history in central Europe makes it often difficult to distinguish between these categories (Scholz 2007). All species occur in similar habitats (as ruderal and segetal annuals), and hence they all can principally co-occur. Since such ruderal sites, commonly occurring near human disturbances, are often dominated by alien species and experience high alien seed input (Chytrý et al. 2008), this is an ideal study system to investigate the effect of several co-occurring alien neighbours and effects of shifted arrival times on species performance. The seeds for the experiment were obtained from our own collections of monocultures in previous experiments or from a botanical garden in our region (except for *Vicia villosa* from a commercial supplier, Table 3.1).

Table 3.1 *Status in Germany, seed origin, range size and year of first record for the five study species. Range size was measured as the percentage of occupied area in grid cells at the scale of 11 x 11 km obtained from the database FlorKart (https://doi.org/10.15468/wnkii7) from BfN and NetPhyD Netzwerk Phytodiversität Deutschlands e.V., deutschlandflora.de and ww.floraweb.de). Year of first record and status were obtained from Jäger (2017); www.floraweb.de.*

Family	Species	Status in Germany	Seed origin	Range size in %	Year of first record
Amaranthaceae	*Amaranthus retroflexus* L.	Alien	Previous experiment	69.5	1815
	Chenopodium album L.	Native	Botanical garden University of Konstanz	92.8	
Asteraceae	*Centaurea diffusa* Lam.	Alien	Previous experiment	4.8	1876
	Lapsana communis L.	Native	Previous experiment	98.0	
Fabaceae	*Vicia villosa* Roth s. l.	Alien	Revierberatung Wolmersdorf GmbH & Co. KG, Nindorf, Germany	66.4	1808
	Trifolium campestre Schreb.	Native	Botanical garden University of Konstanz	92.7	
Poaceae	*Panicum capillare* L.	Alien	Previous experiment	13.1	1890
	Setaria pumila (Poir.) Roem & Schult.	Native	Botanical garden University of Konstanz	52.3	
Solanaceae	*Nicandra physalodes* (L.) J. Gaertn.	Alien	Previous experiment	17.2	1782
	Solanum nigrum L.	Native	Previous experiment	84.5	

Experimental Design

We carried out a common garden experiment during the growing season in 2019 at a field station of the University of Hohenheim (48°42’45.2”N, 9°11’23.6”E) in Stuttgart, Germany (400 m a.s.l; mean annual temperature 2019: 10.6 °C; total annual precipitation 2019: 856.5 mm). The experiment consisted of two parts, one assessing the effect of having one or two alien neighbour species on a native target species (“neighbour experiment”) and the other investigating the effect of arriving earlier or later than an alien or native neighbour species (“priority experiment”). For the experiment we established a total of 360 pots in a completely randomized design. The pots were filled with 10 L of field soil (total soil carbon content 1.77% [0.66% inorganic, 1.11% organic]; total soil nitrogen content 0.059%; 85.5 kg NO3-N/ha and 0.45 kg NH4-N/ha; 205.4 mg P2O5/kg; soil texture of 9.7% clay, 71.9% sand, 18.4% silt) and equipped with a drip watering system.

For the two parts of the experiment, seeds of either one, two, or three of the study species were sown per pot, always using a total of 20 seeds per pot, with three replicates for each species or species combination (see below). We established simultaneously sown combinations of an alien and a native species (25 possible combinations with three replications resulting in 75 pots with ten seeds per species), serving as “one neighbour”-treatment for the neighbour experiment and as “simultaneous”-treatment for the priority experiment. To answer the first question on the effect of number of alien neighbour species on a native species, we added a treatment with two alien neighbour species, using all 35 possible species combinations with differing alien neighbour functional groups (i.e. forb-grass, forb-legume, legume-grass, with three replicates totalling 105 pots, using five seeds of each alien species and ten seeds of the native species, all sown simultaneously). Additionally, we used monoculture pots of each native target species (sown with 20 seeds, totalling 15 pots) as an intraspecific competition control treatment. To answer the second question on the priority effects of alien and native species, we used all 25 pairwise combinations from the “one neighbour” treatment, whereas we used two additional treatments, with each of the species either arriving first or second (totalling 150 pots). For this, we sowed the alien (or the native) species three weeks later than the native (or the alien). We sowed seeds of the neighbour experiment as well as the first arrivals of the priority experiment on 22 May 2019 and harvested all plants on 14 Sep 2019 (16 weeks later). Weeds emerging from the seedbank were removed before sowing and regularly during the experimental period.

Data Collection

We counted germinated seedlings six weeks after first sowing (three weeks after second sowing in the priority experiment, respectively) on 26 June 2019. To obtain a more natural population-level experiment and include all life-stages of the individuals we did not replace non-germinated seeds. To measure plant performance, we considered aboveground biomass as proxy for competitive ability and number of flowerheads as a measure of reproductive output and proxy for fitness for annual species (Gaudet and Keddy 1988; Jelbert et al. 2015). Further we observed flowering onset, as phenology is presumably acting independently of general resource strategies (Craine et al. 2012). We recorded flowering of each species per pot weekly throughout the experimental period. Before harvest, we counted the number of individuals as measure for final establishment and the total number of flower heads per pot. We then cut all plants at ground level and dried the aboveground biomass of each species per pot at 70 °C for 72 h.

Statistical Analysis

All statistical analyses were performed using the software R (version 4.0.4, R Core Team 2021). To assess the effect of neighbour treatments on target plants, for biomass and number of flowerheads the relative performance of individuals in interspecific competition (i.e. pots containing two or three species) was calculated as the log response ratio (lnRR, log(performance in mixture/performance in monoculture)) (Weigelt and Jolliffe 2003) which represents the ability to tolerate the respective interspecific treatment compared to growing in monoculture (intraspecific competition). For a better estimate of species overall performance, we used pot-level performance values (i.e. total aboveground biomass per species per pot and total number of flowerheads per species per pot) and divided the performance values in monoculture by two to correct for the double amount of seeds added in monoculture pots per species compared to interspecific treatments. Positive values of the lnRR represent better performance in the respective interspecific treatment while negative values represent better performance in monoculture. In the priority-experiment, we used the (log transformed) absolute performance measures to compare differences in biomass and number of flowerheads depending on arrival time. We further considered flowering onset and establishment success (after six weeks and at harvest) as absolute performance measures (only in interspecific treatments) for both parts of the experiment. To ensure that each pot actually represents the species combination it originally was assigned, we included only pots in the analysis where at least one individual of all species that were sown germinated (removal of 85 pots in the neighbour-experiment and 116 pots in the priority experiment for all performance measures except establishment). For the neighbour experiment, we only considered the native species as target, which is growing together with one or two alien species. For the priority experiment, both species in the pot were considered as target species (whereby we analysed the native and alien species separately). We performed a control analysis on average performance per individual in a pot and found no qualitative differences to the population-level (pot) measures which are reported hereafter.

For the neighbour experiment, our main interest was the effect of number of alien neighbour species (one vs two) and differences among target species. We analysed each normally distributed performance measure (lnRR biomass, lnRR flowerheads, flowering onset) with a linear model, while for initial and final establishment we used a generalized linear model with a quasibinomial distribution due to overdispersion. We included the number of alien neighbour species and target species identity as factors, as well as their interaction. To assess the importance of the explanatory factors, we performed backwards step-wise model simplification using F-tests (employing Type III Sum of Squares to account for the unbalanced dataset due to high mortality in some species-combinations) to obtain the minimum adequate model, containing only significant terms (whereby we retained marginally significant effects, i.e. $P<0.1$). Given that we found a negative effect of having two alien neighbour species (see below), we additionally tested if this effect might be due to more neighbour biomass being produced per pot when aliens grow in a two-species neighbour mixture compared to when growing with a single neighbour. To this end, we calculated the expected biomass in the two-neighbour treatment by adding for the two neighbour species the average of the respective one-neighbour treatment, divided by two (for each neighbour species combination separately). We then performed a one-tailed paired t-test across the seven alien neighbour species combinations comparing the mean biomass production by neighbours in two-neighbour treatment with the expected biomass.

For the priority experiment, our main interest was the order of arrival (first, second, simultaneous) and differences between alien and native targets. We analysed each normally distributed performance measure (log-transformed biomass, log-transformed flowerheads, flowering onset) with a linear mixed effects model within the R package lme4 (Bates et al. 2010), while for initial and final establishment we used a generalized linear model with a quasibinomial distribution due to overdispersion. We included the fixed effects of order of sowing and target species identity as well as their interactions. We used neighbour species identity as a random effect, except for the models for initial and final establishment due to singular fit issues. To assess the importance of fixed effects, we performed backwards step-wise model simplification using likelihood ratio tests (F-tests for establishment i.e. quasibinomial models) to obtain the minimum adequate model, containing only significant terms.

3.4. Results

Neighbour experiment

Native target species differed in response to growing with two alien neighbour species compared to only one in terms of lnRR biomass. While over all species the effect of two neighbours compared to one was negative (Table 3.2), we find *Chenopodium album* and *Lapsana communis* to strongly suffer from having two alien neighbour species (Figure 3.1) while *Solanum nigrum* shows a weak negative response. *Setaria pumila* and *Trifolium campestre* showed positive lnRR, indicating better performance in interspecific competition with alien neighbours than in intraspecific competition (although note that for *T. campestre*, performance varied greatly). Despite these apparent species-specific differences, the interaction between species identity and number of alien neighbour species was not significant for lnRR biomass. However, species identity as a main effect was significant for all response measures. To further investigate what caused potential negative effects of having two alien neighbour species on native biomass, we checked whether more neighbour biomass was produced when growing with two neighbours than expected from comparisons with the average biomass of the respective single neighbour treatments. The average biomass produced in the two neighbour treatment was higher than the expected average, which was significant across the seven alien neighbour species combinations (t=4.47; P=0.002; N=7), suggesting a more than additive effect on the target individuals when growing with two compared to one alien neighbour species (Supplementary material Ch 3, Figure A2).

Table 3.2 *Results of model selection in the neighbour experiment, showing the significant effects (including marginally significant effects i.e. P<0.1) retained in the minimum adequate models. For each model of the five performance measures (lnRR biomass, lnRR flowerheads, initial and final establishment, flowering onset), the significant effects are shown with their respective test statistic and adjusted R2 of the models. Adjusted R2 for initial and final establishment was obtained using a Kullback-Leibler-divergence-based method, extended to quasi models by Zhang et al.(2017) using the R package rsq (Zhang, 2021).*

Performance measure	Significant effects	Test statistic	R^2_{adj}
lnRR biomass	Species	F(4, 93)=7.5; *P*<0.001	0.21
	No. alien neighbour species	F(1, 96)=3.8; *P*=0.053	
lnRR flowerheads	Species	F(4, 95)=11.3; *P*<0.001	0.30
Initial establishment	Species	F(4, 176)=67.5; *P*<0.001	0.60
Final establishment	Species	F(4, 176)=53.8; *P*<0.001	0.55
Flowering onset	Species	F(4, 57)=8.0; *P*<0.001	0.32

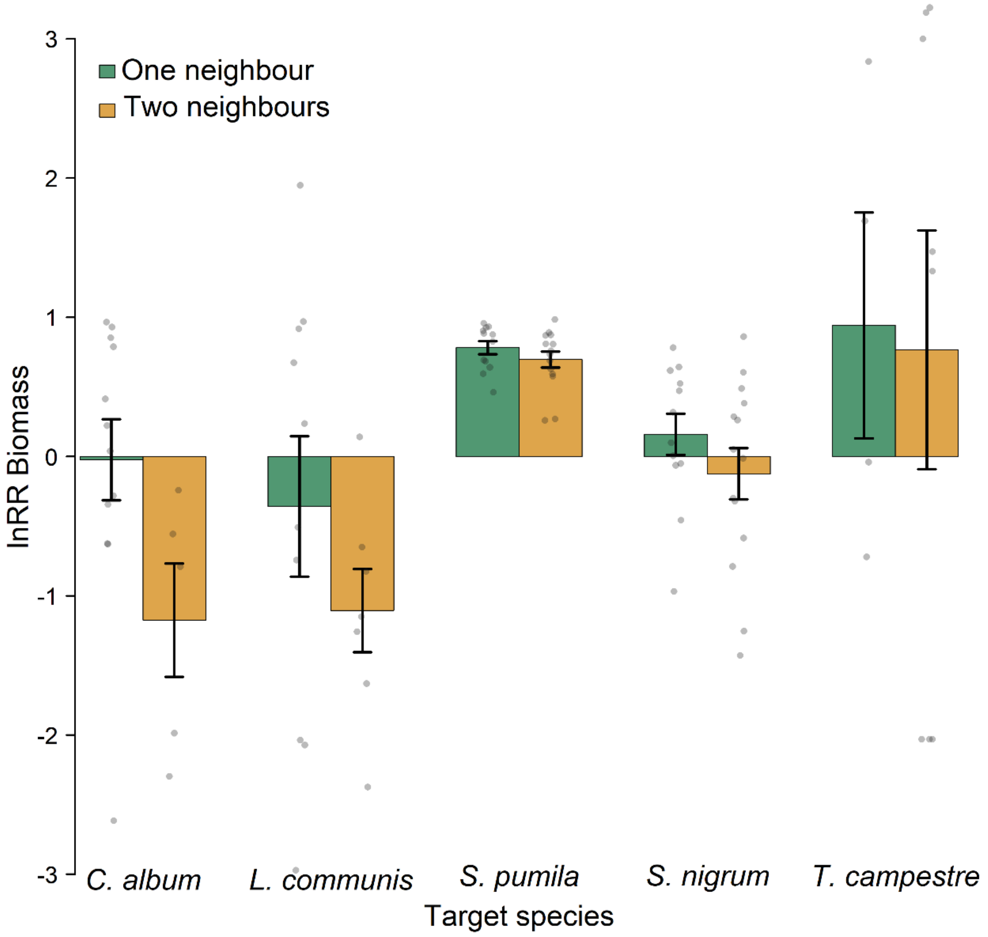

Figure 3.1 *The effect of one vs. two alien neighbour species treatment on the performance of five native target species measured as lnRR biomass. Bars are means ± SE; grey dots depict individual data points. For sample sizes see Appendix, Supplementary material Ch 3, Table A1.*

Priority experiment

In the second part of the experiment, looking at priority effects, the order of arrival and species identity was relevant for all performance measures for both alien and native target species (Table 3.3). Alien species generally performed better when arriving first (mean ± SE: 9.29 ± 1.35 gram of dry biomass; percent change compared to simultaneous arrival: + 45.6 %) compared to arriving simultaneously (6.38 ± 0.74 g) with a neighbour species, while when arriving second (3.27 ± 0.66 g; - 48.7 %) species performed worse than arriving simultaneously (Figure 3.2). Native species suffered on average even more from late arrival (2.45 ± 0.60 g; - 64.7 %) compared to simultaneous arrival (6.95 ± 0.75 g), whereas average differences to arriving first (6.00 ± 0.88 g; - 13.7 %) are small. Similarly, for alien species the number of flowerheads was on average similar between arriving first (mean ± SE: 7.56 ± 1.49 number of flowerheads; percent change compared to simultaneous arrival: - 1.7 %) and arriving simultaneously (7.69 ± 0.98) but smaller for arriving second (3.24 ± 0.86; - 57.9 %). In contrast, for native species we find an interaction of order of arrival and species identity (Table 3.3). While *S. pumila* showed no difference between early and simultaneous arrival, compared to producing less flowerheads when arriving late compared to simultaneous (Figure 3.2), *S. nigrum* benefits from arriving first compared to simultaneous, while we see no difference between late and simultaneous arrival. The other three native species (*C. album, C. diffusa* and *T. campestre*) had no apparent differences of order of arrival (note that for *T. campestre* no individuals of late arrival were recorded).

Table 3.3 *Results of model selection of the priority experiment, showing the significant effects (P<0.1) retained in the minimum adequate models. For each model of the five performance measures (biomass, flowerheads, initial and final establishment, flowering onset) the significant fixed effects are shown with their respective test statistic and R2 of the models (marginal and conditional for biomass, flowerheads and flowering, adjusted R2 for initial and final establishment). Adjusted R2 for initial and final establishment was obtained using a Kullback-Leibler-divergence-based method, extended to quasi models by Zhang et al. (2017) using the R package rsq (Zhang 2021).*

Performance measure	Status	Significant fixed terms	Test statistic	R^2 marginal/adjusted	R^2 conditional
Biomass	Alien	Order	χ^2(df=2, N=109)=30.1; *P*<0.001	0.34	0.42
		Species	χ^2(df=4, N=109)=29.4; *P*<0.001		
	Native	Order	χ^2(df=2, N=107)=36.6; *P*<0.001	0.51	0.56
		Species	χ^2(df=4, N=107)=61.5; *P*<0.001		
Flowerheads	Alien	Order	χ^2(df=2, N=109)=49.7; *P*<0.001	0.63	0.70
		Species	χ^2(df=4, N=109)=102.1; *P*<0.001		
	Native	Order*Species	χ^2(df=7, N=109)=14.8; *P*=0.040	0.57	0.60
Initial establishment	Alien	Order	F(2, 222)=107.7; *P*<0.001	0.61	
		Species	F(4, 218)=35.3; *P*<0.001		
	Native	Order	F(2, 222)=64.4; *P*<0.001	0.61	
		Species	F(4, 218)=56.9; *P*<0.001		
Final establishment	Alien	Order*Species	F(8, 216)=2.5; *P*=0.012	0.56	
	Native	Order*Species	F(8, 216)=3.2; *P*=0.002	0.62	
Flowering onset	Alien	Order*Species	χ^2(df=6, N=85)=24.1; *P*<0.001	0.81	0.81
	Native	Order*Species	χ^2(df=7, N=77)=24.1; *P*<0.001	0.34	0.43

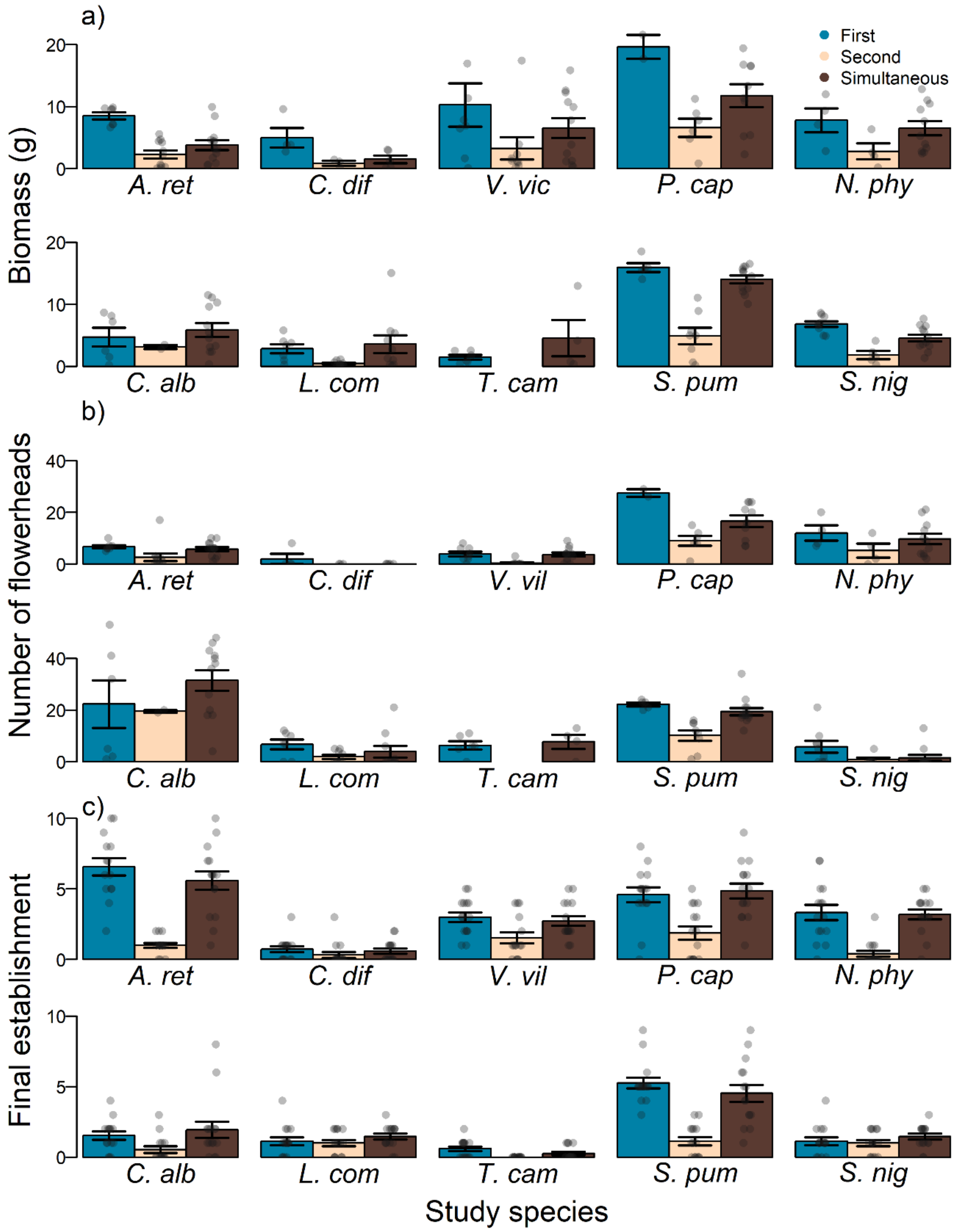

Figure 3.2 *The effects of order of arrival (arriving first, second or simultaneously with a neighbour) of the respective species on a) biomass, b) number of flowerheads, and c) final establishment (number of individuals established from 10 sown seeds). Top panels a) – c) depict the five alien target species and the bottom panels the confamilial native target species as listed in Table 3.1. Bars are means ± SE.*

Furthermore, for final establishment and flowering onset the effect of order of arrival also depended on species identity, for both alien and native species (i.e. significant interaction, Table 3.3). For most species we see no apparent difference between first and simultaneous arrival in number of established individuals at harvest, but lower establishment of second arrival compared to simultaneous arrival. However, *A. retroflexus* additionally showed higher establishment for first arrival compared to simultaneous, while *C. diffusa* and *L. communis* showed no effect of order of arrival (Figure 3.2). These trends were also identified for initial establishment (Appendix, Supplementary Material Ch 3, Figure A3), while species differed much in the effect of order of arrival on flowering onset where no clear trend could be observed (Figure A3). Interestingly, confamilial species pairs often performed similarly regardless of their status (Figure 3.2, Figure A3). Although we could not statistically test this, visual inspection showed only minor differences between alien and native species (Figure A4) in response to arriving early or late compared to simultaneous for most response variables. The main exception is for biomass production, whereby we find alien species (but not natives) to benefit from arriving first compared to simultaneous arrival.

3.5. Discussion

One alien neighbour vs two alien neighbour species

In the first part of our study, investigating the effects of number of alien neighbour species on a native plant, we found that native plants overall suffered more when having two alien neighbour species compared to one, producing relatively less biomass. A closer inspection of native target species showed a strong negative effect for the forbs *Chenopodium album* and *Lapsana communis* and a weak effect for *Solanum nigrum*. The strong negative effect multiple alien species can have on natives has been highlighted in a recent review (Kuebbing and Nuñez 2016) and involves a number of explanations. For instance, Flory and Bauer (2014) and Cushman et al. (2011) found experimental evidence for alien species facilitating the growth of other invasive species. Furthermore, across 190 alien species pairs, facilitation was observed in a quarter of cases (Ferenc and Sheppard 2020). Such facilitation between multiple alien species might then even lead to an accelerated negative effect on the native (‘invasional meltdown’, Simberloff and von Holle 1999). To date this hypothesis is not unequivocally supported.

Rather than accelerated effects of co-occurring alien species on natives, more commonly studies find additive effects of multiple alien species, such as Braga et al. (2020) find in aquatic ecosystems when comparing native communities with communities invaded by one, two or three alien species. Importantly, even if multiple aliens compete with rather than facilitate each other, effects of asymmetric competition may still lead to higher impacts on natives: if alien species have a higher negative effect on natives than on co-occurring aliens, this would lead to stronger negative effects on natives. This was also shown by a meta-analysis from Kuebbing and Nuñez (2016), who found alien species to have a nearly two times stronger negative effect on native species than on co-occurring other alien species. Another explanation why having two neighbour species compared to one is worse for the target might simply be due to a sampling effect, with two neighbours it is more likely that a good competitor is amongst the neighbours. There is also a higher chance of potentially greater overlap of niche space resulting in decreased performance. Which of the proposed mechanisms is at play is not possible to determine from our study design. However, we find more biomass per pot being produced by all neighbour plant combinations if two alien neighbour species are present compared to one and thus a more than additive negative effect of two species being present. Such increased neighbour biomass production may explain why some species experience strong negative effect of more than one neighbour species.

However, although the overall effect of the two neighbour treatment was significantly negative for biomass, we found strong species-specific effects for all response measures. The finding that particularly the native target species *C. album* and *L. communis* are strongly negatively affected (with strong negative log response ratios when growing with two alien neighbours) may be surprising given that these are highly successful species, abundant and widespread in Germany as well as particularly *C. album* being successful neophytes elsewhere (CABI 2021) . In contrast, we find positive log response ratios for the native target species *Setaria pumila* (a grass) and *Trifolium campestre* (a legume), and for both a minor difference between treatments (one vs two alien neighbours). Generally, in line with such positive log response ratios, stronger intraspecific competition is more commonly expected compared to interspecific competition (Adler et al. 2018) when neglecting the often-assumed negative effect of alien competitors. In a similar experimental set-up by Ferenc and Sheppard (2020) such stronger intraspecific competition (compared to interspecific competition by other aliens) was found for a few alien species (eg. *Panicum capillare*, *Bidens pilosa*, *Cosmos bipinnatus*, *Vicia villosa)*, usually considered to be dominant or legume species. As *S. pumila* is a neophyte in North America and by being a grass species considered to be more competitive and dominant in resource uptake (Bloor et al. 2008), this might explain stronger intra- compared to interspecific competition. For *T. campestre*, log response ratios varied greatly and sample sizes were low, suggesting that these results should not be over-interpreted.

The factors that are related to these species-specific differences in the strength of the various native-alien and alien-alien interactions with their consequences should be further investigated in future studies. One potentially interesting avenue for further studies that our data hints at is the disappearance of potential positive effects of legume neighbours when growing with multiple aliens.

Growing with only the legume *Vicia villosa* affected biomass and flowerheads of natives positively, whereas this effect diminished when a second alien neighbour was added (Supplementary material Ch 3, Figure A5). The positive effect in the one-neighbour treatment could be either simply because *V. villosa* is a weak competitor (although, in a previous study we found it to be one of the dominant competitors, (Ferenc and Sheppard 2020)), or because as a nitrogen-fixing legume (Tate 1995) it may facilitate co-occurring species (Temperton et al. 2007). There are two possible explanations why this positive effect disappears when growing together with a second alien species. First, the second alien species might have had strong negative effects on the alien legume, so that the positive effect of nitrogen-fixation on the native plant was much reduced. In line with this explanation, we found that the alien legumes produced less biomass in the two-neighbour treatment than what would be expected from the single-neighbour treatment (Supplementary material Ch 2, Figure A6). The second possible explanation is similar to aforementioned asymmetric competition effects (Kuebbing and Nuñez 2016), whereby the second alien neighbour species benefits much more from the nitrogen-fixation, or from exploiting a weak competitor (accordingly, biomass of 3 of the 4 non-legume alien neighbour species was considerably higher in the two-neighbour treatment when growing with the legume than expected from the single-neighbour treatments, see Figure A7). Given that we only had one legume in this study we cannot infer whether these are species-specific or functional group effects, however we suggest that this interesting pattern should be more thoroughly investigated in future studies shedding light on the role of (legume) facilitation in multi-species invasion scenarios.

Arriving first, second or simultaneously

Concerning the second part of our study, how the order of arrival in the pot affects plant performance, we find a strong disadvantage of arriving second compared to simultaneously. Regardless of the short difference of only three weeks, the species arriving second produced less biomass and frequently less flowerheads. Also, fewer individuals germinated and were present at harvest when sown later than the neighbour. Differences between arriving first versus simultaneously with a neighbour were however less pronounced, and beneficial effects of early arrival mostly observed in alien species. Similarly Stuble and Souza (2016) showed a strong disadvantage of arriving later and only a small advantage of arriving early, while Dickson et al. (2012) and Goodale and Wilsey (2018) found strong advantages of arriving early especially for alien species. This suggests, along with Körner et al. (2008), who investigated priority effects of native species, altered arrival times can greatly affect species development and ultimately may alter community composition in subsequent years. Notably we used annual ruderal plant species in our experiment, in contrast to Dickson et al. (2012) or Goodale and Wilsey (2018) who used perennial species. Experiments commonly find annual ruderal species to be less competitive (Grime 1977) and hence such species might generally suffer more if a competitor is already established. Accordingly, we generally found a stronger disadvantage of arriving late (difference between late sown species and simultaneous treatment) compared to an advantage of arriving early (difference between early and simultaneously sown species), at least for natives.

One possible explanation for the commonly observed lack of advantage of arriving early might be favourable abiotic conditions in the experiment. Abiotic conditions such as nutrient availability (Kardol et al. 2013) or water depth in vernal pools (Collinge and Ray 2009) have been shown to pose a large effect on the magnitude of priority effects. However, we note that the greater magnitude of effects for arriving late compared to arriving first may also be due to the fact that species arriving later experienced a shorter experimental duration than early sown species as all species were harvested at the same time. Ideally, one might have accounted for this by using a late sown simultaneous control, as some previous studies (Delory et al. 2019a) did. Nevertheless, we expect the short difference of three weeks between sowing events to have a small effect in our specific study system, as we used annual ruderal species which finish their life cycle within a short time span. While accounting for the difference in experimental duration would avoid confounding effects with the treatment of arrival time, with our approach we intended to mimic a more natural scenario: the end of the season will be the same for all individuals present in a certain site, regardless of differences in arrival time, similarly to the approach used by Dickson et al (2012). Additionally, our main interest was the response of species with different invasion status, whereby aliens and natives were equally influenced by the shorter experimental duration.

Although we could not directly statistically compare priority effects of aliens versus natives, responses of both status groups overall appear to be similar. However, in terms of biomass production, more alien compared to native species benefitted from arriving earlier compared to simultaneous (Figure A4). This is in line with previous studies finding invasive species benefitted more from arriving earlier (Dickson et al. 2012; Goodale and Wilsey 2018). On the other hand, both alien and native species suffered from priority effects by an early arrived species, but the magnitude of this effect was on average even stronger for natives. Principally, differences might arise due to differing magnitudes of priority effects of the early arriving plant on the later arriving ones, or by a higher tolerance of such effects by the later arriving plant, as a previous study found (Stuble and Souza 2016). Notably, published studies comparing aliens and natives may be biased to some degree as studies often use common alien (invasive) species that are well-known for their strong impacts (Hulme et al. 2013; but see Stuble and Souza 2016), but also mostly use rare natives to investigate effects by alien species (Vilá and Weiner 2004). Zhang and van Kleunen (2019) compared the competitive ability of common and rare aliens versus common and rare natives and could not find an overall advantage of alien species. They showed that common natives would outcompete rare natives while common natives did not differ from common aliens, a result also in line with a previous study by Dawson et al. (2012). As we used common native species and their alien counterparts, this finding of commonness vs rarity being more relevant than alien vs native status might explain why we did not find great differences in priority effects between alien and native species in our study. Nevertheless, we find species-specific differences in magnitude of response to timing of arrival for some performance measures, and a tendency of related species to respond similarly to the treatment. While most species suffer from arriving second compared to simultaneous, only some species benefit from arriving early. The aliens *A. retroflexus*, *C. diffusa* and *P. capillare* as well as the native *S. nigrum* perform better when arriving first compared to simultaneously, which could hint at lower competitive ability for these species. However, even though *P. capillare* was greatly negatively affected by arriving second, it still produced the most biomass out of all species. In contrast, *C. diffusa* generally had a low rate of establishment, which may also explain why it has the lowest range size of all alien species used in this experiment.

Conclusions

This study investigated two important yet not fully understood aspects of alien plant invasion. We found exacerbated negative effects of alien plants on at least some native species when growing together with two alien plant species compared to only one alien neighbour species, highlighting the importance of taking multiple invasions into account for management measures. The species-specific differences we found suggest interesting avenues for further research. Of particular importance could be the finding that the species showing strong responses were forbs that may suffer more from multiple alien species presence, while beneficial facilitative effects of certain neighbour species can disappear in the presence of multiple aliens. Although aliens more often benefitted from arriving first compared to simultaneous, we observed a general disadvantage of arriving second compared to simultaneous in a pot for both aliens and natives. Future studies might confirm whether these comparatively small differences between aliens and natives are indeed due to using common native species rather than rare species as often in such experiments. Our research provides insight into small-scale dynamics of invasion by co-occurring alien species from different families and different times of arrival. We observed relevant patterns affecting species interactions and proposed possible mechanisms behind them even in the comparably small set of species we investigated. Therefore, we expect future studies with a larger number of species to further enlighten the role of species-specific interactions or possible functional group effects such as by nitrogen-fixing legumes in sites invaded by multiple aliens and to achieve generalisations concerning the response of multiple alien and native plants to arrival time.

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Chapter 4 Legume effects in a native community invaded by alien Asteraceae in a multi-species comparison

4.1. Abstract

Facilitation has been a long-neglected type of interaction but received more attention recently. Legumes are commonly involved in facilitative interactions due to their nitrogen fixation. Facilitative interactions are so far underappreciated yet potentially important for biological invasions, especially given increasing numbers of alien species. In a common garden experiment using 30 annual Asteraceae species (neophytes, archaeophytes, plus some natives), grown in communities with or without legume presence, we measured functional traits and fitness in focal Asteraceae, as well as nitrogen characteristics of Asteraceae and two native community phytometer species. We investigated how legume presence affects relationships between trait and nitrogen concentration and Asteraceae fitness; and whether mechanisms of facilitation in legume presence and its effects on aboveground performance differ among native phytometer, neophyte and archaeophyte Asteraceae using the $\delta^{15}N$ natural abundance method. Lower specific leaf area was associated with higher aboveground biomass and seed production, with a stronger effect in legume absence. Nitrogen concentration had a positive relationship with biomass, but did not generally increase seed production. Our results hint at N facilitation for the native grass phytometer *Festuca rupicola* when growing in legume presence, whereas the forb *Potentilla argentea* and 27 alien Asteraceae species did not indicate facilitative effects. Intriguingly, direct legume facilitation in native phytometer species was only detected when growing with archaeophytes neighbours, not with neophytes. This hints at varied mechanisms of competition for nitrogen between natives and alien species of different residence time and deepens the understanding of altered facilitative leguminous effects in alien species presence.

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4.2. Introduction

Invasive species can have major negative effects on ecosystems and communities and thereby pose substantial threats to biodiversity (Vilà et al. 2011). Among the most important drivers for alien species introductions is the increasing global connectivity causing large-scale trade and transport (Hulme 2009), which results in deliberate and accidental spread of organisms (Hulme et al. 2008). The number of alien species is predicted to continue to increase and pose even more severe impacts in the future (Pyšek et al. 2020).

The negative effects of alien plant species on native communities are considered to be related to altered soil structure, microbial community and nutrition such as carbon and nitrogen (N) availability in alien species presence and thereby affecting community composition and performance (Evans et al. 2001; Ehrenfeld 2003; Miki 2012; Zhang et al. 2021). Furthermore, a meta-analysis by van Kleunen et al. (2010) showed that invasive species are more commonly associated with increased performance-related measures such as size and fitness, as well as an enhanced nutrient uptake of alien species (Dassonville et al. 2008). N availability is thus a crucial factor for plant invasions, and may be increased by eutrophication or legume presence. Legume presence improves N availability due to their ability to fix atmospheric N due to symbiosis with root bacteria called rhizobia (Burris and Roberts 1993), which can have direct and indirect effects on neighbouring plants. Since legumes usually depend much less on soil N than non-leguminous species, they face less competition with neighbouring plants and can even have facilitative effects on these (Temperton et al. 2007). Direct facilitation occurs when non-legumes directly benefit from N transferred via mycorrhiza from legumes (Frey and Schüepp 1993; Montesinos-Navarro et al. 2016). In contrast, we here refer to indirect facilitative effects when neighbouring species have additional soil N available as legumes use atmospheric N instead (Bessler et al. 2012). Additionally, even more N can become available in the soil when above- or belowground parts of legumes are decomposed and mineralized or via root exudation (Tomm et al. 1995; Høgh-Jensen and Schjoerring 2001; Paynel et al. 2001; Li et al. 2016). Generally, effects of soil N availability on plant performance have been studied with different experimental approaches. Previous studies artificially added N to the soil in the field (Wilson and Tilman 1991; Huang et al. 2016; Yelenik et al. 2017) or in pot experiments (Broadbent et al. 2018) to investigate the response of species performance or outcome of species interactions to elevated N availability. Results commonly show species-specific effects. Furthermore, functional traits (Yelenik et al. 2017) or competitive ability (Broadbent et al. 2018) explained differences between alien and native species performance in response to N availability. While these studies simulated an abiotic addition of N to ecosystems and address the effects of eutrophication on species interactions and performance, it is difficult to draw conclusions for the context of altered N availability due to N fixation by legumes, because they neglect the biotic component of increased N availability such as potentially synchronous competition and facilitation due to legume presence.

Previous studies with legumes showed the species identity of the legume as well as the non-legume species plays a role in the magnitude of facilitation observed (Høgh-Jensen and Schjoerring 2000; Spehn et al. 2002; Montesinos-Navarro et al. 2017), mainly due to effectiveness of N fixation of the legume. However, such species-specific effects have rarely been investigated regarding the relative importance of direct and indirect N facilitation mechanisms (but see Temperton et al. 2007) which have been termed “N sharing” (direct transfer of N from the legume to non-legume) or “N sparing” (taking up soil N that is available since the legume is fixing N and does not depend on soil N). To qualitatively distinguish between direct and indirect facilitation, the natural abundance method can be used. The δ^{15}N signal of N-fixing legumes is closer to the atmospheric δ^{15}N and therefore δ^{15}N signals of species relying on direct facilitation are more similar to atmospheric δ^{15}N, too (Högberg 1997). Since biotic and abiotic factors of the study system can influence δ^{15}N, one cannot quantify fixed N from δ^{15}N alone. However, δ^{15}N can be used to qualitatively estimate the transfer of legume-fixed N to non-fixing plants (Høgh-Jensen and Schjoerring 1994).

Mechanisms of facilitation may be particularly important regarding invasion of grasslands. Among the main limiting resources in temperate grasslands is N (Vitousek and Howarth 1991; LeBauer and Treseder 2008). Legumes present a key functional group in these systems as they can fix atmospheric N and improve N availability (Mulder et al. 2002). This has been found to influence N dynamics in ecosystems and increase community productivity (Spehn et al. 2002; Hille Ris Lambers et al. 2004; Palmborg et al. 2005) and diversity (Loreau and Hector 2001; Spehn et al. 2005; Temperton et al. 2007). Interactions involving legume presence could facilitate the establishment of native species in a native community (Mwangi et al. 2007).

Even though facilitative interactions in the context of alien species only recently gained more attention, previous work raised the concern that alien species establishment can have a great impact on competition and N dynamics, as seen in the example of an invasive legume species (*Myrica faba*) in Hawai’i (Vitousek and Walker 1989). Conversely, facilitative effects of natives may also promote alien species establishment (Maron and Connors 1996; Cavieres et al. 2005; Lucero et al. 2019; Cavieres 2021). However, experimental studies have also found that legume presence might buffer negative effects of alien species and decrease invasibility of communities (Eisenhauer and Scheu 2008). One explanation for this could be higher functional diversity given legumes presence, as diversity is well known to improve community resistance against alien species invasion (Elton 1958; Tilman 2004; Tilman et al. 2014). Facilitative interactions are hypothesized to be more important in abiotically stressful and species-poor habitats, described as the stress gradient hypothesis (Bertness and Callaway 1994), which was recently extended by Cavieres (2021) to the context of community invasibility (increased invasibility in stressful habitats). However, facilitative effects of native on alien species have also been described in mild environments (McIntire and Fajardo 2014). Nevertheless, few studies investigated in a community context whether a) alien invaders benefit from facilitation by native legumes and b) the presence of alien invaders alters facilitative interactions between native legumes and native non-leguminous species.

To investigate underlying drivers of species interactions it is important to use multi-species approaches to make generalized statements (van Kleunen et al. 2014). Since plant species can be very diverse it became common to characterise plants based on functional traits which can be morphological, physiological or phenological characteristics that influence fitness (Violle et al. 2007). These traits are subject to selection and environmental filtering by responding to biotic and abiotic factors that affect a plant’s fitness components (Lavorel and Garnier 2002; Brendel et al. 2021). Further, traits can be used to describe fundamental trade-offs between ecological strategies of species as they can affect resource uptake and investment of plants (Lavorel and Garnier 2002). Traits have been shown to influence ecosystem properties such as diversity by affecting interactions among species and ultimately species compositions (van der Plas et al. 2020). This in turn can affect community stability and susceptibility to alien species invasion (Hooper et al. 2005).

Alien species in Europe are commonly divided based on residence time into two status groups: neophytes (i.e. introduced to Europe after 1492) and archaeophytes (introduced since the advent of agriculture in the Neolithic). These groups may differ in their response to legumes and effects on communities as they often occur in different habitats (Pyšek et al. 2005; Chytrý et al. 2008). Neophytes are usually adapted to highly disturbed but nutrient-rich habitats. Archaeophytes were mainly introduced from Southern Europe and the Mediterranean Basin, before the present high levels of eutrophication and might thereby be better adapted to nutrient-poor habitats. Given the differing habitats one could expect neophytes to require more N and therefore strongly compete for it, which leads to less competitive species having less N available. Contrastingly, archaeophytes are adapted to nutrient-poor habitats and would therefore compete less strongly for N. In legume presence in general more N should be available; therefore, neophytes are expected to take up this additional N, while archaeophytes due to their lower requirement of N, may enable a native community to benefit from such additional N by legume N fixation. Furthermore, competition, but also facilitation, can be subject to co-evolutionary mechanisms (Bronstein 2009) that only build up over time and therefore might lead to differences among alien species of variable residence times. The context of alien species invasion has so far often been neglected when studying mechanisms of legume facilitation, especially regarding potentially differing effects of alien species depending on their characteristics and history of introduction, except in the context of N fixing legume trees on Hawai'i (Vitousek and Walker 1989).

Here, we present the results of a multi-species common garden experiment designed to further elucidate the effects of legumes on alien species invasion, as well as considering the role of functional traits and alien species status for responses to legumes, focusing on aboveground effects. We pose four research questions: (i) Do alien species reach higher fitness in communities with a legume, and does this differ depending on alien status? (ii) Does the relationship between functional traits and alien species fitness depend on the presence of a legume? (iii) Do plant N characteristics determine fitness of alien species? (iv) Is there evidence of direct or indirect legume facilitation and do these mechanisms differ between neophytes, archaeophytes and native community species?

4.3. Methods

Study system

We included 30 annual Asteraceae plant species occurring in Germany in the experiment (Table 4.1). To compare differences in potential N facilitation across a wide range of Asteraceae, we used 3 native, 13 archaeophyte and 14 neophyte species (the latter including 6 casual and 8 established neophytes). We included a few native species in this species set as well to test if alien responses and performance are fundamentally different, but only used a small number of natives as there are not many annual native Asteraceae in Germany and our main focus was on alien species. All species belong to the species-rich Asteraceae family that holds a high number of established alien species (Hanspach et al. 2008). We selected annual species which enabled us to measure lifetime reproductive output (as total seed weight produced per plant) within the experimental season and serves as the best measure for fitness. We selected species from ruderal or segetal habitats, using seeds mostly collected from wild populations in the south-west of Germany (close to the experimental field site) in 2015 and partly complemented by seeds from botanical gardens (Table 4.1).

Table 4.1 *Overview of the focal Asteraceae species.*

Species	Status in Germany	Seed origin	Ellenberg N value	Included for question
Anthemis arvensis L.	Archaeophyte	Wild populations; Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany	6	1,2,3,4
Bidens ferulifolia L.	Neophyte	Botanical garden University of Dresden; Botanical garden University of Hohenheim	NA	1,2,3,4
Bidens pilosa L.	Neophyte	Botanical garden University of Dresden; Botanical garden University of Hohenheim; Botanical garden University of Konstanz	NA	1,2,3,4
Calendula arvensis M.Bieb.	Archaeophyte	Wild populations	6	1,2,3,4
Calendula officinalis L.	Archaeophyte	Wild populations	NA	1,2,3,4
Callistephus chinensis (L.) Nees	Neophyte	Wild population; Botanical garden University of Dresden; Botanical garden University of Tübingen	3[1]	1,2,3,4
Centaurea diffusa Lam.	Neophyte	Wild populations	3	1,2,3,4
Cosmos bipinnatus Cav.	Neophyte	Wild populations	NA	1,2,3,4
Crepis capillaris (L.) Wallr.	Archaeophyte	Wild populations	4	1,2,3,4
Cyanus segetum Hill.	Archaeophyte	Wild population	NA	1,2
Dittrichia graveolens (L.) Greuter	Neophyte	Wild populations	5	1,2,3,4
Erigeron annuus (L.) Pers	Neophyte	Wild population	8	1,2,3,4
Erigeron canadensis L.	Neophyte	Wild populations	7[1]	1,2,3,4
Erigeron sumatrensis Retz.	Neophyte	Wild population	7[1]	1,2,3,4
Galinsoga quadriradiata Ruiz & Pav.	Neophyte	Wild populations	8	1,2,3,4
Glebionis coronaria (L.) Cass. Ex Spach	Neophyte	Wild populations	NA	1,2,3,4
Glebionis segetum (L.) Fourr.	Archaeophyte	Wild populations; Botanical garden University of Dresden	5	1,2
Guizotia abyssinica (L.f.) Cass.	Neophyte	Botanical garden University of Dresden; Botanical garden University of Bonn	NA	1,2,3,4
Helminthoteca echioides (L.) Holub	Archaeophyte	Wild populations	6	1,2,3,4
Lactuca serriola L.	Archaeophyte	Wild populations	4	1,2,3,4
Lapsana communis L.	Native	Wild populations	7	1,2,3
Matricaria chamomilla L.	Archaeophyte	Wild populations	5	1,2
Matricaria discoidea DC.	Neophyte	Wild populations	8[1]	1,2
Pulicaria vulgaris Gaertn.	Native	Wild populations	7	1,2,3
Rudbeckia hirta L.	Neophyte	Botanical garden University of Dresden; Botanical garden University of Hohenheim	5	1,2,3,4
Senecio viscosus L.	Native	Wild populations	4	1,2,3
Senecio vulgaris L.	Archaeophyte	Wild populations	8	1,2
Sonchus asper (L.) Hill	Archaeophyte	Wild populations	7	1,2,3,4
Sonchus oleraceus (L.) L.	Archaeophyte	Wild populations	8	1,2,3,4
Tripleurospermum inodorum (L.) Sch.Bip.	Archaeophyte	Wild populations	6	1,2,3,4

Ellenberg values indicate nitrogen availability in common habitats of the respective species, whereby low values indicate typically nutrient poor habitats and high values nitrogen-rich habitats, ranging from 1-9 (with values marked with 1 taken from Domina et al., 2018; Ellenberg & Leuschner, 2010). Due to seedling mortality *Sonchus asper* occurred in only one pot and *Cyanus segetum* in no pot in the leguminous community.

Experimental set-up

The experiment was established in March 2016 on an experimental field site at the University of Hohenheim, Germany (48°43’02.1”N 9°11’03.1”E, 400 m a.s.l; mean annual precipitation: 698 mm; mean annual temperature: 8.8 °C). The experimental design follows Brendel et al. (2021) who studied monocultures of a larger set of Asteraceae species within the same set-up. We filled 50L pots (50 cm upper diameter, 38 cm lower diameter, 40 cm height, 0.159 m^2 soil surface area) with local soil of sandy-loamy texture (70 % sand, 14 % clay, and 16 % silt) and low nutrient content (0.07 % nitrogen and 1.79 % carbon). Before filling the pots with soil, we added a layer of expanding clay to improve drainage. All pots were sufficiently watered throughout the experiment with a drip watering system. We established 120 community pots, 30 per community with two replicates per focal species (with measurements based on 12 individuals, see below) of which three pots could not be used in the end due to focal species seedling mortality. As our aim is to make generalized statements, we used a multi-species approach and have therefore less replicates per species, but more different species, thus increasing the accuracy of estimating performance in the two treatment groups (van Kleunen et al. 2014; Kreyling et al. 2018) and ultimately to answer our research questions However, this implies that we can only draw limited conclusions regarding any species-specific results.

We followed fitness of the 30 focal species in two different communities: a non-leguminous community consisting of Central European grassland species and a leguminous community consisting of the same grassland species but complemented with a dominant legume. To establish these communities, we sowed a seed mixture of 12 native perennial species (plus the legume in the leguminous community) at the end of April 2016 at an overall density of 3 g/m^2. The communities were sown including four grasses and eight forbs, of which three grasses and seven forbs successfully established (Supplementary information, Table A1). All species are considered as mesic to dry calcareous grassland species (*Festuco-Brometea*) occurring in semi-open and ruderal habitats (Ellenberg 1988), similar to our focal species. For the leguminous community we added *Medicago lupulina* to the existing seed mixture. We adjusted the number of seeds per community species according to their individual seed mass to make a trade-off between constant seed mass vs. seed number across species (see detailed information on species composition in Supplementary material Ch 4, Table A1).

Two weeks after the community species were sown in the pots, the 30 focal species were sown in germination trays in greenhouses next to the common garden facility. We transplanted focal species into the established communities by late June 2016 (6 weeks after seeds were sown in germination trays and 8 weeks after community-mixtures were sown in pots). Six seedlings of a species were used for each of two replicate pots for all species-community combinations, and planted in spatially explicit positions (Supplementary material Ch 4, Figure A1). Thereby we cover intraspecific variation both due to chance as well as due to potentially varying distances to legume neighbours. Moreover, to cover a wider range of intraspecific variation, we aimed at including three different populations per species, each population represented by two individuals per pot. We note that due to low germination ability, we could only transplant five individuals for *Cyanus segetum* per pot. Dead individuals were replaced until the second week after transplanting and we recorded initial height of the seedlings and initial community cover (with 5 % accuracy).

To assess fitness of the Asteraceae in the communities, we quantified aboveground biomass and total seed weight for all focal individuals. Twenty weeks after transplanting the Asteraceae (October 2016), we harvested, dried (at 70°C for 72 hours), and weighed the aboveground biomass of all focal individuals separately. To quantify total seed weight, we counted the number of capitula of each individual before harvest. To then obtain the total seed weight, we used data from Brendel et al. (2021) who sampled ten intact capitula of each species growing in monoculture pots in the same location and year to measure the average number of seeds per capitula. We extrapolated this to the total number of capitula per individual and used the data on individual seed mass (see below) to obtain total seed weight.

Functional trait measurements

The fitness of focal native and alien species in a native community (with or without legume presence) was related to their functional trait values. In particular, we measured plant height, specific leaf area (SLA), and seed mass, which represent the key axes of plant ecological strategies (Westoby 1998).

Plant height is associated with competitive strength for light (Westoby 1998; Pérez-Harguindeguy et al. 2013), whereas SLA is positively correlated with relative growth rate, describing different resource use strategies of water and N. Higher SLA values indicate investment in growth and rapid resource acquisition (exploitative strategy), while lower SLA values point at investments in leaf storage tissues and more conservative resource use (conservative strategy, Pérez-Harguindeguy et al. 2013).

Seed mass forms an inherent part of reproductive effort (Pérez-Harguindeguy et al. 2013) and shows a reciprocal relationship with seed output. Additionally, while seedlings of large-seeded species tend to have a higher survival (Westoby 1998), small-seeded species have an advantage in increased fecundity (Henery and Westoby 2001). All in all, these three traits provide information about the overlap between resource use strategies of different species and their relative competitive ability in terms of competitive response and biotic resistance (Conti et al. 2018; Castillo et al. 2021).

To measure functional traits of the focal Asteraceae species, we established two additional monoculture pots of each focal species (each population represented by two individuals per pot). Trait measurements follow the standard protocols of Pérez-Harguindeguy et al. (2013) and are described in Brendel et al. (2021), whereby the Asteraceae trait data is available from the TRY database (Kattge et al. 2020). To measure traits of the community species, we established two additional monocultures in 15L pots (33 cm upper diameter, 26 cm lower diameter, 24.5 cm height, 0.08 m^2 soil surface area). In these pots, we initially sowed a sufficient amount of seeds and then thinned out the seedlings to three individuals (which reflects the same number of individuals per unit soil surface area as for the Asteraceae monoculture transplants).

Overall, an ANOVA followed by TukeyHSD tests showed that although neophytes generally had slightly lower SLA than archaeophytes, neither neophytes nor archaeophytes differed from natives. Height and seed mass overlap between status groups (Supplementary material Ch 4, Figure A2).

Stable isotope analyses

To answer the third question on the relative importance of direct and indirect N facilitation for species in the experiment, we conducted stable isotope analyses of leaves of the focal Asteraceae and *M. lupulina*. Since the response to N-facilitation can be very species-specific and different between forbs and grasses (Temperton et al. 2007), we further used the two most abundant community species *Potentilla argentea* (forb) and *Festuca rupicola* (grass) as phytometer species. The phytometer method enables to use standardised plants in the community as an integrated measure of effects of the community, and has often been used to investigate the effects of habitats on plant communities and species, and is encouraged to be used more commonly (Clements and Goldsmith 1924; Dietrich et al. 2013). Due to practical reasons and logistical constraints, we analysed bulk samples at pot level of a random subset of pots (focal individuals were sampled from 90 pots, while data of the legume *Medicago lupulina*, and the community phytometer species *Potentilla argentea* and *Festuca rupicola* was collected from 15, 35 and 27 pots respectively). We used six harvested leaves from the focal and phytometer individuals and, if present, the legume species. Leaf samples were dried at 70°C for 72 hours and ground to fine powder. About 2 mg per sample were weighed into tin capsules for solids to determine N concentration with an elemental analyzer (Euro AE 3000 via CAP 40 autosampler, HEKAtech GmbH, Wegberg, Germany) and $\delta^{15}N$ (‰) with a mass spectrometer (Delta plus CP with a ConfloIV interface, Thermo Finnigan MAT, Bremen, Germany). Glutamin was used as the internal standard for $\delta^{15}N$ analyses. The isotopic composition is expressed in relation to atmospheric N_2 using USGS40 as secondary standard (Reston Stable Isotope Laboratory, Reston, Virginia, USA):

$$\delta 15\mathrm{N} = \frac{R_{sample}}{R_{atmosphere} - 1} * 1000$$

with R representing the $^{15}N/^{14}N$ ratio. This method allows to approximately distinguish between actively N-fixing plants and non-N-fixing plants. The $\delta^{15}N$ signal of N-fixing legumes is closer to the atmospheric $\delta^{15}N$ as microbes discriminate against heavy isotopes during fractionation of N. Therefore compounds of the heavier isotope are enriched in the soil (Högberg 1997), and species depending on soil N take up more of the heavy isotope (i.e. have higher $\delta^{15}N$). Since biotic and abiotic factors of the study system such as rooting depth, mycorrhizal symbioses or the type of soil-N used can influence $\delta^{15}N$, one cannot quantify fixed N from $\delta^{15}N$ alone. However, $\delta^{15}N$ can be used to estimate the transfer of legume-fixed N to non-fixing plants (Høgh-Jensen and Schjoerring 1994). Together with the change in N concentration of the plants we can derive qualitative changes caused by legume presence in the N cycle of the respective species. We focused on comparing values between different treatments to observe relative qualitative differences, similar to the approach taken by Temperton et al (2007). We point out that using the 15N natural abundance methods to quantify relative importance of direct and indirect legume facilitation has limitations (Handley and Scrimgeour 1997; Peoples et al. 2015), which we addressed insofar possible in our experimental design as well as in careful interpretation of the results. The absolute amount of transferred N cannot be quantified accurately with this method (Gehring and Vlek 2004). Due to the lack of labelling transferred N and measures of soil N compositions, it cannot be proven that the N a plant takes up really originates from legumes or other sources, neither how much of the changes in $\delta^{15}N$ is possibly due to fractionation (Peoples et al. 2015). However, by using a common garden pot experiment we controlled for differences in soil conditions between pots as the soil was sieved and mixed. Regarding the influence of fractionation on $\delta^{15}N$ due to N transfer and uptake, we assume this affects all species and pots equally and should therefore not alter the outcome of our overall results, additionally we accounted for focal species identity in our statistical models (see explanations below) to correct for differences due to individual species effects. Importantly, we collected all samples at the same time to avoid potential differences due to timing of the measurement of $\delta^{15}N$ status. Additionally, we use a large sample size of 30 different focal species to better estimate the general effect of legume presence. N facilitation by legumes is a complex process consisting of several interacting factors, which have not yet been fully understood. Given common logistical constraints in such large common garden experiments, using $\delta^{15}N$ data paired with N concentrations of plants (Högberg 1997) provides an insight into potential facilitative mechanisms between alien species and native communities as basis for further investigations.

Data analyses

To assess the first question on overall differences between focal species status and community type, we performed a linear mixed-effects model for each fitness measure on individual level and focal species identity as random effect. Then, to address the second question on whether functional traits can explain focal Asteraceae species fitness in communities with vs. without legume presence, we analysed the response variables aboveground biomass (log-transformed) and total seed weight (log+1-transformed) as well as the (logit-transformed) ratio of reproductive biomass (weight of seeds and all other reproductive organs of flowerhead) to total biomass (as measure of resource allocation) by using linear mixed-effects models. These models included log-transformed SLA, height and seed mass and their interaction with community type (with and without legume presence) as fixed effects. We also added initial focal plant height and initial community cover as covariates to account for initial differences in plant growth and competition. We used pot identity and species identity as random effects. This analysis was carried out both at individual and pot level for aboveground biomass and total seed weight (whereby for analyses on pot level, aboveground biomass and total seed weight were averaged across the focal individuals per pot and pot identity was not used as random effect; since this led to the same qualitative results we present the latter in the Supplementary information).

To investigate the third question about the effects of N concentration on fitness measures in focal species growing with or without legume presence, we analysed the mean aboveground biomass (log-transformed), mean total seed weight (log+1-transformed) and additionally also the ratio of reproductive biomass to total biomass (logit-transformed) of the focal individuals per pot as response variables. We used linear mixed-effect models that contained fixed effects of logit-transformed N concentration, community type and their interaction as well as a random effect of species identity.

Regarding the final question of direct and indirect legume facilitation we compared whether legume presence affects the measures of N concentration and $\delta^{15}N$ ratio of focal species and native phytometers. An increased N concentration in legume presence would generally hint at N facilitation by the legume, while a simultaneous decrease of $\delta^{15}N$ could indicate direct legume facilitation. For this fourth question and the respective analyses we excluded native focal species due to low sample size and only compared archaeophyte and neophyte groups. We separately analysed focal species and phytometer species. To firstly assess whether the added legume *M. lupulina* really fixed N in the experimental pots we compared it with the other native species (i.e. phytometer species) and performed one ANOVA for N concentration and one for $\delta^{15}N$ as response variable and legume presence as explanatory variables (sensu Temperton et al. 2007). Since the N level measures of non-leguminous phytometers are confounded when a legume is present, we only used measures from non-leguminous communities for phytometers. We compared these with *M. lupulina* measures (which could only occur in leguminous communities). In a second step we analysed the effect of legume presence on focal species on pot level. We performed linear mixed-effect models for the two response variables N concentration and $\delta^{15}N$ with community type (leguminous or not) and focal status group (archaeophyte or neophyte) and the interaction, using species identity as a random effect. In a third step we analysed the effects of community type and focal species status group on each phytometer species. We used linear mixed-effect models for logit-transformed N concentration and $\delta^{15}N$ as response variable, and community type and the status group of the respective neighbouring focal species and their interaction as explanatory variables and focal species identity as random effect.

For all linear mixed-effect models, we performed backwards step-wise model simplification using likelihood ratio tests to obtain the minimum adequate model containing only significant terms (whereby we retained marginally significant effects, i.e., $P < 0.1$). All statistical analyses were carried out using the software R (version 4.0.4 R Core Team 2021) and the package lme4 (Bates et al. 2010) for linear mixed-effects models.

4.4. Results

Effect of legume presence on focal Asteraceae species fitness

Regarding overall fitness, status did not significantly affect biomass production, but neophyte species produced more seed weight than archaeophytes and natives. Legume presence had a negative effect on biomass production across all status groups, while natives had a considerably lower and neophytes tended to have a lower seed production in leguminous communities than non-leguminous communities (Table 4.2, Figure 4.1). These results thus do not suggest legume facilitation of alien Asteraceae.

Table 4.2 *Results of statistical tests on how alien species fitness (response variables biomass and total seed weight, both log-transformed) depend on alien status and the interaction with community type. In both models species identity was included as random effect. All relevant terms after model simplification are listed with their respective χ^2 test statistic. R^2 values (calculated with the MuMIn package, Bartón, 2019) describe how much of the overall variation is explained by the explanatory variables (R^2_m) and the explanatory variables and random effects together (R^2_c).*

Response variable	Sample size	Explanatory variables	Test statistic	R^2_m	R^2_c
Log biomass	604	Community type	χ^2_{3df}=4.9; P=0.026	0.005	0.42
Log (Total seed weight+1)	631	Status*community type	χ^2_{2df}=12.3; P=0.002	0.07	0.39

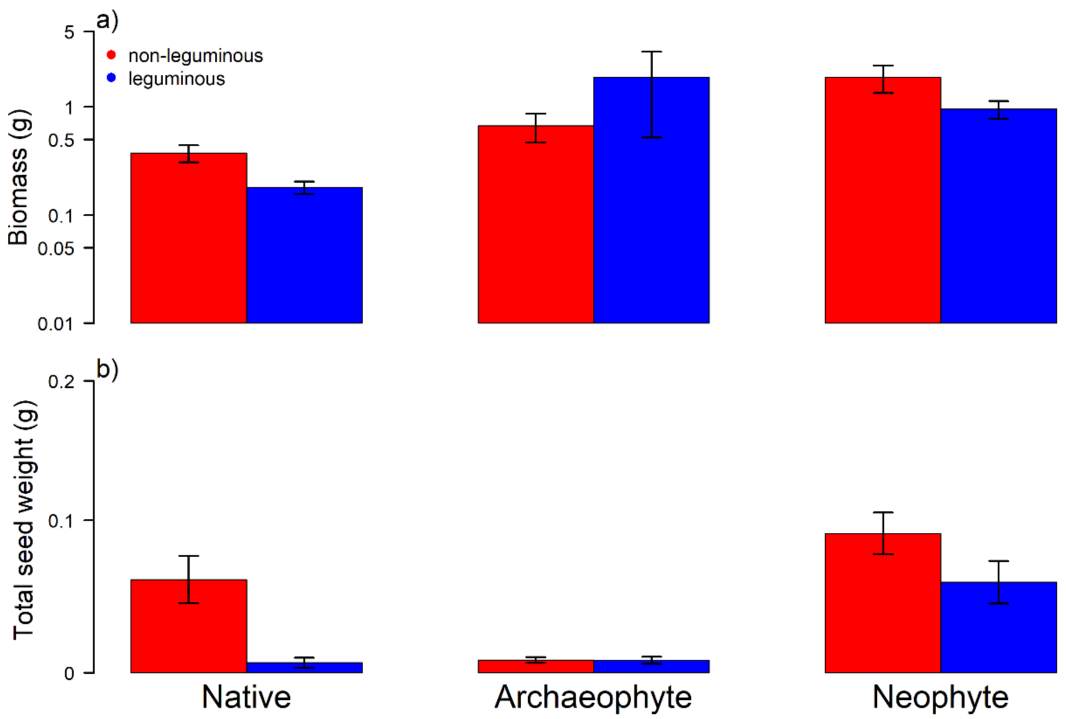

Figure 4.1 *Effect of alien status of focal Asteraceae and treatment on response variables biomass (a) and total seed weight (b). Barplots show mean ± SE; y-axes are log-transformed.*

Relationship between focal Asteraceae species fitness and functional traits

SLA showed a significant negative relationship with biomass production and a significant interaction with community type. Species with higher SLA tended to have lower biomass in non-leguminous communities, while little change was observed in leguminous communities (Table 4.3, Figure 4.2). Height showed a significant positive relationship with biomass production across both community types, taller height led to higher biomass production. SLA was the only trait that showed a significant negative relationship with total seed weight. Species with higher SLA had lower seed production, this effect was stronger in non-leguminous communities (Table 4.3, Figure 4.2).

Table 4.3 *Results of statistical tests on how focal Asteraceae species fitness (response variables biomass and total seed weight at focal individual level, both log-transformed) are affected by functional traits and their interaction with community type. All relevant terms after model simplification are listed with their respective χ^2 test statistic. R^2 values (calculated with the MuMIn package, Bartón, 2019) describe how much of the overall variation is explained by the fixed effects (R^2_m) and the fixed and random effects together (R^2_c).*

Response variable	Sample size	Explanatory variables	Test statistic	R^2_m	R^2_c
Log biomass	604	SLA*community	χ^2_{2df}=7.8; *P*=0.005	0.13	0.49
		Height	χ^2_{2df}=3.8; *P*=0.050		
		Initial height	χ^2_{1df}=11.6; *P*<0.001		
Log (Total seed weight+1)	631	SLA*community	χ^2_{2df}=8.3; *P*=0.004	0.11	0.41
		Initial height	χ^2_{1df}=15; *P*<0.001		

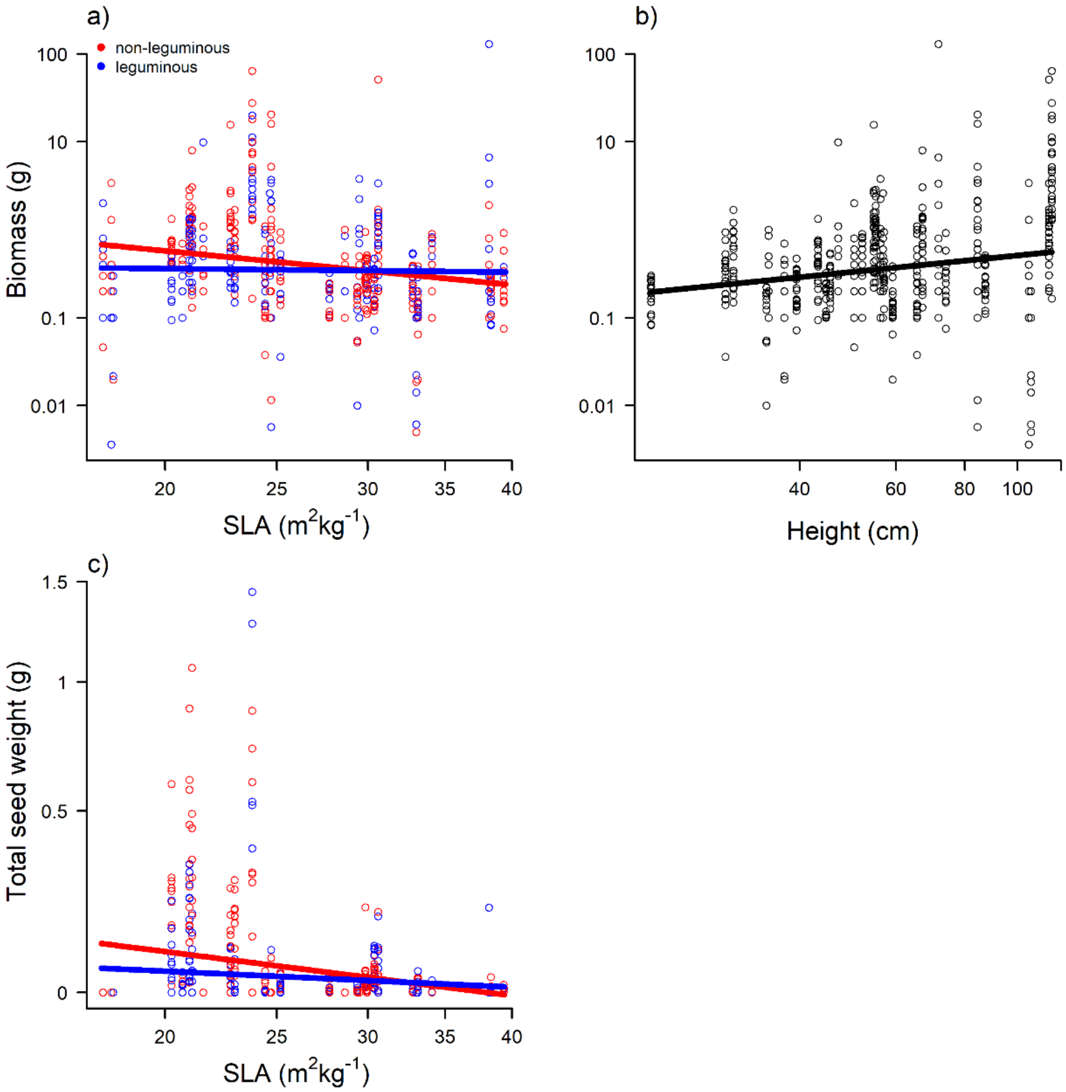

Figure 4.2 *Predictions of the relevant trait effects (a and c, SLA; b height) based on the statistical model for the response variables biomass (upper panels) and total seed weight (lower panel) at focal individual level. Circles indicate raw data points. Axes are log-transformed. Different colours indicate different community types: non-leguminous (red) and leguminous (blue).*

Furthermore, the biomass allocation of reproductive to total biomass changed in response to SLA and height (Supplementary information Ch 4, Table A2, Figure A3). Specifically, we found a positive relationship of SLA and the biomass allocation ratio, meaning a relatively higher allocation towards reproductive biomass for higher SLA values. This effect was stronger when growing with legumes. Height showed a negative relationship with the resource allocation ratio, indicating a higher allocation towards reproductive biomass for shorter plants. Note that we found qualitatively the same results for alien species fitness when using the pot-level dataset, except for an additional significant positive relationship of seed mass and total seed weight (Supplementary information Ch 4, Table A3, Figure A4).

Relationship between N concentration and fitness of alien Asteraceae species

Aboveground biomass of our focal species was positively related to N concentration, and across all experimental pots, focal species produced more biomass in communities without a legume (Table 4.4, Figure 4.3a). For total seed weight, the effect of N concentration interacted with community type. When growing with a legume species, individuals with high N concentration performed better, whereas in non-leguminous communities low N concentration individuals tended to produce more seeds (Table 4.4, Figure 4.3b).

Table 4.4 *Results of statistical tests on how focal Asteraceae species fitness (response variables biomass and total seed weight at pot level, both log-transformed) is affected by N concentration depending on community type (leguminous or non-leguminous). All relevant terms after model simplification are listed with their respective χ^2 test statistic. R^2 values (calculated with the MuMIn package, Bartón, 2019) describe how much of the overall variation is explained by the fixed effects (R^2_m) and the fixed and random effects together (R^2_c).*

Response variable	Sample Size	Explanatory variables	Test statistic	R^2_m	R^2_c
Log Mean biomass per pot	90	N concentration	χ^2_{1df}=17.2; *P*<0.001	0.19	0.64
		Community	χ^2_{2df}=4.5; *P*=0.033		
Log (Mean total seed weight+1 per pot)	90	N concentration *Community	χ^2_{2df}=3.3; *P*=0.071	0.03	0.75

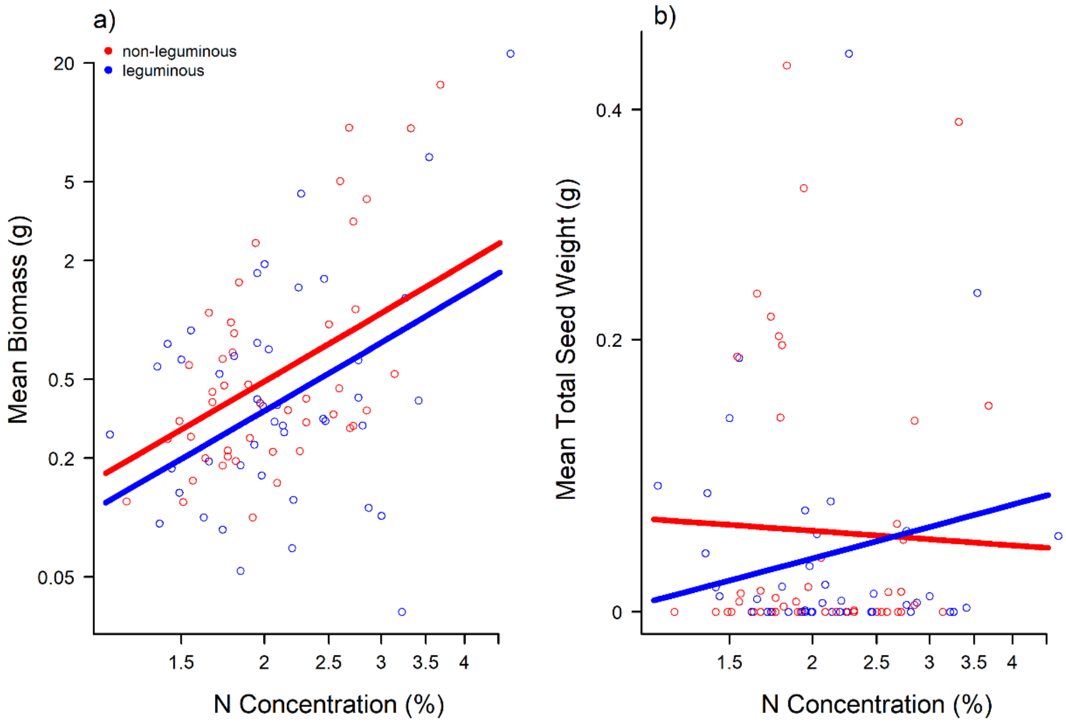

Figure 4.3 *Predictions of the effect of N concentration based on the statistical model for the response variables biomass (a) and total seed weight (b) at pot level. Circles indicate original data points. Axes are log-transformed. Different colours indicate different community types: non-leguminous (red) and leguminous (blue).*

Regarding biomass allocation, we found a positive relationship with N concentration but only in communities with a legume (Supplementary information Ch 4, Table A2, Figure A5). However, in contrast to biomass, the seed weight and the resource allocation model explained very little variance in the data (Table 4.4, Supplementary information Ch 4, Table A2).

Investigating if legume facilitation is differently affected by status or community type for neophyte, archaeophytes or phytometer species

We found evidence for N fixation of legumes as $\delta^{15}N$ values of legumes were significantly lower, and N concentration was higher for legumes compared to the native phytometer species *P. argentea* and *F. rupicola* in non-leguminous communities (Figure 4.4). The low $\delta^{15}N$ value (close to 0 ‰) together with higher N concentration than the other non-leguminous species indicates active N fixation (Högberg, 1977) and enables potentially facilitative effects on other species present.

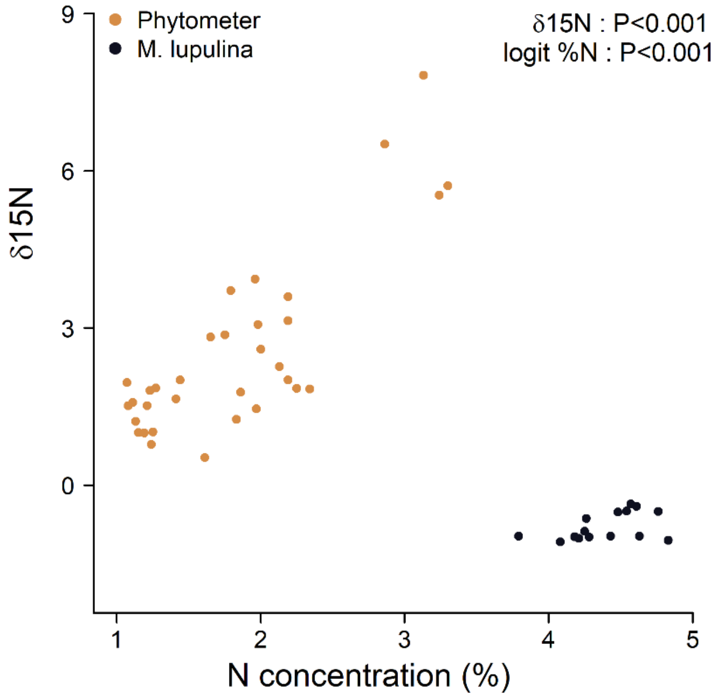

Figure 4.4 *The relationship between N concentration and $\delta^{15}N$ for the phytometer species Festuca rupicola and Potentilla argentea (orange) and the legume species Medicago lupulina (black). Depicted is data from non-leguminous treatment for the phytometer species and from leguminous treatment for M. lupulina. Significant differences between groups were tested with ANOVA.*

However, we could not detect an effect of legume presence on alien focal species, neither on N concentration nor $\delta^{15}N$ values which would indicate facilitative interactions with a legume. Focal species of the status archaeophyte were found to have marginally higher N content than neophytes (χ^2_{1df}= 2.9; *P*= 0.09), but this did not depend on legume presence (Supplementary material Ch 4, Figure A6). The proportion of legume biomass of the whole community had no effect on N concentration or $\delta^{15}N$ (unpublished data).

For the examined native phytometer species we found effects of legume presence and focal neighbour status. For *F. rupicola* we found a marginally significant positive effect of legume presence on N concentration and marginally significant negative effect if the focal species present is a neophyte compared to an archaeophyte (Table 4.5, Figure 4.5). For *P. argentea* we found (marginally) significant interactions between legume presence and status of the focal neighbour species present on N concentration and $\delta^{15}N$ (Table 4.5). We found higher levels of N concentration in *P. argentea* in legume presence compared to absence when growing together with a neophyte (Figure 4.5b), but not with archaeophytes. Regarding $\delta^{15}N$ we find a trend of decreasing values in legume presence when growing with an archaeophyte (hinting at direct legume facilitation), while we find higher $\delta^{15}N$ values in legume presence when growing with a neophyte (Figure 4.5d).

Table 4.5 *Results of statistical tests on how phytometer species nitrogen level (N concentration and $\delta^{15}N$) is affected by legume (treatment leguminous or non-leguminous) and alien neighbour species presence (archaeophyte or neophyte) and their interaction. All relevant terms after model simplification are listed with their respective χ^2 test statistic. R^2 values (calculated with the MuMIn package, Bartón, 2019) describe how much of the overall variation is explained by the fixed effects (R^2_m) and the fixed and random effects together (R^2_c).*

Phytometer	Response variable	Explanatory variables	Test statistic	R^2_m	R^2_c
F. rupicola	N%	Treatment	χ^2_{1df}=3.8; *P*=0.05	0.23	0.28
		Neighbour status	χ^2_{1df}=3.6; *P*=0.057		
	δ^{15}N	*ns.*	-	-	-
P. argentea	N%	Treatment* Neighbour status	χ^2_{1df}=6; *P*=0.014	0.26	0.52
	δ^{15}N	Treatment*Neighbour status	χ^2_{1df}=3.2; *P*=0.076	0.09	0.09

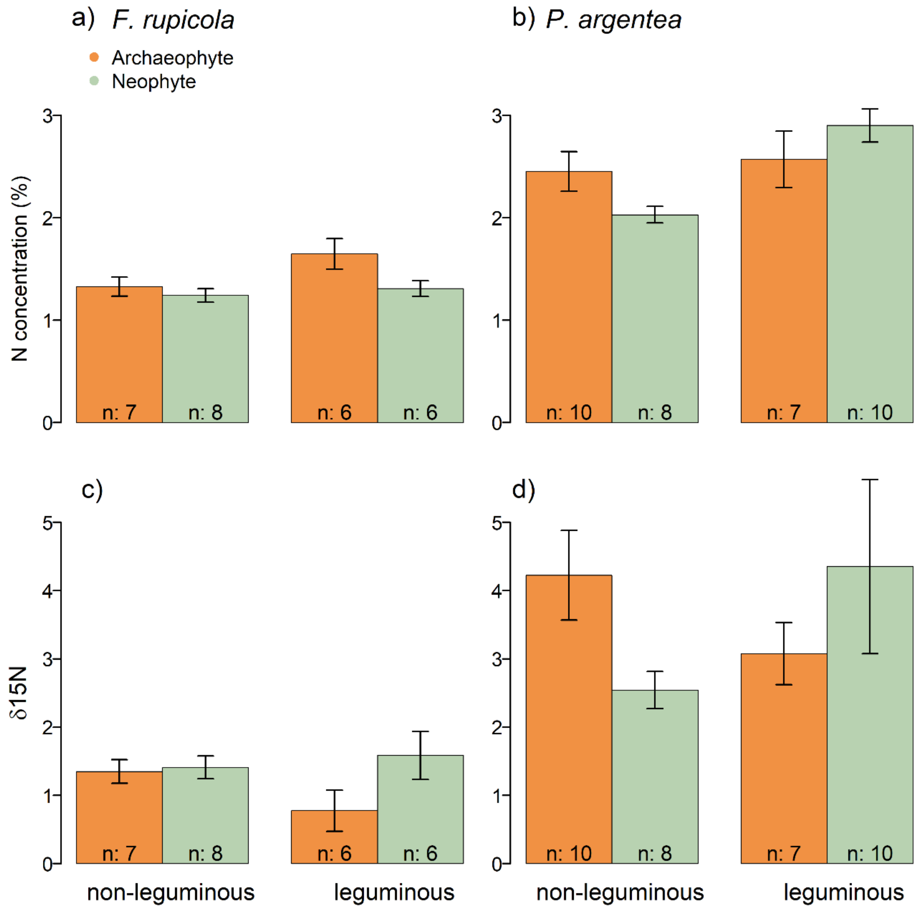

Figure 4.5 *Barplots depicting N concentration of the phytometer species in top panels, as well as $\delta^{15}N$ values in lower panels. F. rupicola is represented in panels a) and c) while P. argentea is in panels b) and d). Orange bars indicate the neighbouring focal Asteraceae was an archaeophyte while green bars indicate neophyte neighbours. Bars show mean ± SE, sample sizes are indicated.*

4.5. Discussion

Although legume presence did not benefit fitness of alien focal species overall, we found that the presence of legumes affected how traits determine fitness. Specifically, fitness advantages of species with low SLA could only be observed in absence of legumes. Higher nitrogen concentration was associated with higher aboveground biomass of alien species, whereas its effect on seed production depended on legume presence. Alien Asteraceae species N concentration and $\delta^{15}N$ were not affected by legume presence, therefore in this study we could not identify facilitative effects by legumes on alien species. However, we found that the effect of legume presence on native community species N concentration and $\delta^{15}N$ depended on species identity and type of alien neighbour species (status).

Legume presence alters how SLA determines plant fitness

Our analyses reveal a link between functional traits and fitness measures of the Asteraceae species used in the experiment. Importantly, some trait effects differed with community type, highlighting that the factors that influence plant fitness and interactions depend on the presence (or absence) of a legume species. We found that alien (and native) Asteraceae plants with lower SLA had higher fitness by producing more aboveground biomass and more seeds, an effect which was also shown by previous alien species studies (Conti et al. 2018; Ferenc and Sheppard 2020). These effects were weaker or almost absent in legume presence, which could be due to a relief of competition for N as low SLA values typically indicate a more conservative resource use strategy (Pérez-Harguindeguy et al. 2013), which in our experiment might have only been advantageous under lower nutrient availability and potentially higher competition for N uptake, that is in absence of legumes. However, SLA can also influence responses to competition for other resources such as water due to a more conservative resource use strategy (Pérez-Harguindeguy et al. 2013). Yet, in our experiment, water was not a limiting resource due to artificial irrigation. Nevertheless, the presence of a legume can pose synchronous facilitative and competitive effects on neighbouring plants as previous studies have shown (Henneron et al. 2020), for example regarding competition for light. Previous studies have shown that legume presence and thereby the nutrient availability can affect competition outcome (Klabi et al. 2014), which is not unique for alien species but was also shown for natives (Ordonez and Olff 2013). However, some studies found no effect of SLA in response to increased soil N, which indicates how functional traits determine fitness can vary (Yelenik et al. 2017). The ratio between reproductive and total biomass was positively related to SLA, with a stronger effect in communities with a legume. This indicates an effect of legume presence on the proportion of reproductive biomass produced by a plant. The optimal allocation theory predicts the resource investment into organs that are relevant to capture the most limited resource (Bloom et al. 1985; Weiner 2004). Regarding a relatively higher investment into reproductive compared to vegetative biomass in legume presence, this might hint at reduced competition for resources due to increased N availability, allowing relatively more reproductive biomass to be produced for species with high SLA, that is species that invest less in robust leaves.

We found height to have a positive effect on aboveground biomass regardless of legume presence reflecting a general advantage in competition for light, the taller the plant is (Westoby 1998), as previous studies have also shown (Kraft et al. 2015; Ferenc and Sheppard 2020). Notably, taller height did not increase seed production as the best measure of fitness for annual plants (given that almost all our individuals survived during the experiment), however. This shows that because of demographic trade-offs, the effects of traits on individual vital rates such as growth, reproduction or survival can yield misleading results when aiming to assess trait effects on performance (Laughlin et al. 2020). Although height influenced biomass it did not increase total seed weight. If our study had only assessed biomass as a performance measure as commonly done in experimental studies, this might result in an overinterpretation of the importance of height for invasion success.

We note that due to measuring functional traits in low-density monocultures (in the same environmental setting), our analyses do not account for intraspecific trait variation due to growing with native communities. However, we expect this bias to have only small effect on the conclusions we draw, as generally trait variation between species is expected to be larger than within species. Indeed, we found in a previous study that individual-level trait measurements could not explain more variation in species performance than species-level trait averages (Ferenc and Sheppard 2020).

Higher nitrogen concentration is associated with higher aboveground biomass in both communities whereas effects are weak and vary for seed production

We find a strong positive correlation between aboveground biomass and N concentration of the Asteraceae focal individuals. This is in line with a previous study by Spehn et al. (2002) who investigated effects of legume presence of grassland species assemblages. Although N concentration has a positive relationship with biomass production in leguminous and non-leguminous communities in the same manner, contrasting to their study, we found a slightly higher biomass production of alien (and native) Asteraceae in absence of a legume. This could be explained by an increased competition for other resources such as light in legume presence, since we see higher total community cover (as measures of overall competition pressure) in legume presence (unpublished data). Regarding biomass allocation towards reproductive and vegetative aboveground biomass, we found a positive relationship to N concentration but only in communities with a legume. This can indicate reduced competition for N in legume presence, therefore more resources can be allocated towards reproductive output, however the variance being explained in this model was very low (Supplementary material, Ch 4, Table A2). We note the importance for future studies to elucidate effects of legume presence on belowground biomass, which might shed more light on explaining resource allocation patterns in response to legume presence. We found no consistent effects of N concentration on seed production, with the amount of variance explained in this model also being low (Table 4.4). For the annual plants that we considered here, this lack of effect on seed production suggests N concentration might not have a direct effect on plant fitness. As we also note above, this finding raises awareness to consider performance measures more closely linked to intrinsic growth rates to improve predictions of population dynamics (Laughlin et al. 2020; Brendel et al. 2021).

No aboveground evidence of direct and indirect nitrogen facilitation for alien focal species

Generally, we can expect that the legume fixed N in our experiment, as *M. lupulina* had higher N concentrations as well as lower $\delta^{15}N$ compared to the native phytometer species growing without legumes in the same abiotic conditions, indicating the uptake of fixed atmospheric N. Similar evidence has Generally, we can expect that the legume fixed N in our experiment, as *M. lupulina* had higher N concentrations as well as lower $\delta^{15}N$ compared to the native phytometer species growing without legumes in the same abiotic conditions, indicating the uptake of fixed atmospheric N. Similar evidence has been shown by Temperton et al. (2007) and Spehn et al. (2002) who measured lower $\delta^{15}N$ of legume species compared to non-legume forbs or grasses, interpreting this as indication for N-fixation (Högberg 1997). In these studies, legumes were shown to rely on N fixation when grown in competition with other dominant species such as grasses. The latter can better exploit soil N with their extensive root system enabling them to take up N very efficiently (Oelmann et al. 2007) and benefit from N facilitation more than forbs (Temperton et al. 2007), whereas legumes being weaker competitors rather fix N than compete for soil-N (Brophy et al. 1987).

We found no indication of legumes facilitating the alien Asteraceae species. Specifically, alien Asteraceae growing in legume communities did not benefit in terms of biomass or seed production, with even a slightly negative effect of legume presence (Table 4.2). This can indicate synchronous facilitative and competitive effects, as shown in previous studies. A high nutrient acquisition or fixation can lead to high biomass production and therefore stronger competitive effects as well as altered microbial activity (Henneron et al. 2020). Across our Asteraceae species, we found species-specific differences in fitness in response to the presence or absence of a legume (Supplementary material Ch 4, Figure A7). While many species only show little difference between growing in legume presence or absence, other species show clear negative trends in legume presence (*Bidens ferulifolia*, *Callistephus chinensis*, *Matricaria discoidea*, *Pulicaria vulgaris*, *Tripleurospermum inodorum*) and only two show a positive tendency (*Glebionis segetum*, *Matricaria chamomilla*). This raises the importance of using a multi-species approach in order to achieve generalizable conclusions (van Kleunen et al. 2014).

Furthermore, legume presence did neither increase N concentration (indicating direct or indirect facilitation) nor could we observe a change towards lower values in $\delta^{15}N$ (indicating direct facilitation) of alien Asteraceae species. These results did also not differ between neophytes and archaeophytes. Residence time and other differences in species characteristics of neophytes versus archaeophytes due to their differing introduction history and environmental affinity (Pyšek et al. 2005; Chytrý et al. 2008) thus did not appear to affect the aliens themselves regarding legume facilitation (although their effect on natives differed, see below). For both groups, not finding facilitative effects could indicate that alien species did not need to rely on legume presence in our experiment as they were not limited by N, probably due to efficient or enhanced resource uptake (Dassonville et al. 2008). As we planted individuals in a specific pattern in the pots (Supplementary material Ch 4, Figure A1) and sowed the legume species randomly, we assume all focal species should have principally been able to receive N from legumes. Even if individual distance to the closest legume might have varied, previous studies showed N transfer occurs over distances such as 20 cm (Brophy et al. 1987), while our pots had a radius of 25 cm. An additional explanation for relying less on legume presence could be that alien species are very efficient in turning N into biomass, rather than being very efficient in N uptake, as a study by Parepa et al. (2019) on N-use efficiency of invasive knotweed showed: actual N uptake between alien and native species was similar (in absolute values) but alien plants could produce biomass more efficiently. A similar change in N-use efficiency as opposed to a change in total N uptake was also shown for different species responding to elevated CO_2 in some, but not all, experiments (Davey et al. 1999; Calfapietra et al. 2007; Finzi et al. 2007; Wang and Wang 2021).

Another reason for the lack of facilitation in our Asteraceae species might be because previous work showed that facilitative effects get stronger over time, either because mycorrhizal connection and microbial associations need longer to build or some legumes only provide facilitative effects after they die (Carino and Daehler 2002), therefore one season might not be enough to show facilitative interactions (Mulder et al. 2002). Furthermore, we note since efficiency of N fixation can depend on the legume species identity (Spehn et al. 2002), that other legume species might pose stronger effects than *M. lupulina*. Another reason for blurred effects could be bi- or multidirectional N transfer in mycorrhizal networks (Carlsson et al. 2009), for example N in small shares can also be transferred from a non-legume to the legume or between non-legumes. To elucidate N cycles in communities in even more depth, investigations of belowground mechanisms during the experimental period are necessary (Fernandez et al. 2022). Such knowledge enables us to understand N facilitation in more detail and add to the mechanistic understanding of altered N cycling in the presence of different alien plant species. A recent meta-analysis showed strong impacts of invasive species on soil systems, by altering microbial communities as well as nutrient cycling (Zhang et al. 2019), which may facilitate alien plant invasion. Additionally, a study by Huang et al. (2016) suggested the impact of alien plant species presence on soil N cycle and microbial activity to be more severe than artificially increased N levels. However, an understanding of changes of belowground biomass and microorganism composition in the context of leguminous communities invaded by alien species of various residence times is lacking. Given the effects we found when using aboveground measures, the investigation of belowground mechanisms appears to be a promising research avenue for further elucidating potential facilitative effects of legumes and alien species invasion.

Relative importance of direct and indirect nitrogen facilitation for native phytometer species

In contrast to the alien Asteraceae, legume presence affected N concentration and $\delta^{15}N$ of native phytometer species, although in different ways. The native grass phytometer species *F. rupicola* showed an overall increased N concentration in legume presence, which was also found for other species in previous studies (Spehn et al. 2002; Temperton et al. 2007). In contrast to Temperton et al. (2007) who studied the related species *Festuca pratensis*, we did not simultaneously find a significant decrease of $\delta^{15}N$, which indicates direct N transfer, the so-called N sharing. However, Figure 4.5 does suggest a tendency of overall decrease in $\delta^{15}N$ in leguminous communities, which might nevertheless be biologically relevant if it can be statistically confirmed with more data points in future studies, as the lack of effect in our study could be due to small sample size and therefore not enough data. When looking closer at the alien Asteraceae species neighbouring the phytometer, we find neighbour status to affect N concentration of *F. rupicola*. When growing with archaeophyte neighbours, N concentration increased more in legume presence than when growing with neophyte neighbours. One possible explanation is the inhibition of native mutualists such as mycorrhiza by neophytes as shown previously (Stinson et al. 2006; Zubek et al. 2016), which can lead to lower nutrient acquisition of native species.

However, these effects seem to be highly species-specific, since we found an overall increased N concentration in legume presence also for our second phytometer species *P. argentea*, but here we find the status of the neighbouring focal individual to interact with legume treatment. While N concentration in *P. argentea* with archaeophyte neighbours is similar with and without legume presence, for neophyte neighbours we found lower N concentration in the absence of legumes. This could hint at stronger N limitation of *P. argentea* in neophyte presence, since only when growing with a legume it showed similar N concentration as with archaeophytes. Lower $\delta^{15}N$ values in leguminous communities hint at N sharing when growing with archaeophyte neighbours, but due to the similar N concentration in both treatments, and little explained variance in the model, we expect this to not contribute substantially to changes in N concentration. However, potential N sparing when growing with neophytes seems to be more effective than potential N sharing when growing with archaeophytes. Nevertheless, our findings indicate a trend of differing importance of N facilitation pathways, potentially being affected by the types of alien species that co-occur. In general, the response to N-facilitation can be very species-specific and especially strongly differs between grasses and forbs (Temperton et al. 2007), due to different N use strategies (Kahmen et al. 2006) which might explain our species-specific findings, although we cannot generalize from findings of only two phytometers. Nevertheless, we consider the species as good approximation for the communities in our experiment as they were among the most abundant and occurred in every pot.

Conclusions

Our results indicate a negative effect of SLA on aboveground biomass of alien species, which was weaker when growing with legumes, likely due to a relief from competition for N in legume presence. However, we could not identify legume presence to have an impact on how other functional traits such as height or seed mass affect alien plant fitness. Plant N concentration was positively related with aboveground biomass but not necessarily with total seed weight. This finding raises awareness to consider better fitness proxies than solely biomass, since especially for annual species as in our experiment, seed production is a very important fitness component. We found N characteristics of native community phytometer species to be affected by legume presence, but also type of neighbouring alien Asteraceae species, since we found a more sensitive reaction to the presence of neophytes than archaeophytes. In contrast, the alien species themselves did not benefit from legume presence or show differences in response to N facilitation between the status groups. Our results show the importance of investigating legume facilitation in an invasion context due to differing mechanisms of competition for N between native and alien species of various residence times. By extending knowledge on facilitation mechanisms we can improve predictions of species competition outcome and can make more informed management decisions. As a next step, the quantitative differences of N distribution in native leguminous communities above- and especially also belowground invaded by alien species of different residence time should be investigated to deepen our understanding of facilitative and competitive effects in these systems.

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Chapter 5 General Discussion

5.1. Research gaps

Alien plant species performance, establishment and their impact on native species has been a focus of much research, since in the light of climate change and globalisation, the ever-increasing number of alien species poses severe threats to species, communities and ecosystems. However, most studies do not consider the co-occurrence of multiple alien species in regard to their interactions. These can be competitive or facilitative, which can determine potential effects on native species.

For my thesis, I investigated interactions among multiple co-occurring alien plant species as well as their interactions with native species, to disentangle whether potential positive interactions among alien species, or with native species such as legumes, could ultimately have strong negative impacts on native species and communities. In a first step I investigated interaction effects (competitive or facilitative) among multiple co-occurring alien species, and how these effects can be explained, be it by niche or fitness differences. Secondly, the effects co-occurring alien species pose on natives have to be investigated more thoroughly in order to improve predictions of alien species establishment and management implications. Community assembly is affected by a variety of processes, one of them being the effects of early emerging species on later emerging species in a given environment, so-called priority effects. As alien species commonly emerge and grow earlier than native species, priority effects should be considered especially in the context of alien species establishment. Lastly, I focused explicitly on facilitative interactions by legumes and how their facilitation mechanisms vary in response to species of different residence times. Legumes are a plant functional group widely known for facilitative effects on their neighbour species due to their ability to fix atmospheric nitrogen. However, the outcome of legume facilitation regarding alien species establishment has rarely been studied. Additional nitrogen provided by the legume might facilitate the alien species and lead to even more severe negative effects on the native, but might also reduce resource competition and decrease negative effects on native species. In the following I discuss how my research contributed to increase the knowledge regarding interactions among co-occurring alien plant species and their effects on native species, regarding priority effects on alien and native plant interactions as well as legume facilitation in the context of alien plant invasion.

5.2. Interactions among co-occurring aliens

In chapter 2 I focused on how co-occurring alien species interact. I found the predominant type of interaction to be competition, which is in line with previous research (Kuebbing and Nuñez 2014; Jackson 2015) and contradicting the 'invasional meltdown' hypothesis (Simberloff and von Holle 1999). However, I observed facilitation in 24% of the cases, often when a legume was involved but not exclusively. Given the interesting findings of a quarter of the interactions among co-occurring aliens being facilitations, in my third chapter I extended the testing of only interactions among alien species to the effects on a native species. I found that overall natives suffer more from having two alien neighbours compared to only one, which might hint at alien species facilitation and potential 'invasional meltdown' (Simberloff and von Holle 1999). Although at first glance this may contradict findings from the second chapter, where predominantly competition among alien species occurred, there are a few possible explanations of these findings. For instance, effects of two alien neighbours on a native are likely additive effects of multiple species or asymmetric competition, where negative effects on the native plant are more severe than on the co-occurring alien plant (Kuebbing and Nuñez 2016; Braga et al. 2020). Comparing the interaction outcome of two species with the effect of two species on a third species is not necessarily easy as it was shown previously that mutualistic effects tend to be more context dependent than competitive effects (Chamberlain et al. 2014). Additionally, compared to the second chapter where I investigated all possible pairwise combinations of 20 alien species, for the experiment in the third chapter effects were highly species-specific due to a smaller set of investigated species. The results of both chapters are complex, as for instance I did observe facilitation in some cases in the second chapter, while I also found facilitative effect of one alien species on the native if the alien was *Vicia villosa* in the third chapter. The positive effect of *V. villosa* disappeared when adding a second alien species. This can either be due to negative effects of the second alien species on the alien *V. villosa,* resulting in weaker facilitative effects; or alternatively due to previously mentioned asymmetric competition. These findings show a range of possible interaction outcomes when considering alien species co-occurrence, the importance for considering context dependence for species interactions and the need for investigating underlying mechanisms that drive interaction outcomes.

To explain underlying mechanisms of variation in species interaction and go beyond a species-specific level, I employed a trait-based approach and compared it to a phylogenetic approach and trait hierarchies. I found trait hierarchy related to competitive ability to be the best predictor of interaction outcome. However individual traits also explained a lot of the variance in the data, and showed to be good predictors of interaction outcome. Contrasting to the trait hierarchy models, models containing phylogenetic and multi-trait distances explained very little variance in the data (1-3%), which is in line with recent studies investigating whether interactions could be explained by phylogenetic relatedness (Kunstler et al. 2012; Golivets and Wallin 2018) or a study by Funk and Wolf (2016) comparing multi-variate distance with phylogenetic relatedness and trait hierarchy, who found the latter to explain interaction outcome best. My findings emphasise the importance of considering fitness differences when explaining species interactions (Mayfield and Levine 2010; Kunstler et al. 2012) also in the context of co-occurring alien species. Regarding the Coexistence Theory (Chesson 2000) 'stabilising niche differences' or 'average fitness differences' would explain coexistence of species and ultimately community structure. Since I found average fitness difference to explain the outcome of species interactions best, while phylogenetic and multi-trait distances (describing niche differences) could not explain much variation in the data, the 'stronger' alien species might outcompete the inferior alien species over time within this experimental setting. Since alien species with fitness advantages over native species are expected to pose high impact on native communities (MacDougall et al. 2009), my findings could indicate that the highly competitive species in my experiment could also have strong impact on a native community.

5.3. Priority effects impact alien – native interactions

Priority effects are an important, yet not fully understood, mechanism considering alien species establishment which I investigated more closely in chapter 3. I found a strong disadvantage of arriving late and a smaller advantage of arriving early for natives as well as aliens similar to previous studies (Stuble and Souza 2016). While overall the effects were similar for native and alien species, we found alien species to suffer less from arriving late and benefitting more from arriving early than native species.

The dependence of species performance on order of arrival suggests community composition can be greatly affected by priority effects, even after several years (Weidlich et al. 2017). Thus, temporal shifts of species arrival or emergence may not be neglected when investigating mechanisms of community assembly, especially in the context of alien species invasion. A modern approach to community assembly research is the filter theory. To successfully invade and establish in a community, the species (of a regional species pool which was shaped by historical processes) has to overcome so-called filters, which are shaped by abiotic but also biotic factors (Belyea and Lancaster 1999; Hille Ris Lambers et al. 2012; Kraft et al. 2015a). These filters are subject to change due to feedbacks from interactions in the existing community (Hille Ris Lambers et al. 2012). This theory was further extended to invasion ecology by Pearson et al. (2018), by including filters on an extrinsic level through which an alien species has to arrive in a regional species pool, as opposed to native species that were subject to historical processes. Stabilising niche differences and average fitness differences according to Coexistence Theory (Chesson 2000) are considered to explain community interactions and their feedback on biotic filters (Hille Ris Lambers et al. 2012). Additionally, priority effects can play a role in shaping the filter which ultimately affects whether a subsequent species can establish into the community (Belyea and Lancaster 1999). I found disadvantageous effects of late arrival compared to simultaneous arrival in my experiment, where the temporal shift was only three weeks but still showed to be more severe for native species. Within this framework this could indicate biotic filters which the (in this case native) species has to overcome was more negatively altered by alien species than vice versa. However, in my experiment I used only a single species that was present rather than a community, hence only intraspecific interactions of the early arrived species, rather than also interspecific interactions within a community could impact the establishment of the subsequent species.

Priority effects of native and invasive species are crucial for restoration management. Early establishment of less competitive species can prevent competitive exclusion (Young et al. 2001; Vaughn and Young 2015), such differences in arrival time can have impacts on community structure even after several years (Weidlich et al. 2018). Especially in the context of alien species invasion, priority effects are important to consider since alien species tend to germinate earlier than natives. My results indicate that native plants might suffer more from later arrival than alien species and should therefore be sown early to increase chances of establishment for successful restorations.

Both of these aspects, community assembly and restoration ecology, are important topics in community ecology. I showed how questions in these field can be addressed by using alien species invasion as a model study, with the additional importance regarding the negative impact alien species invasions can have on native species, communities and ecosystems.

5.4. Functional traits explaining alien species performance

As previously discussed, I could explain interactions among co-occurring alien plant species with functional traits. In this section I want to discuss the individual traits I measured in some more detail. Studies commonly advocate to consider intraspecific trait variation (which can be due to genetical or plastic variability) in trait-based studies since it reflects niche-differences in a population or community better than species level averages (Violle et al. 2012; Westerband et al. 2021). Contradicting previous studies (Kraft et al. 2014; Conti et al. 2018; Carmona et al. 2019), for my experiment I found the model with individual level traits to not explain species interactions better than using species-level averages. This might be explained by trait-environment effects (Bennett et al. 2016), since I measured species-level traits by using same populations in same abiotic conditions and as the individuals in competition treatments were exposed to (opposed to previous work using trait measures from databases as Kunstler et al. 2012; Carboni et al. 2015). Using individual trait values measured in interspecific interaction to explain outcomes of this interspecific interaction may be concerning due to circularity between explanatory and response variable. My results therefore suggest, it might not always be commended to use individual-level measures, besides it being very labour intense and therefore unrealistic on larger scales. However, measuring traits on species-level in the same conditions as the experimental treatments might reduce unexplained variance due to environment effects.

I found several traits to contribute to explaining plant performance and thereby competition. Specifically, when growing taller than the co-occurring alien neighbour, biomass and seed number of individuals in mixture increased compared to growing alone in chapter 2. Similarly, growing taller led to higher biomass of the alien focal plants in chapter 4. Plant height is related to competitive ability, since growing taller reflects an advantage in competition for light (Westoby 1998) and has been shown to provide a competitive advantage over the neighbour species (Kraft et al. 2015b). However, only for the experiment in chapter 2 I could show that plant height had an effect on seed production, while I found no such effect for alien species in chapter 4.

Besides plant height, also specific leaf area had relevant effects on performance of alien plants, in both chapter 2 and 4. In chapter 2 I found species having a lower specific leaf area than the co-occurring alien neighbour, increased biomass and seed number in mixture compared to growing alone. A lower specific leaf area led to higher biomass of the alien focal plant in chapter 4. Specific leaf area, in contrast to plant height, also had a negative effect on seed production, which is one of the best measures of performance for annual plants. In this experiment I additionally examined the effect legume presence in the community has on how traits relate to species performance. Interestingly, for specific leaf area I found an impact on performance only when growing without a legume. My findings are consistent with previous work showing a higher competitive ability for individuals with low specific leaf area (Kraft et al. 2015b; Conti et al. 2018), which typically indicates a more conservative resource strategy (Pérez-Harguindeguy et al. 2013). This conservative resource strategy might however only be advantageous under low nutrient availability. When growing in legume presence (chapter 4) I could not find this effect, which can hint at a relief of competition in legume presence due to their ability to fix atmospheric nitrogen. This is in line with earlier studies showing that legume presence and nutrient availability can affect competition outcome (Klabi et al. 2014).

I found initial seed mass to have a positive effect on biomass (chapter 2) and on total seed mass (pot-level-analyses of chapter 4). Seedlings from heavier seeds likely have more resources and are more robust regarding environmental stress (Westoby 1998). According to Violle et al. (2007), functional traits are affecting plant species performance, growth or survival and therefore indirectly their fitness. In our experiments the key to increased fitness appears to be growing tall, having smaller specific leaf area and heavy seeds.

5.5. Legume facilitation in the context of alien species invasion

Positive interactions such as facilitation play an important role regarding species interactions. In this thesis I focus on legume facilitation, which is well-known to occur due to legumes ability to fix atmospheric nitrogen and therefore is an important factor regarding species interactions. I showed in the second chapter of this thesis when investigating pairwise interactions among co-occurring alien plants, it had a positive effect on biomass production of the focal alien plant to grow together with an alien legume neighbour species. Similarly, in the third chapter legume facilitation could be observed to some extent. Although these results might be species-specific and not generalizable for the plant functional group of legumes, I could show that having one alien legume neighbour had a positive effect on biomass of the native species. Both of this is in line with previous studies showing increased productivity when growing together with legumes (Temperton et al. 2007). In the fourth chapter of this thesis, I found a positive correlation of N concentration (approximating nutrient uptake) and biomass. However, I found that alien species biomass was lower in legume presence compared to legume absence. This indicates that additionally there might be competition for other resources besides nitrogen such as light in this experiment. One difference to chapters 2 and 3 is that the legume was a native species in chapter 4, while for chapter 2 and 3 alien legume species were used. Although previous work has shown that native legumes can facilitate alien species (Maron and Connors 1996), in our experiment alien species might not have been limited by nitrogen, or the legume species posed synchronous facilitative and competitive effects (Henneron et al. 2020). Also, opposed to chapter 2 and 3, only the experiment in chapter 4 was performed with a native background community, which might have caused more competition for other resources than the previous experiments. The occurrence or strength of facilitative effects appears to be context dependent, at least when comparing my three experiments. I found some indication for facilitation and increased negative effects on native communities, which might be independent from legume facilitation.

While the effects of legume facilitation on biomass and seed production in chapter 2 were unequivocal, in chapter 3 and 4 the effects on seed production were not as clear. This might mean that fitness consequences could actually be small depending on the context of species interactions, since performance measures linked to intrinsic growth rate have shown to be important when determining species fitness (Laughlin et al. 2020). When investigating the effect of nitrogen concentration on seed production in chapter 4, the model explained little variance overall, therefore more studies are needed to disentangle the drivers of the relationship between nitrogen concentration and performance of alien plants.

Since I found some facilitative interactions with legumes previously, I focused on different mechanisms of nitrogen facilitation on neophyte, archaeophyte and native species. While I could not detect any difference between neophyte and archaeophyte focal species in terms of legume facilitation, they differently affected nitrogen facilitation of the native phytometer species. I found potential indirect legume facilitation for *Festuca rupicola* while results of *Potentilla argentea* hinted at N sharing, however the models explained little variation and since the result seem to be species-specific, conclusions have to be drawn carefully. However, my results hint at differences in legume facilitation pathways for different plant functional groups (grasses vs. forbs) which can be due to different nitrogen use strategies between plant functional groups and thereby explain our findings. Interestingly I found the status group (archaeophyte or neophyte) of the alien neighbour to impact nitrogen dynamics in the pot, although I could not find legume facilitation of the species themselves. Native phytometers were more sensitive to neophyte than archaeophyte presence in terms of nitrogen characteristics. This hints at varied mechanisms of competition for nitrogen between natives and alien species of different residence times and emphasize the importance of extending knowledge on facilitation mechanisms to improve predictions of species competition outcome and make more informed management decisions.

5.6. Implications and future research

In chapter 2 I showed that it is crucial to investigate interactions among co-occurring alien species, since for some species combination facilitative effects were found that can be used to improve management of invasive species. However, in such pot experiments using pairwise species combinations the community context is neglected, which might have a strong impact on species interactions and reflects a more natural setting. Identifying and taking into account context dependence can improve understanding and predictions of interactions (Catford et al. 2022). I therefore conducted an experiment investigating interactions of alien species in a native community (Supplementary material Discussion, 6.4.1). By using a standardised community and one, two or three different alien species invading the community I wanted to test the community invasibility in response to more (or less) similar invaders, as it is commonly assumed that more dissimilar species can invade a community more easily. Additionally, in the light of co-occurring alien species, it is crucial to investigate whether potential facilitative (or competitive) effect among the alien species would promote (or hinder) alien species establishment and whether this can be explained by trait (or phylogenetic) similarity or dissimilarity. While accounting for direct and indirect biotic interaction in a community context is a crucial step to improve predictions, it can be challenging to introduce or establish focal species in a pot experiment (Supplementary material Discussion, 6.4.1), as the artificially established native community might become too competitive for later introduced alien species to establish. The timing of establishment and sowing density is crucial for a successful experiment and should be planned very carefully for future studies. Since weather conditions might also impact establishment of seedlings, additionally or alternatively a study in a more controlled environment such as a greenhouse could be considered.

In chapter 3 I discussed the importance of priority effects and how natives can suffer more from late arrival which is common since invasive species germinate earlier and grow earlier. This is an important conclusion in the context of restoration efforts, as for example to avoid strong negative effects of alien species, one should aim for early sowing of native species rather than leaving the ground bare for longer and sow later. In the context of co-occurring alien species, it is unlikely that several alien species arrive at the exact same moment in a certain location. Therefore, studies should also investigate priority effects among alien species and not only between native and alien species, ideally within a community context aiming at more natural conditions. I aimed at including this in an experiment (Supplementary material Discussion, 6.4.1), by sowing alien species into a native community. In pairwise combinations, species were either sown simultaneously or one prior to the other. This would have enabled me to compare the magnitude of early versus late arrival compared to simultaneous arrival, and whether the effects on the native community would be impacted by the order of sowing. For example, if the early arriving species poses facilitative effects on a later arriving species, they may impact the native community stronger than if the first arriving species poses negative effects on a later species. Additionally, I attempted to predict direction and magnitude of such priority effects by species niche versus fitness differences. However, studying priority effects among co-occurring alien species with a multi-species approach in a pot experiment has shown to be very challenging regarding the timing and density of the experimental background community (Supplementary material Discussion, 6.4.1). The timing of sowing alien species in regard to native species and weather conditions is especially crucial regarding priority effects, and therefore has to be planned carefully in future studies.

Further research is needed in investigating the role of legumes in alien species invasion, disentangle direct and indirect mechanisms of facilitation to draw generalized conclusions. In the third chapter I found the presence of one alien species to have facilitative effects on the native if that happens to be *V. villosa,* which is a legume. When adding a second alien species this effect disappeared. Since only one legume species could be used in this experiment I cannot draw conclusions about general legume effects here. However, I am convinced this is a promising avenue for future studies. To shed light on the mechanisms of this outcome, an experiment could investigate the pairwise combinations of the involved species and single species treatments to quantify the effect a neighbour species has. For example, if the mechanism behind it would be asymmetric competition, the pairwise treatments would show different competition of different magnitude. To avoid observing species-specific effects then additionally an experiment should comprise more species and their possible combinations. Additionally, direct and indirect legume facilitation could be investigated by performing $\delta^{15}N$ and nitrogen content analyses. This would enable to conclude which of the neighbouring species might have benefitted directly from legume presence, and whether this changes between settings of one and two neighbour species and their status (alien or native), since I found some indication for differing facilitation pathways between having archaeophyte or neophyte neighbours in chapter 4. These finding need to be strengthened and further elucidated by more studies. Especially the quantitative differences of nitrogen distribution, but also belowground interactions in native leguminous communities invaded by alien species of different residence time need to receive more attention, to deepen our understanding of facilitative and competitive effects in these systems.

Beyond investigating alien species performance in regard to interactions with co-occurring alien or native species and communities, attention has to be paid to novel environmental conditions as well. Besides novel biotic interactions imposed by alien species on a native community, plant communities are exposed to environmental stress that can be subject to global change. Although these changes have been investigated separately, less studies have focused on combined effects of these components, how they impact alien species establishment and whether interactions among co-occurring alien species and their impact on native communities is subject to combined or synergistic impacts of environmental stress. I tried to test this with a common garden pot experiment, using drought as a component of climate change and manual disturbance as another abiotic factor, that is common in ruderal habitats where the native and alien species I used commonly occur (Supplementary material Discussion, 6.4.2). To test whether one of these factors has a stronger effect and whether combined effects are more than additive, I used a full factorial experimental design. I implemented single species invasions of 14 alien species from 5 different families and compared them to pairwise species invasion. However, the native community that has been established one year prior to the experiment, was too dense for most alien species to establish (except for *Cosmos bipinnatus* and *Panicum capillare*). Future studies should continue to investigate these combined impacts on alien species establishment, but adjust the native community density or perform stronger environmental stress factors.

5.7. Conclusion

I showed the importance of priority effects which might affect community assembly in the context of alien-native species interactions. My specific findings hint at slight advantages of early compared to simultaneous arrival for natives, which suggests to sow native species early in restoration sites to increase the chance of successful establishment when they are exposed to competition with alien species. Interestingly I found similar directions of effects for both, alien and native species in response to arriving earlier or later than another species. Similarly, alien Asteraceae as well as a native forb phytometer species seem to not benefit from native legume facilitation directly in my experiment, opposed to a native grass phytometer species. This might be another hint at alien and native species not necessarily performing differently, but other factors than solely status predicting competition outcome. However, I found indications legume facilitation among native species to be differently affected by alien species of different residence times, which has so far been underrepresented in the studies of alien species interactions. Given the importance of nitrogen availability for plant performance and potentially resulting increase or relief of competition pressure in plant communities, further investigation of nitrogen dynamics in response to invasion by species of different residence times is crucial for improving predictions of competition outcome.

Based on my experimental work, I mostly found competition among co-occurring alien species which was driven by fitness differences rather than niche differences and therefore hints at possible competitive exclusion among co-occurring alien species. My results emphasise the importance of considering hierarchical trait differences linked to competitive ability rather than niche differences based on phylogenetic or multi-trait distances, fortifying recent studies on native and native-alien interactions with similar findings. However, I have not exclusively found competition among co-occurring alien species, but also facilitative effects in pairwise interaction as well as when growing with a native species. This indicates that some alien species combinations can have additive or potentially even more than additive effects on native species and more severely impact native communities than single species invasions.

I showed how using concepts and theories of community ecology can be applied to examples of alien species interaction especially with the novel aspect of multiple co-occurring alien species. Since the continuously increasing rate of alien species arrival in new habitats makes co-occurrence of alien species more likely, it becomes even more important to examine their combined impact on native species, communities and ecosystems.

5.8. References

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Chapter 6 Appendix

6.1. Supplementary material Chapter 2

Appendix 1: Study species

Table A1. *Overview of the study species.*

Family	Species	Seed source	Range size in Germany (%)	First record in Germany
Amaranthaceae	*Amaranthus albus* L.	Botanical garden University of Hohenheim	9.37	1880
	Amaranthus blitoides S. WATSON	Botanical garden University of Dresden and Botanical garden University of Halle	3.47	1893
	Amaranthus retroflexus L.	Botanical garden University of Halle	46.81	1815
	Amaranthus tricolor L.	Botanical garden University of Tübingen	0.02[c]	1950
Asteraceae	*Bidens pilosa* L.	Botanical garden University of Braunschweig	0.11[c]	1926
	Cosmos bipinnatus Cav.	Botanical garden University of Bayreuth	1.74[c]	1980
	Iva xanthiifolia NUTT.	Botanical garden University of Dresden	2.90	1860
Brassicaceae	*Berteroa incana* (L.) DC.	Botanical garden University of Frankfurt	39.64	1594
	Diplotaxis muralis (L.) DC.	Botanical garden University of Ulm	14.82	1750
	Hirschfeldia incana (L.) LAGR.-FOSS.	Botanical garden University of Konstanz	1.88	1850
Chenopodiaceae	*Atriplex sagittata* BORKH.	Botanical garden University of Krefeld	26.61	1750
	Bassia scoparia (L.) VOSS	Monoculture of previous experiment[a]	5.61	1750
Fabaceae	*Medicago polymorpha* L.	B & T World Seeds, Paguignan, France	0.98	1820
	Vicia villosa ROTH	Voitsauer Wildblumensamen, Kottes-Purk, Austria	39.68	1808
Poaceae	*Anthoxanthum aristatum* BOISS.	Botanical garden University of Dresden	15.40	1850
	Apera interrupta (L.) P. BEAUV.	Botanical garden University of Konstanz	0.65	1950
	Eragrostis minor HOST	Monoculture of previous experiment[b]	23.69	1782
	Panicum capillare L.	Botanical garden University of Potsdam	4.83	1890
Solanaceae	*Datura stramonium* L.	Botanical garden University of Ulm	26.72	1584
	Nicandra physalodes L. (GAERTN.)	Botanical garden University of Greifswald	6.06	1782

Range size in Germany (% of occupied grid cells at the scale of 6 x 10 arcmin, i.e. 11 x 11 km; database FlorKart (https://doi.org/10.15468/wnkii7) by BfN and NetPhyD Netzwerk Phytodiversität Deutschlands e.V., www.deutschlandflora.de and www.floraweb.de) and year of first record (Jäger, 2017; www.floraweb.de) are given.

[a] Originally obtained from Botanical garden University of Gießen
[b] Originally obtained from Botanical garden University of Mainz
[c] Considered as casual neophyte

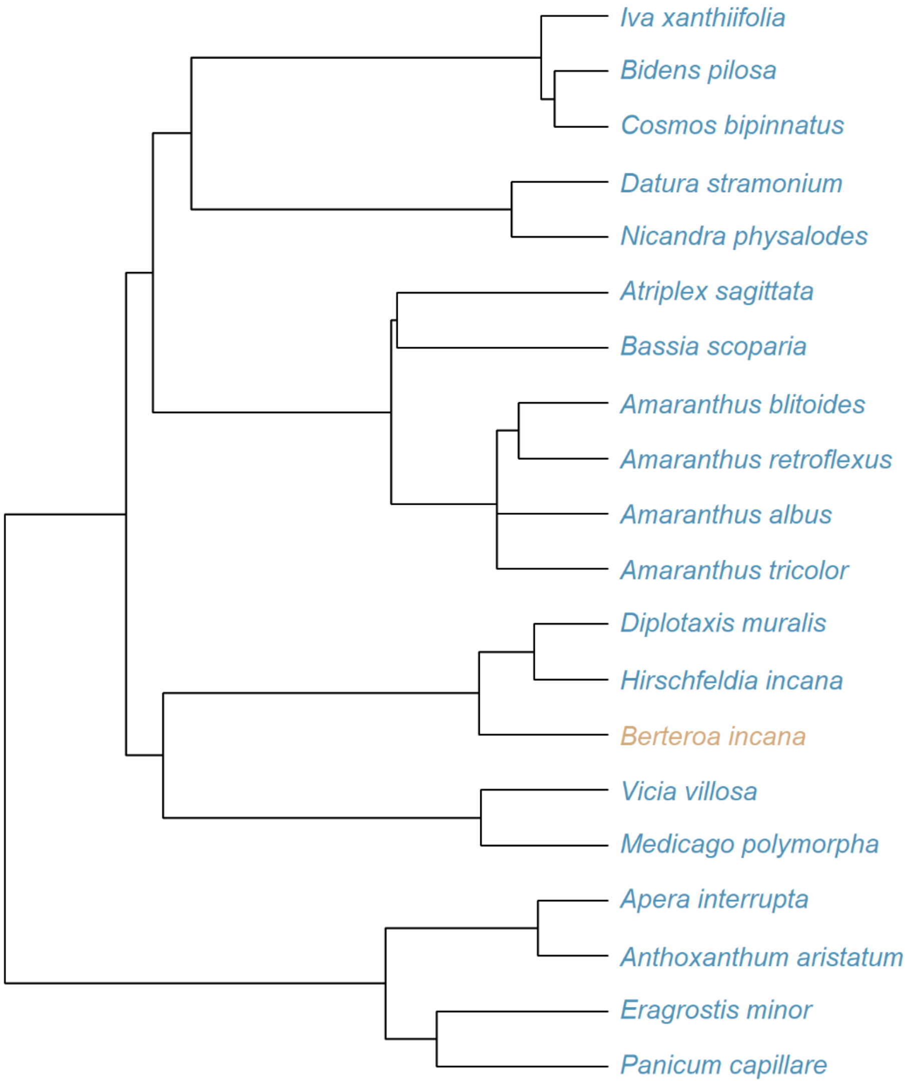

***Figure A1** Phylogenetic tree of the study species. Data was acquired from the Daphne database, a phylogeny of European plant species (Durka & Michalski, 2012). Species in blue were included in all analyses, Berteroa incana (orange) had to be excluded for seed measures as most individuals did not set seeds within the experimental period.*

Appendix 2: Trait selection and data collection

To assess variation in plant functional traits, we measured five different traits of each experimental plant: Firstly, specific leaf area (SLA) reflects differences in resource acquisition strategies, high values indicate high photosynthetic rate and an exploitative strategy, whereas low values indicate leaf longevity and a more conservative resource use (Pérez-Harguindeguy et al., 2013; Westoby, 1998). For SLA measures, we scanned two randomly chosen leaves per plant during the first week of flowering per species. Fresh leaves were scanned with a resolution of 600 dpi and measured using ImageJ Fiji software (Schindelin et al., 2012) and divided it by the dry mass of the leaf (dried for 72 h at 70 °C) (following Pérez-Harguindeguy et al., 2013). Secondly, plant height reflects competitive ability for light, as higher plants reach better light conditions (Westoby, 1998). However, this also comes with the cost of sustaining morphological structures and transporting nutrients and water over longer distances (Westoby, 1998). We measured maximum height of each individual plant at harvest. Thirdly, seed mass reflects the chance of successful spread and seedling establishment, whereby seedling growing from heavier seeds have a higher chance of survival, but it is also costlier to produce heavy seeds (Westoby, 1998). We measured seed mass before sowing (weighing 6 x 50 seeds with a high-precision balance). Fourth, as flowering phenology is considered an important ecological trait and was suggested to act independent from general resource strategies (Craine et al., 2012) we recorded the opening of the first flower of each individual weekly. Fifth, root:shoot ratio is an important measure of resource allocation in plants. Relatively higher root mass suggests an improved ability of resource uptake (Lloret et al., 1999) which is especially advantageous when competing for nutrients in limiting environments. In nutrient rich environments higher allocation to aboveground biomass is expected in order to compete for light (Tilman, 1985). To measure root biomass, we washed and dried (for 72 h at 70 °C) the roots of control individuals at harvest. Additional to these five quantitative traits, we classified the species in plant functional groups (grass, forb, legume). Plant functional groups can be of great use as they cover several traits and can thereby also incorporate traits which are more difficult to measure (Cornelissen et al., 2001).

As niche differences of species might be better represented in multi-dimensional space (Kraft, Godoy, et al., 2015), we calculated multi-trait distance from the species-level average trait values (SLA, height, seed mass, flowering onset and root:shoot ratio) of control individuals. We calculated the Gower distance of the five logarithmized and scaled traits as well as the categorical trait plant functional group using the function daisy in the R package cluster (Maechler et al., 2018). To obtain phylogenetic distances we used Daphne, a phylogeny of European plant species (Durka & Michalski, 2012). This phylogeny was reduced to the study species using the R package picante (Kembel et al., 2010, see Figure A1), and we then derived the cophenetic distance between species pairs with the R package ape (Paradis et al., 2004).

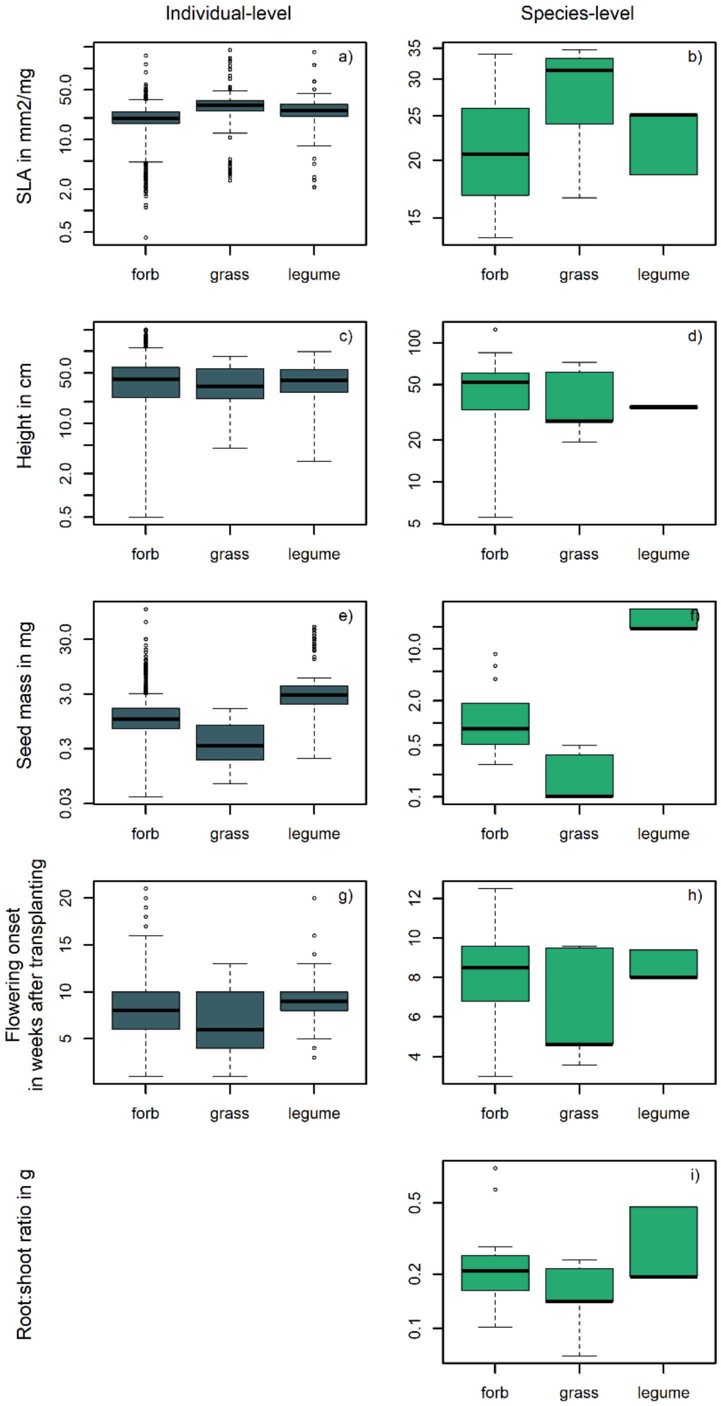

Figure A2 *Trait variation across functional groups (including 14 forb, 4 grass, and 2 legume species) of the target plant shown for specific leaf area (SLA) (a,b), maximum height (c,d), seed mass (e,f), flowering onset (g,h) and root:shoot ratio (i). Dark blue boxplots show individual trait values, green boxplots show species-level averages per functional group. All traits except for flowering onset are displayed on logarithmic y-axis.*

Appendix 3: Alien species competition versus facilitation

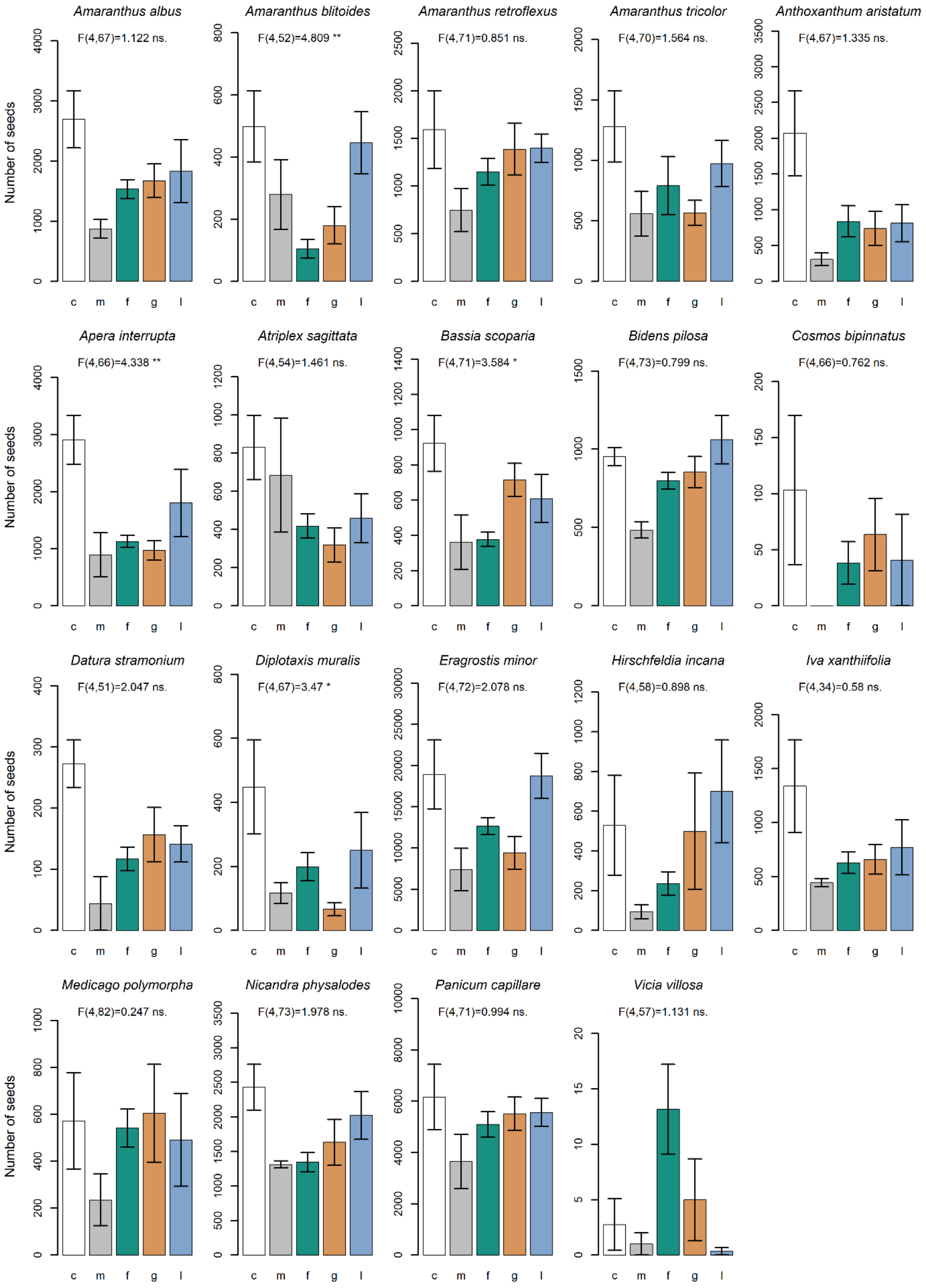

Figure A3 *Mean seed number (± 1 standard error) per species depending on competition treatment. White bars represent the control (plants growing alone in the pot, 'c'); grey bars the monoculture treatment (plants grow with a conspecific individual, 'm'); coloured bars represent the mixture treatment with the neighbour being a forb (green, 'f'), a grass (orange, 'g') or a legume (blue, 'l'). Significance of competition treatment effect on log-transformed seed number was tested using ANOVA. F-statistics and significance of p-values (indicated by asterisk; *=p<0.05; **=p<0.01; ns. indicates no significance) are displayed.*

Appendix 4: Niche differences (phylogenetic or multivariate) versus functional trait hierarchies

Table A2 *Posterior mean values and credible intervals of the four random effects (phylogeny, target species identity, species combination and pot identity, nested in combination). Values for the two most relevant models, using the target trait and trait hierarchy model for both response variables explaining relative performance in interspecific competition compared to growing alone (lnRR_inter/control for biomass and seed number) for species-level trait average models (as reported in Table 2.1) and individual-level trait models (Table 2.2) respectively.*

Response variable (sample size)	**Model**		**Phylogeny** post.mean (95% credible interval)	**Species ID** post.mean (95% credible interval)	**Combination** post.mean (95% credible interval)	**Combination: Pot ID** post.mean (95% credible interval)
lnRR_inter/control biomass (N=1262)	Target trait	Average traits	0.56 (0.002 - 1.826)	0.29 (0.002 - 0.784)	0.31 (0.192 - 0.435)	0.01 (0.002 - 0.023)
	Trait hierarchy		0.28 (0.002 - 0.777)	0.14 (0.003 - 0.348)	0.08 (0.020 - 0.152)	0.01 (0.001 - 0.023)
lnRR_inter/control seed number (N=1117)	Target trait	Average traits	0.91 (0.002 - 3.797)	0.73 (0.002 - 1.793)	0.25 (0.072 - 0.438)	0.02 (0.002 - 0.057)
	Trait hierarchy		0.27 (0.002 - 1.062)	1.06 (0.382 - 2.001)	0.05 (0.002 - 0.130)	0.02 (0.002 - 0.057)
lnRR_inter/control biomass (N=571)	Target trait	Individual traits	3.93 (0.003 - 14.97)	2.27 (0.003 - 5.199)	0.03 (0.003 - 0.064)	0.02 (0.002 - 0.036)
	Trait hierarchy		0.34 (0.002 - 1.289)	0.39 (0.003 - 0.835)	0.05 (0.002 - 0.122)	0.02 (0.002 - 0.051)
lnRR_inter/control seed number (N=565)	Target trait	Individual traits	0.33 (0.002 - 1.528)	0.65 (0.014 - 1.260)	0.05 (0.004 - 0.105)	0.03 (0.002 - 0.082)
	Trait hierarchy		0.19 (0.002 - 0.597)	0.13 (0.003 - 0.301)	0.04 (0.002 - 0.096)	0.02 (0.002 - 0.046)

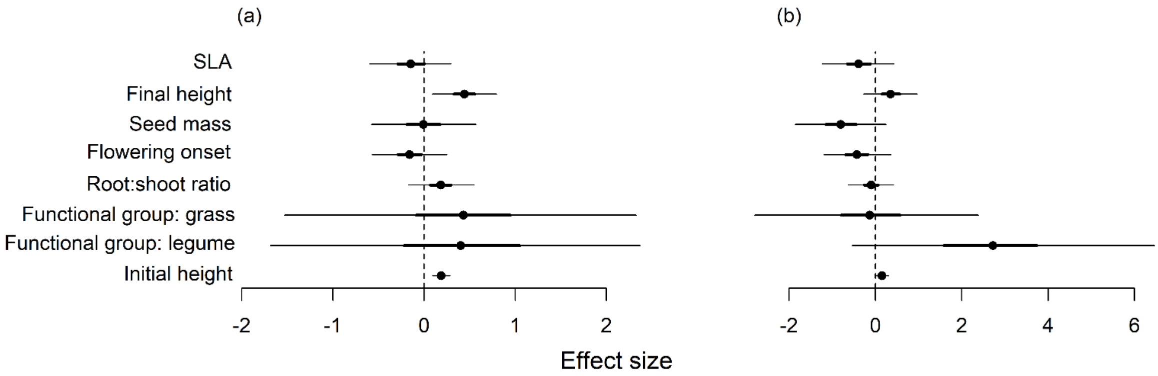

Figure A4 *Results of the target trait model to explain relative performance in interspecific competition compared to growing alone (lnRR_inter/control): effects of specific leaf area (SLA), final height, seed mass, flowering onset, root:shoot ratio and the effect of being a grass or a legume (relative to a forb) and initial height on log response ratio biomass (a) and log response ratio seed number (b). All numerical traits are log-transformed and scaled to a mean of 0 and standard deviation of 1 (except for flowering onset which is only scaled). Positive effects indicate that a higher value of the respective trait leads to more biomass (respectively seeds) in interspecific competition (compared to control single individuals). Effects are considered relevant if the 95% credible interval does not overlap zero. Dots show the posterior mean, wide lines indicating one standard deviation (68% credible interval), while narrow lines indicate the 95% credible interval.*

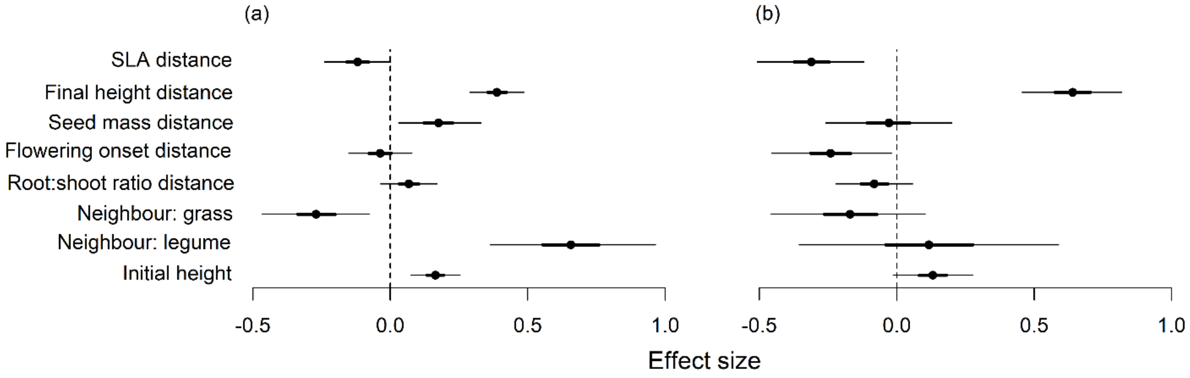

Figure A5 *Results of the trait hierarchy model to explain relative performance in interspecific competition compared to intraspecific competition (lnRR_inter/intra): effects of specific leaf area (SLA) distance, final height distance, seed mass distance, flowering onset distance, root:shoot ratio distance and the effect of having a grass or a legume as neighbour (relative to a forb) and initial height of the individual on log response ratio biomass (a) and log response ratio seed number (b). All numerical traits are log-transformed and scaled to a mean of 0 and standard deviation of 1 (except for flowering onset which is only scaled). Positive effects indicate that a higher value of the respective trait leads to more biomass (respectively seeds) in interspecific competition (compared to individuals growing in intraspecific competition). Effects are considered relevant if the 95% credible interval does not overlap zero. Dots show the posterior mean, wide lines indicating one standard deviation (68% credible interval), while narrow lines indicate the 95% credible interval.*

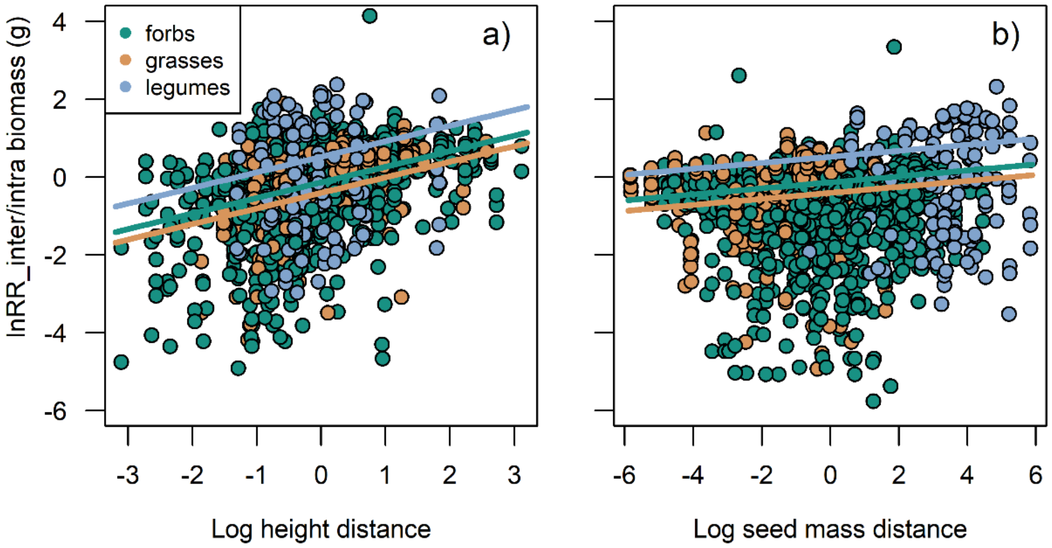

Figure A6 *Predictions of trait effects on the log response ratio biomass of alien plants growing in interspecific competition compared to growing in intraspecific competition (lnRR_inter/intra). Displayed are the predicted effects of the two relevant traits from the trait hierarchy model: maximum height distance (a) and seed mass distance (b) (both log-transformed and scaled to a mean of 0 and standard deviation of 1; predictions for each trait effect shown with the other numeric traits fixed at their mean). Different colours depict the different functional groups: forbs (green), grasses (orange) and legumes (blue). Positive effects indicate that a higher value in the respective trait of the target individual compared to its neighbour leads to more biomass production in interspecific competition (compared to growing in intraspecific competition).*

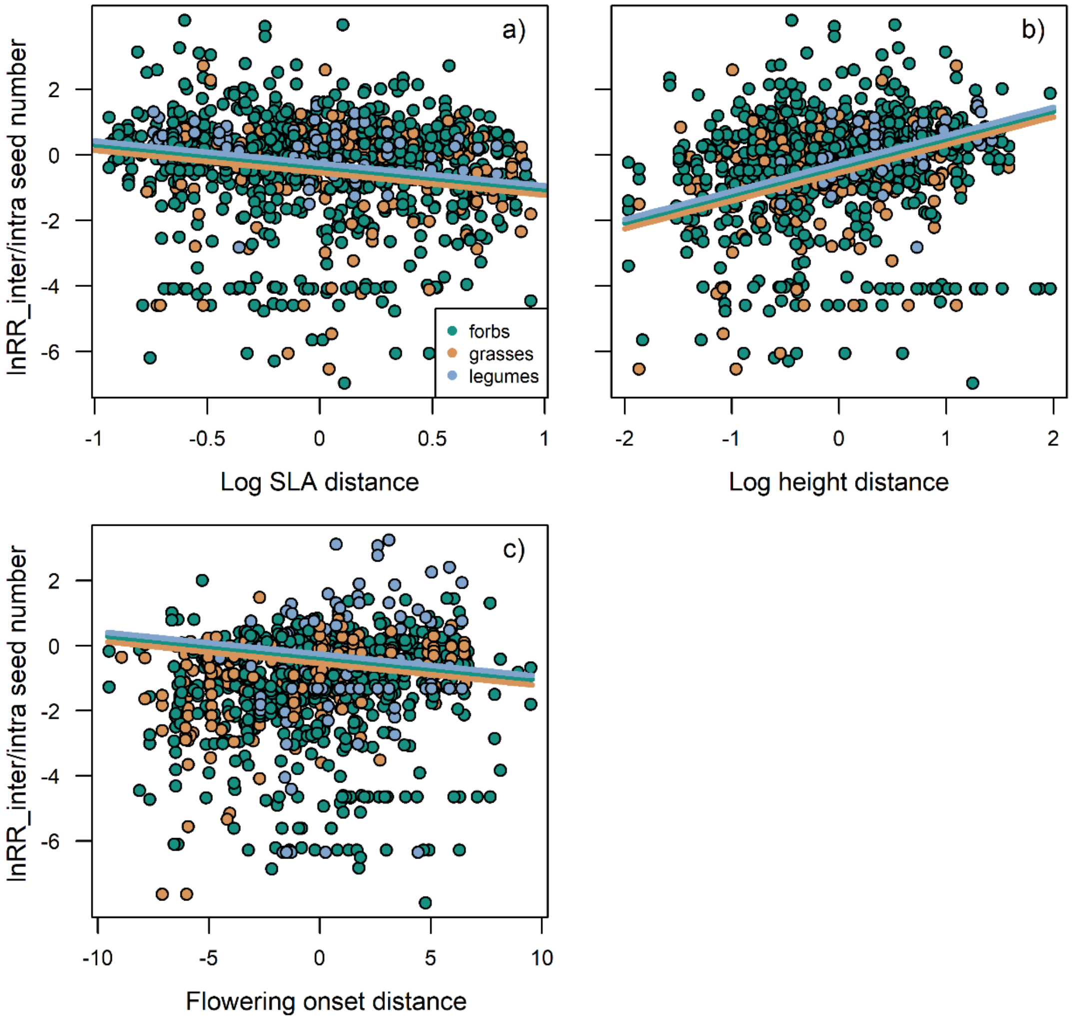

Figure A7 *Predictions of trait effects on the log response ratio seed number of alien plants growing in interspecific competition compared to growing in intraspecific competition (lnRR_inter/intra). Displayed are the predicted effects of the three relevant traits from the trait hierarchy model : specific leaf area (SLA) distance (a), maximum height distance (b) and flowering onset distance (c) (log-transformed and scaled to a mean of 0 and standard deviation of 1, except for flowering onset which was only scaled; predictions for each trait effect shown with the other numeric traits fixed at their mean). Different colours depict the different functional groups: forbs (green), grasses (orange) and legumes (blue). Positive effects indicate that a higher value in the respective trait of the target individual compared to its neighbour leads to higher seed production in interspecific competition (compared to growing in intraspecific competition).*

Appendix 5: Individual-level traits versus species-level trait averages

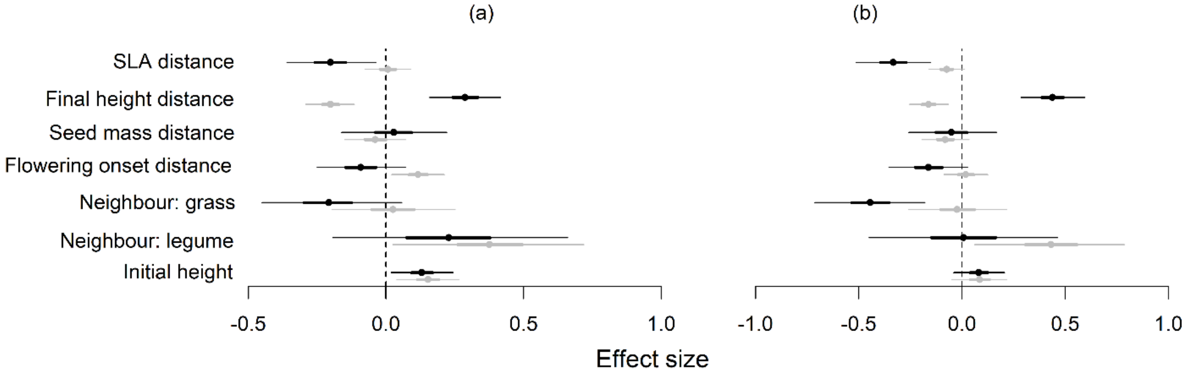

Figure A8 *Results of the trait hierarchy model to explain relative performance in interspecific competition compared to growing alone (lnRR_inter/control) based on species-level trait averages (black) compared to individual-level traits (grey): effects of specific leaf area (SLA) distance, final height distance, seed mass distance, flowering onset distance, the effect of having a grass or a legume as neighbour (relative to a forb) and initial height on log response ratio biomass (a) and log response ratio seed number (b). All numerical traits are scaled and log-transformed (except for flowering onset which is only scaled). Positive effects indicate that a higher value of the respective trait leads to more biomass (respectively seeds) in interspecific competition (compared to control single individuals). Effects are considered relevant if the 95% credible interval does not overlap zero. Dots show the posterior mean, wide lines indicating one standard deviation (68% credible interval), while narrow lines indicate the 95% credible interval.*

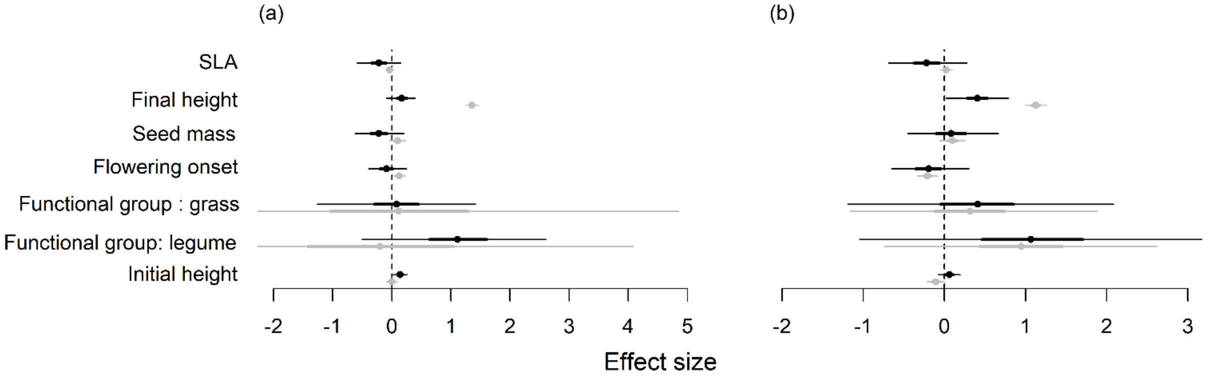

Figure A9 *Results of the target trait model to explain relative performance in interspecific competition compared to growing alone (lnRR_inter/control) based on species-level trait averages (black) compared to individual-level traits (grey): effects of specific leaf area (SLA), final height, seed mass, flowering onset, the effect of being a grass or a legume as (relative to a forb) and initial height on log response ratio biomass (a) and log response ratio seed number (b). All numerical traits are scaled and log-transformed (except for flowering onset which is only scaled). Positive effects indicate that a higher value of the respective trait leads to more biomass (respectively seeds) in interspecific competition (compared to control single individuals). Effects are considered relevant if the 95% credible interval does not overlap zero. Dots show the posterior mean, wide lines indicating one standard deviation (68% credible interval), while narrow lines indicate the 95% credible interval.*

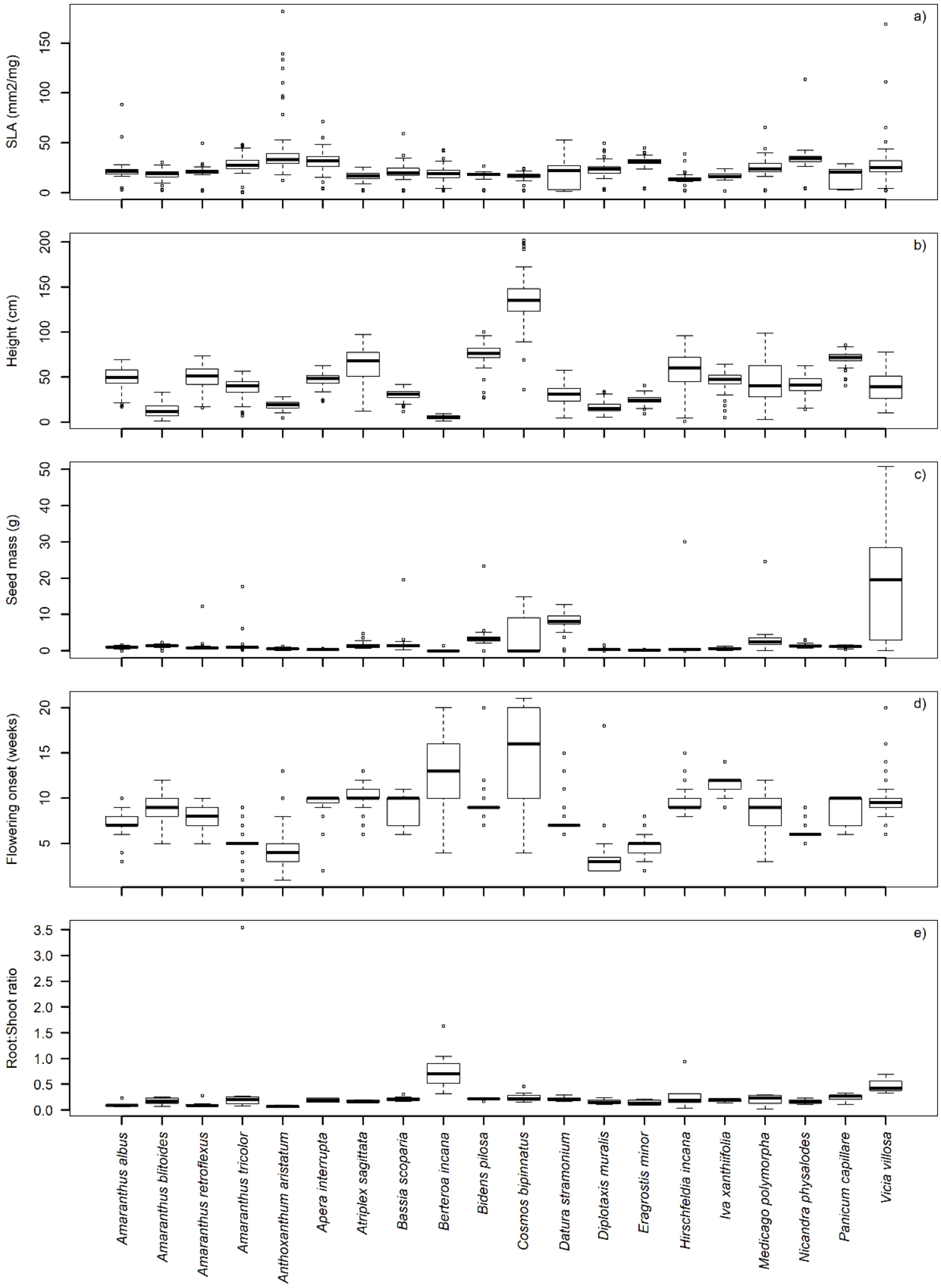

Figure A10 *Intraspecific trait variation for the five focal traits across all study species, shown for specific leaf area (SLA) (a), seed mass (b), maximum height (c), root:shoot ratio (d) and flowering onset (e). Note that for root:shoot ratio, measurements were only taken for control plants growing alone, while for the other traits variation is shown across individuals of all competition treatments.*

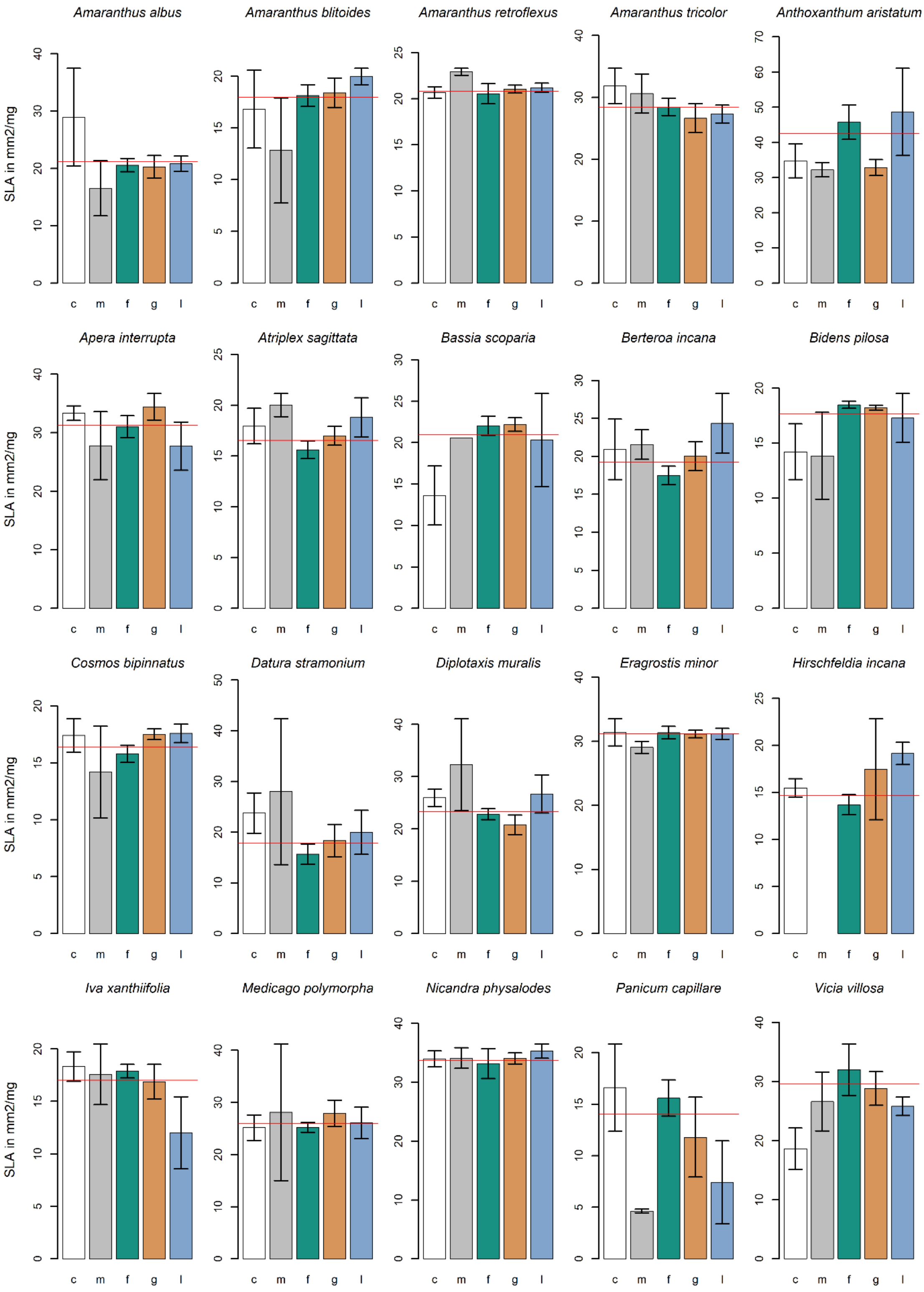

Figure A11 *Mean specific leaf area (SLA) in mm2/mg (± 1 standard error) per species depending on competition treatment. White bars represent the control (plants growing alone in the pot, 'c'; note that this value (logarithmized) is used as the species-level trait average in the models); grey bars the monoculture treatment (plants grow with a conspecific individual, 'm'); coloured bars represent the mixture treatment with the neighbour being a forb (green, 'f'), a grass (orange, 'g') or a legume (blue, 'l'); the red line represents the mean trait value across all competition treatments. Values for monoculture treatment of Hirschfeldia incana could not be assessed, therefore no bar is displayed.*

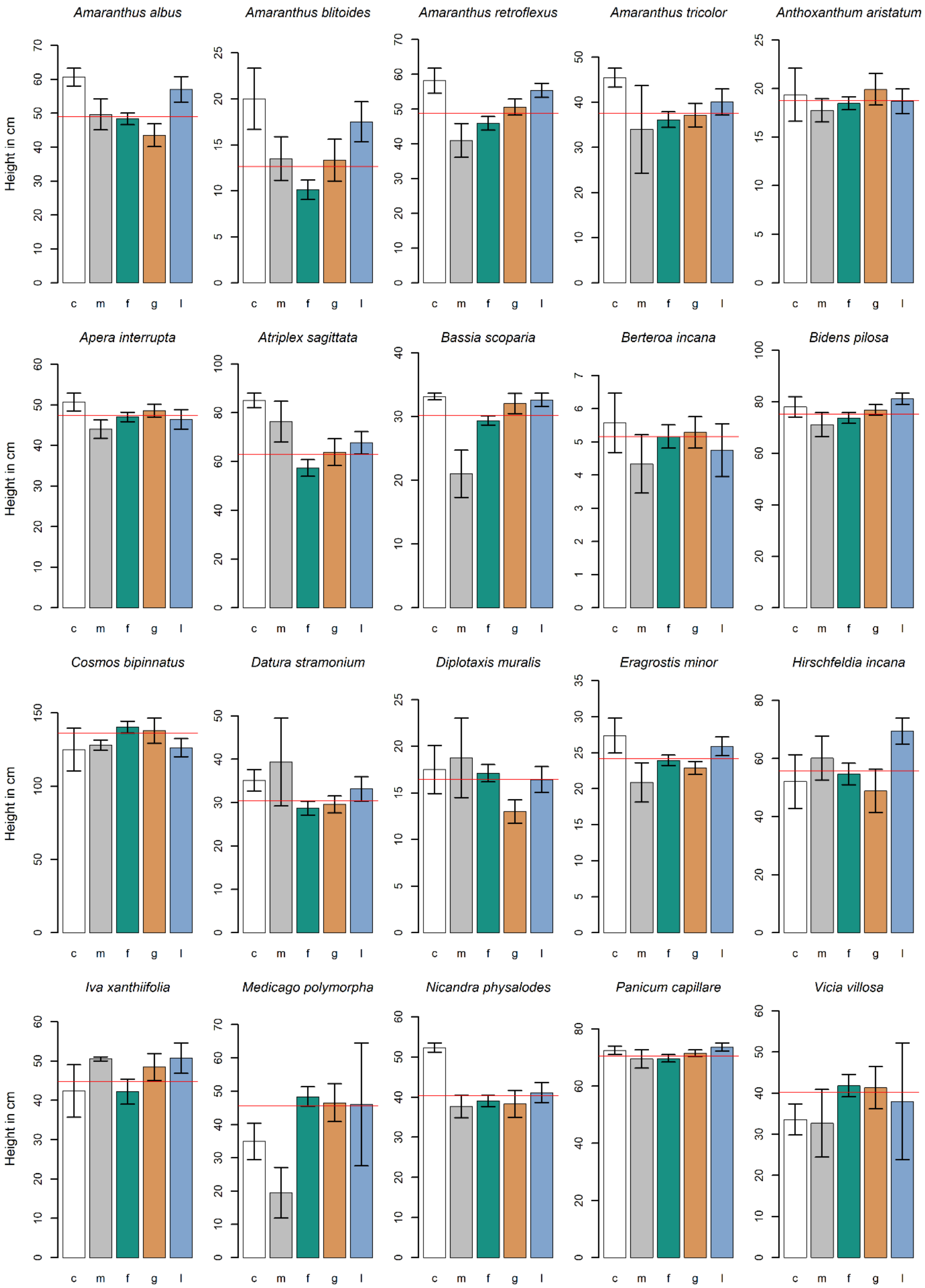

Figure A12 *Mean height in cm (± 1 standard error) per species depending on competition treatment. White bars represent the control (plants growing alone in the pot, ‘c’; note that this value (logarithmized) is used as the species-level trait average in the models); grey bars the monoculture treatment (plants grow with a conspecific individual, ‘m’); coloured bars represent the mixture treatment with the neighbour being a forb (green, ‘f’), a grass (orange, ‘g’) or a legume (blue, ‘l’); the red line represents the mean trait value across all competition treatments.*

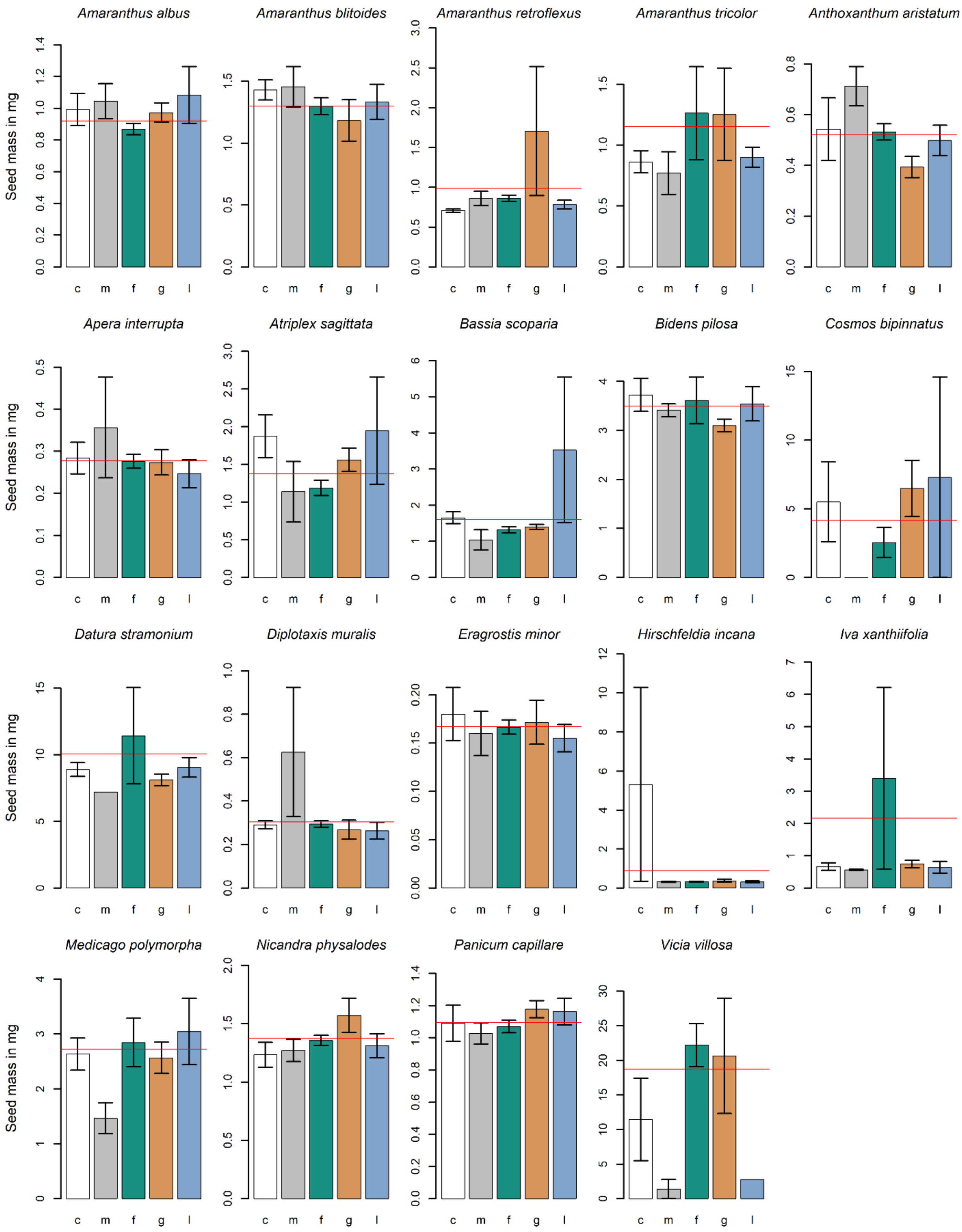

Figure A13 *Mean seed mass in mg (± 1 standard error) per species depending on competition treatment. White bars represent the control (plants growing alone in the pot, 'c'; note that this value (logarithmized) is used as the species-level trait average in the models); grey bars the monoculture treatment (plants grow with a conspecific individual, 'm'); coloured bars represent the mixture treatment with the neighbour being a forb (green, 'f'), a grass (orange, 'g') or a legume (blue, 'l'); the red line represents the mean trait value across all competition treatments. Due to missing values, monoculture bar of Cosmos bipinnatus could not be displayed. As Berteroa incana was excluded from all seed analyses it is not displayed in this figure.*

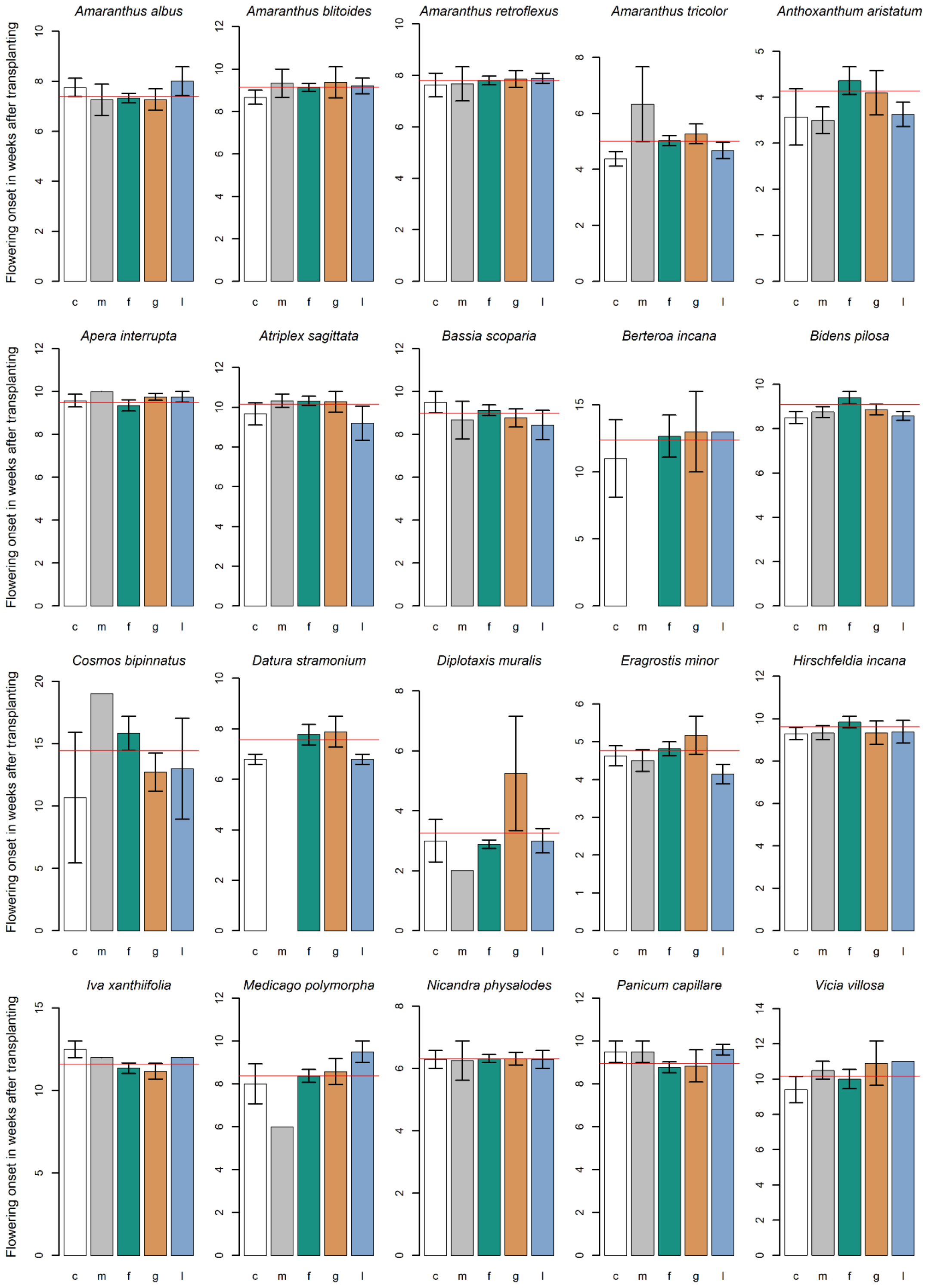

Figure A14 *Mean flowering onset in weeks after transplanting (± 1 standard error) per species depending on competition treatment. White bars represent the control (plants growing alone in the pot, 'c'; note that this value is used as the species-level trait average in the models); grey bars the monoculture treatment (plants grow with a conspecific individual, 'm'); coloured bars represent the mixture treatment with the neighbour being a forb (green, 'f'), a grass (orange, 'g') or a legume (blue, 'l'); the red line represents the mean trait value across all competition treatments. Due to missing values, monoculture treatments of Datura stramonium and Berteroa incana could not be displayed.*

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6.2. Supplementary material Chapter 3

Appendix 1

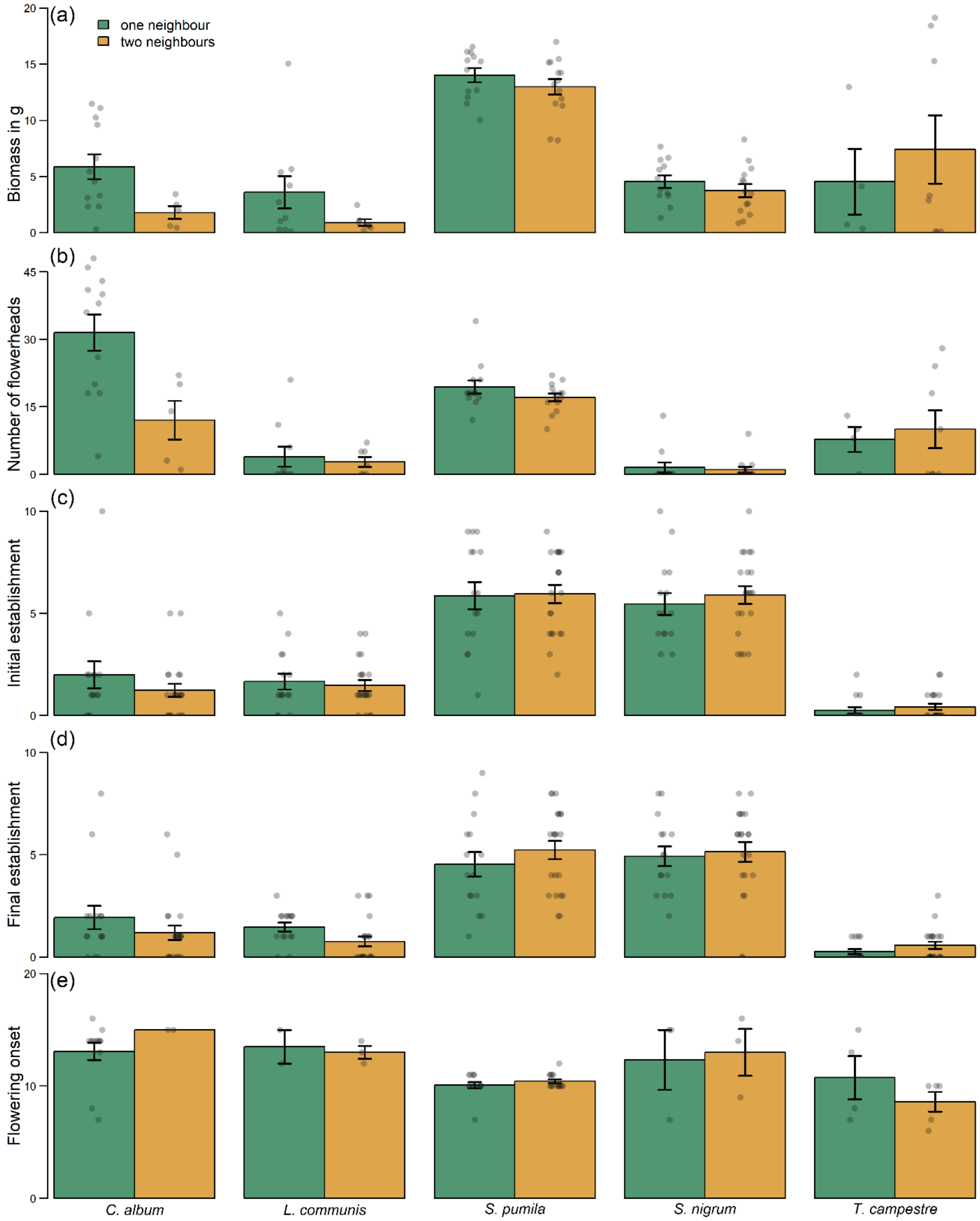

Figure A1 *Species-specific results of the neighbour-experiment. Barplots (means ± SE) show the effect of treatment (one vs two alien neighbour species) on the raw data for the performance measures a) biomass, b) number of flowerheads, c) initial and d) final establishment as well as e) flowering onset for the native target species Chenopodium album, Lapsana communis, Setaria pumila, Solanum nigrum, and Trifolium campestre. Grey dots depict individual datapoints. For sample sizes see Table A1.*

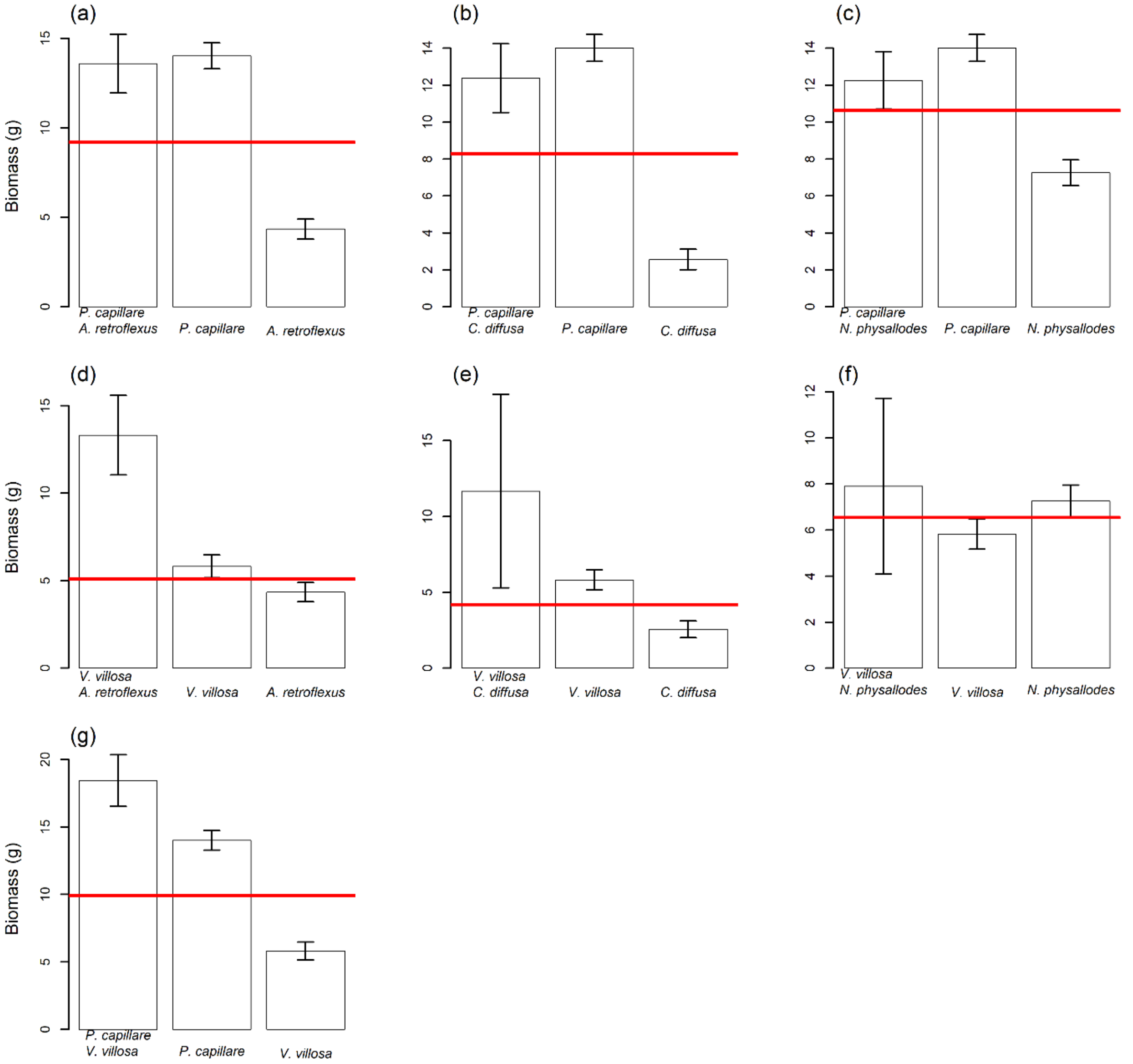

Figure A2 *Neighbour biomass in the neighbour-experiment. Barplots (means ± SE) show the biomass production (g) of alien neighbours. Each panel a) – g) shows data of a specific alien neighbour species combination (first bar) and the two corresponding single neighbour species treatments (second and third bar). The red line indicates the expected biomass production from the average of the single neighbour species treatments. The actual biomass reached in the two-neighbour treatment (first bar) always exceeds the expected biomass. Note that in all neighbour treatments, a total of ten alien seeds were sown.*

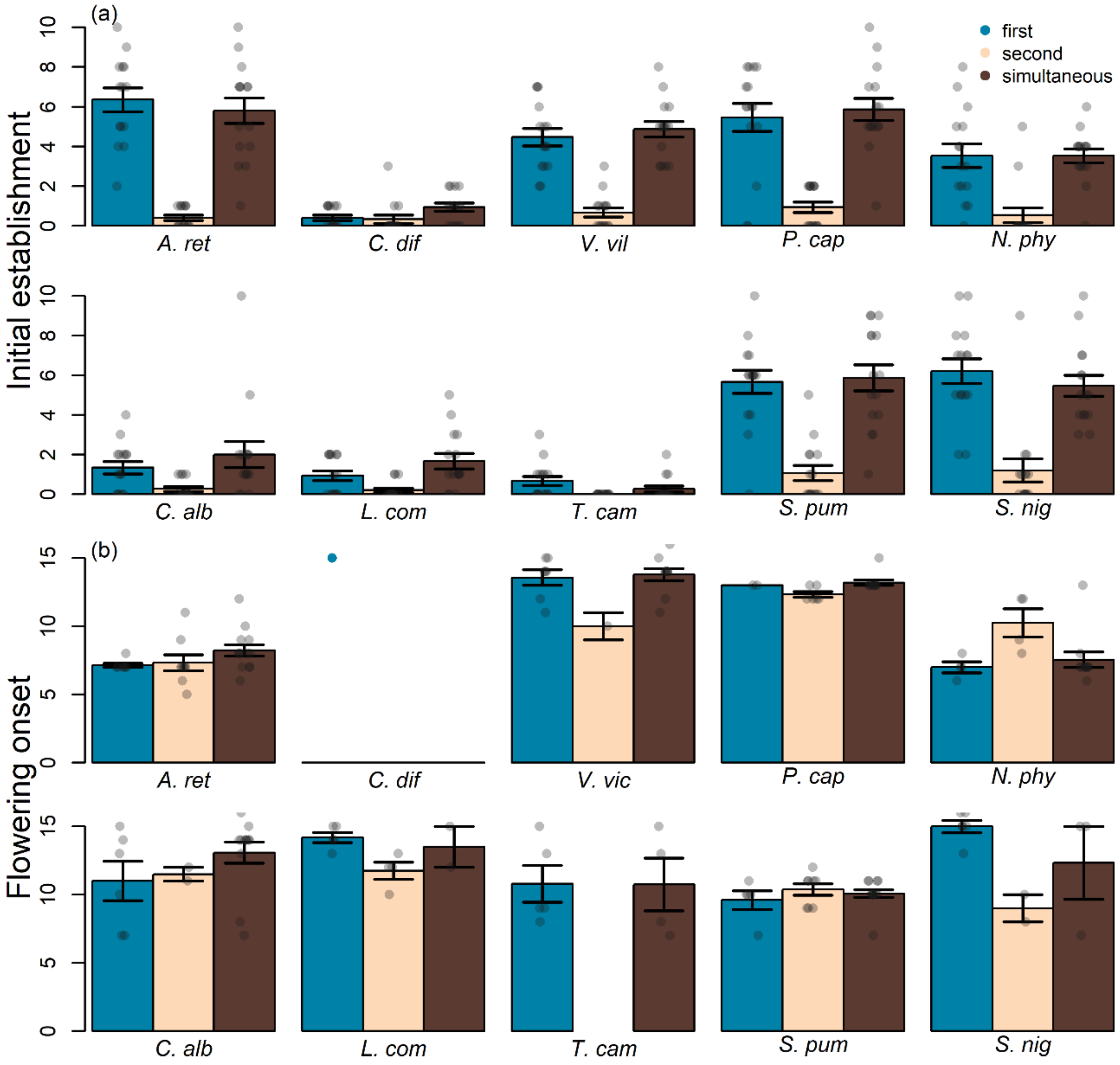

Figure A3 *Results of the priority-experiment. Barplots (means ± SE) show the effect of order of arrival (arriving in the pot first, second or simultaneously with a neighbour) of the respective species on the performance measures a) initial establishment (number of individuals established three weeks after sowing from 10 sown seeds) and b) flowering onset. Top panels for a) and b) depict the five alien target species and the bottom panels the confamilial native target species as listed in Table 3.1. Dots depict individual datapoints. For sample sizes see Table A2.*

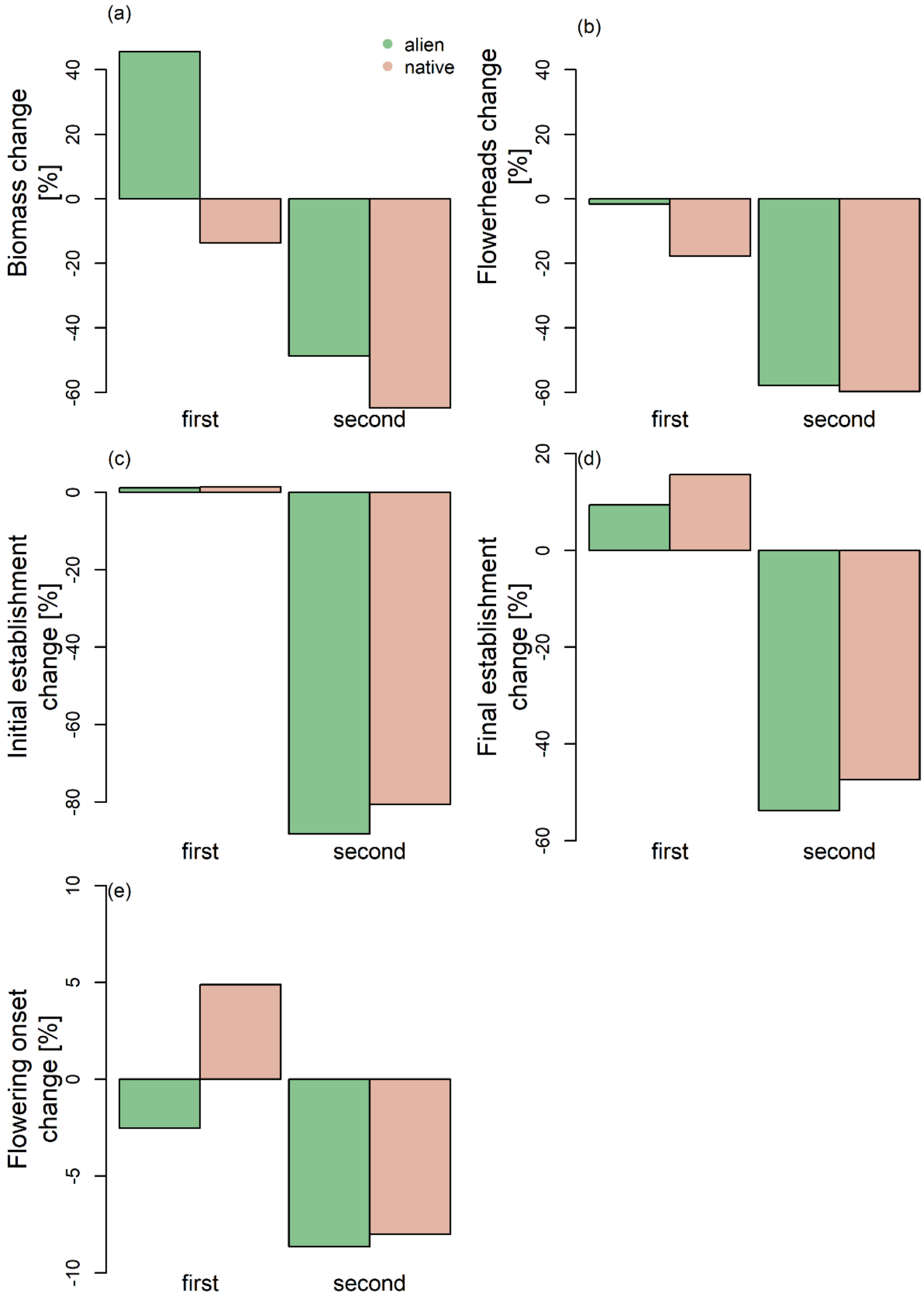

Figure A4 *Results of the priority experiment: comparison of the magnitude of effects on alien versus natives. Barplots show the average percentage change of arriving first (second, respectively) relative to simultaneous arrival for the five performance measures a) biomass, b) number of flowerheads, c) initial establishment, d) final establishment and e) flowering onset.*

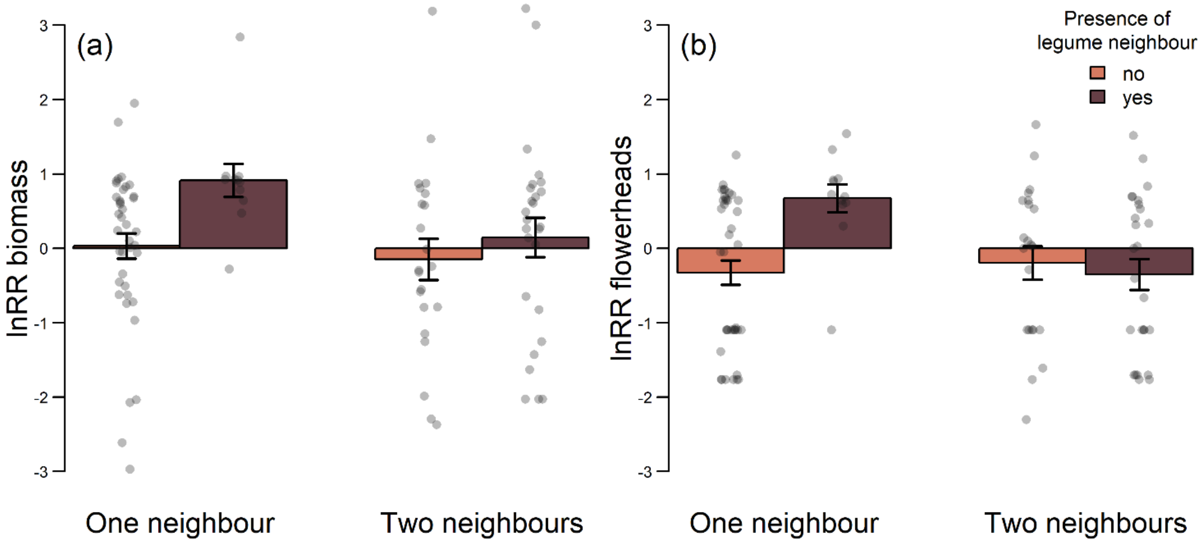

Figure A5 *Results of the neighbour-experiment with regard to presence of a legume neighbour. Barplots (means ± SE) show the effect of treatment (one vs. two alien neighbour species) and presence of the legume neighbour Vicia villosa on the performance measures a) lnRR biomass and b) lnRR flowerheads. Grey dots depict individual data points, with sample sizes 39, 11, 22, 26 (a, from left to right) and 39, 12, 22, 26 (b).*

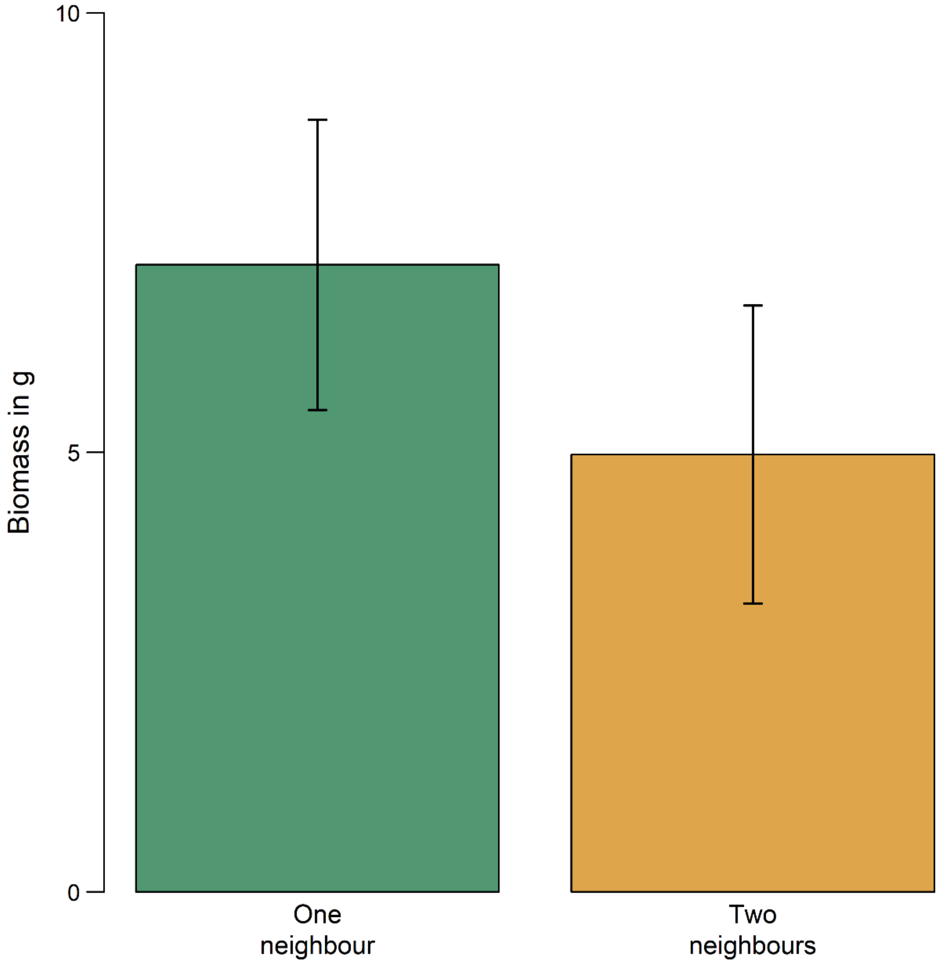

Figure A6 *Results of the neighbour experiment: possible mechanisms for the disappearance of the beneficial legume effect with two alien neighbour species. Barplots (means ± SE) depict alien legume biomass (V. villosa) either alone with the native (biomass divided by two due to double amount of seeds of the alien species, green bar) or with a second alien neighbour (orange bar), averaged across all neighbours and natives.*

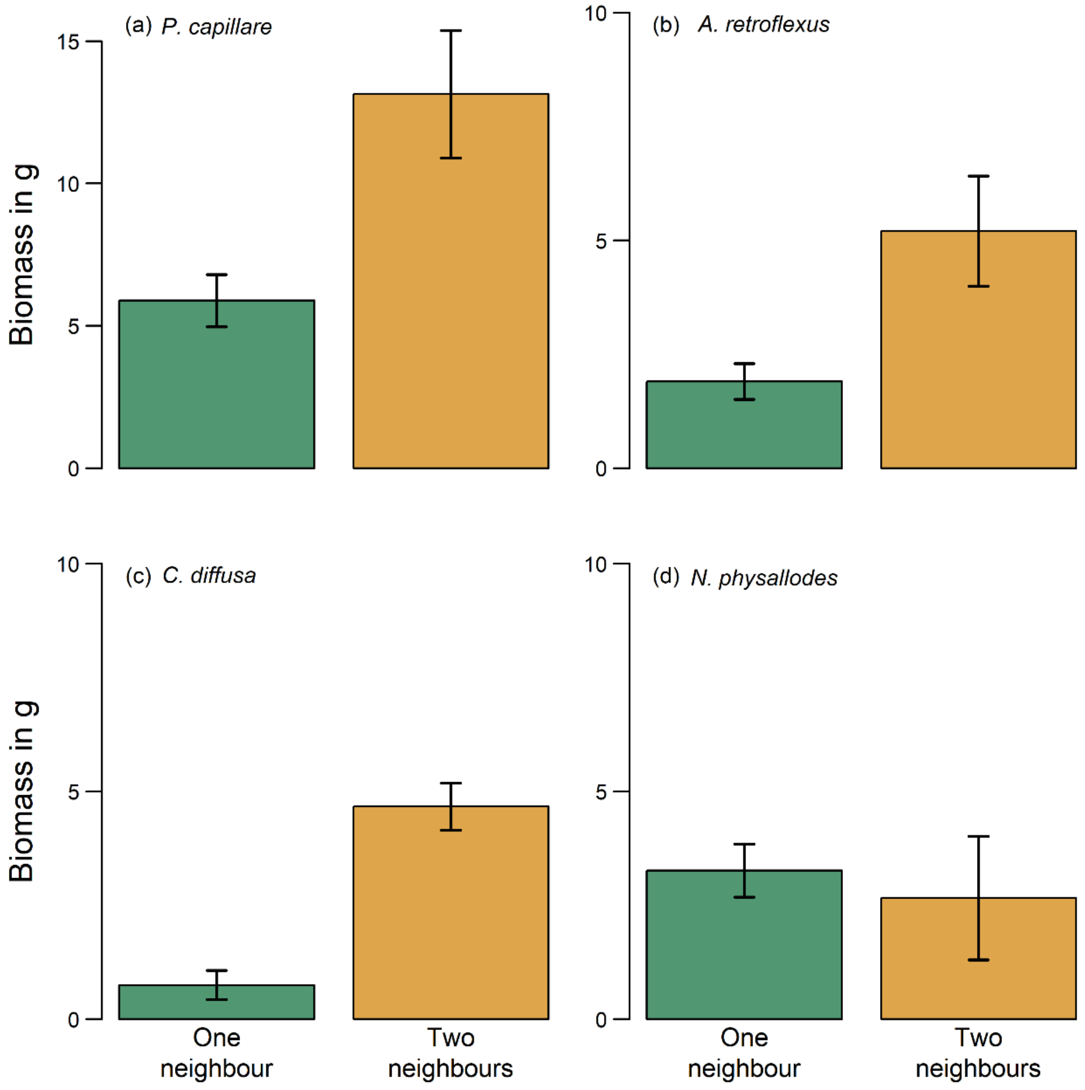

Figure A7 *Results of the neighbour experiment: possible mechanisms for the disappearance of the beneficial legume effect with two alien neighbour species. Barplots (means ± SE) depict biomass of the alien neighbour (a-d), averaged across all native target species, when growing with only the native (biomass divided by two due to double amount of seeds of the alien species, green bars), or with an additional alien neighbour, specifically with the legume V. villosa (orange bars).*

***Table A1** Number of observations used for analysis of the neighbour-experiment part for each species for the five performance measures*

Species	Number of neighbour species	Biomass	Number of flowerheads	Initial establishment	Final establishment	Flowering onset
Chenopodium album	One neighbour	12	12	15	15	12
	Two neighbours	5	5	21	21	2
Lapsana communis	One neighbour	10	10	15	15	2
	Two neighbours	7	7	21	21	3
Setaria pumila	One neighbour	12	13	15	15	13
	Two neighbours	14	14	21	21	14
Solanum nigrum	One neighbour	12	12	15	15	3
	Two neighbours	14	14	21	21	3
Trifolium campestre	One neighbour	4	4	15	15	4
	Two neighbours	8	8	21	21	5

***Table A2** Number of observations used for analysis of the priority effects part for each species in each order of arrival treatment for the five performance measures*

Species	Order of arrival	Biomass	Number of flowerheads	Initial establishment	Final establishment	Flowering onset
Amaranthus retroflexus	first	7	7	14	14	7
	second	11	11	15	15	9
	simultaneous	13	13	15	15	13
Centaurea diffusa	first	4	4	15	15	1
	second	3	3	15	15	0
	simultaneous	5	5	15	15	0
Nicandra physalodes	first	4	4	15	15	4
	second	4	4	15	15	4
	simultaneous	11	11	15	15	11
Panicum capillare	first	2	2	15	15	2
	second	6	6	15	15	6
	simultaneous	10	10	15	15	10
Vicia villosa	first	8	8	15	15	7
	second	9	9	15	15	1
	simultaneous	12	12	15	15	10
Chenopodium album	first	6	6	15	15	6
	second	2	2	15	15	2
	simultaneous	12	12	15	15	12
Lapsana communis	first	7	7	15	15	5
	second	9	9	15	15	4
	simultaneous	10	10	15	15	2
Setaria pumila	first	5	5	15	15	5
	second	8	8	15	15	8
	simultaneous	12	13	15	15	13
Solanum nigrum	first	9	9	15	15	6
	second	5	6	15	15	2
	simultaneous	12	12	15	15	3
Trifolium campestre	first	6	6	15	15	5
	second	0	0	14	14	0
	simultaneous	4	4	15	15	4

6.3. Supplementary material Chapter 4

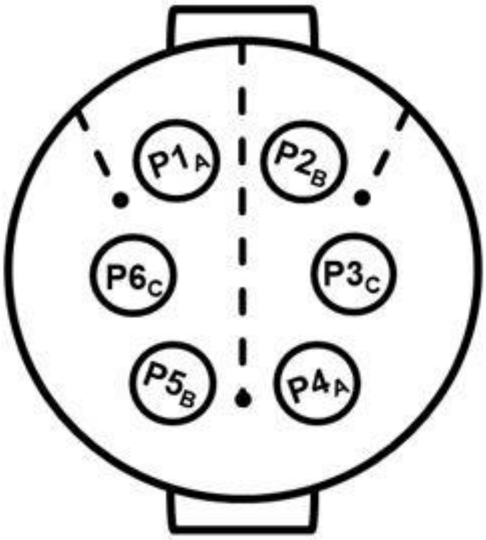

Figure A1 *Experimental set-up of focal Asteraceae individuals within the native community. The sketch depicts the placement of the 6 focal plant individuals (P1-P6) originating from (ideally) 3 different wild populations (A-C). Black dots and dashed lines indicate the placement of the irrigation system.*

Table A1 *Initial set up of leguminous and non-leguminous communities. A general recommendation of the seed supplier (Rieger-Hofmann GmbH) for sowing density is $3g/m^2$ this translates to 477mg/pot in our experiment. The table presents individual seed weights, the number of seeds per species and the total seed weight per species per pot. Species marked grey were sown initially but did not germinate during the experimental period. Ellenberg values (Ellenberg & Leuschner, 2010) indicate nitrogen availability in common habitats of the respective species, where low values indicate typically nutrient poor habitats and high values nitrogen-rich habitats. The letter "x" indicates highly variable nitrogen level at occurrences of the species.*

		leguminous		Non-leguminous		
Community species	Weight/seed (mg)	Seed number	Total seed weight (mg)	Seed number	Total seed weight (mg)	Ellenberg indicator Nitrogen
Bromus erectus Huds.	5.40	24	129.60	29	156.60	3
Campanula rapunculus L.	0.02	84	1.68	100	2.00	4
Carex flacca Schreb.	0.83	24	19.92	29	24.07	4
Elymus repens (L.) Gould	3.00	24	72.00	29	87.00	7
Euphorbia cyparissias L.	2.20	20	44.00	25	55.00	3
Falcaria vulgaris Bernh.	2.00	20	40.00	25	50.00	x
Festuca rupicola L.	0.50	16	8.00	20	10.00	2
Galium verum L.	0.50	42	21.00	50	25.00	3
Potentilla argentea L.	0.10	42	4.20	50	5.00	1
Salvia pratensis L.	1.80	20	36.00	25	45.00	4
Silene nutans L.	0.30	42	12.60	50	15.00	3
Verbascum lychnitis L.	0.10	42	4.20	50	5.00	3
Medicago lupulina L.	2.20	40	88.00	-	-	x

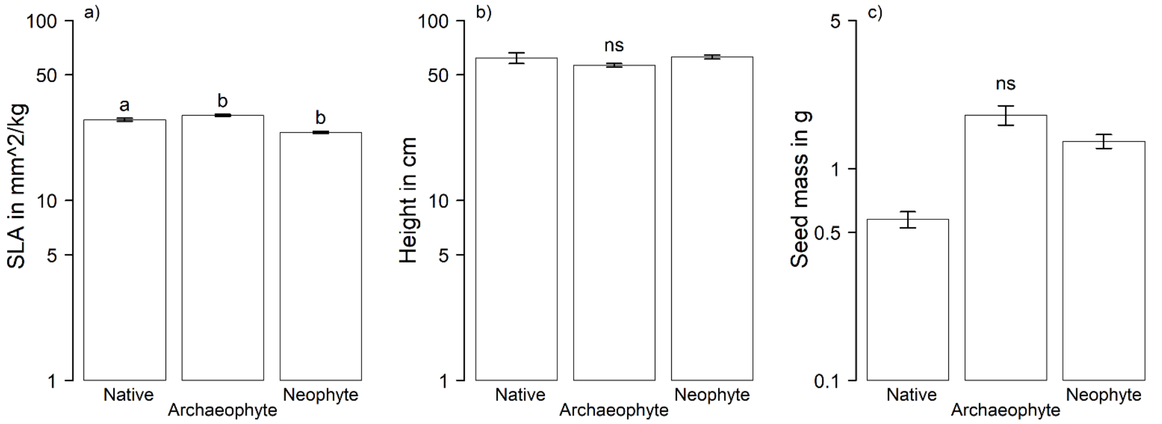

Figure A2 *Differences among status groups of the traits specific leaf area SLA (a), height (b) and seed mass (c). Barplots depict species-level trait values on log-transformed y-axes. Different letters indicate significant differences (P<0.05) among status groups tested with ANOVA and TukeyHSD test. Barplots show mean ± 1 SE.*

***Table A2** Results of statistical tests on how allocation of resource investment of focal Asteraceae species in reproductive to total aboveground biomass is affected by functional traits and their interaction with community type. All relevant terms after model simplification are listed with their respective χ^2 test statistic. Logit transformation was performed with the car package (Fox & Weisenberg, 2019), R2 values (calculated with the MuMIn package, Bartón, 2019) describe how much of the overall variation is explained by the fixed effects (R^2_m) and the fixed and random effects together (R^2_c).*

Response variable	Sample size	Explanatory variables	Test statistic	R^2_m	R^2_c
$logit \frac{\text{reproductive biomass}}{\text{total aboveground biomass}}$	602	SLA* community	χ^2_{2df}=10.6; *P*=0.001	0.14	0.73
		Height	χ^2_{2df}=2.8; *P*=0.095		
		Initial height	χ^2_{1df}=46.5; *P*<0.001		
$logit \frac{\text{reproductive biomass}}{\text{total aboveground biomass}}$	90	N concentration *community	χ^2_{2df}=5.4; *P*=0.02	0.01	0.92

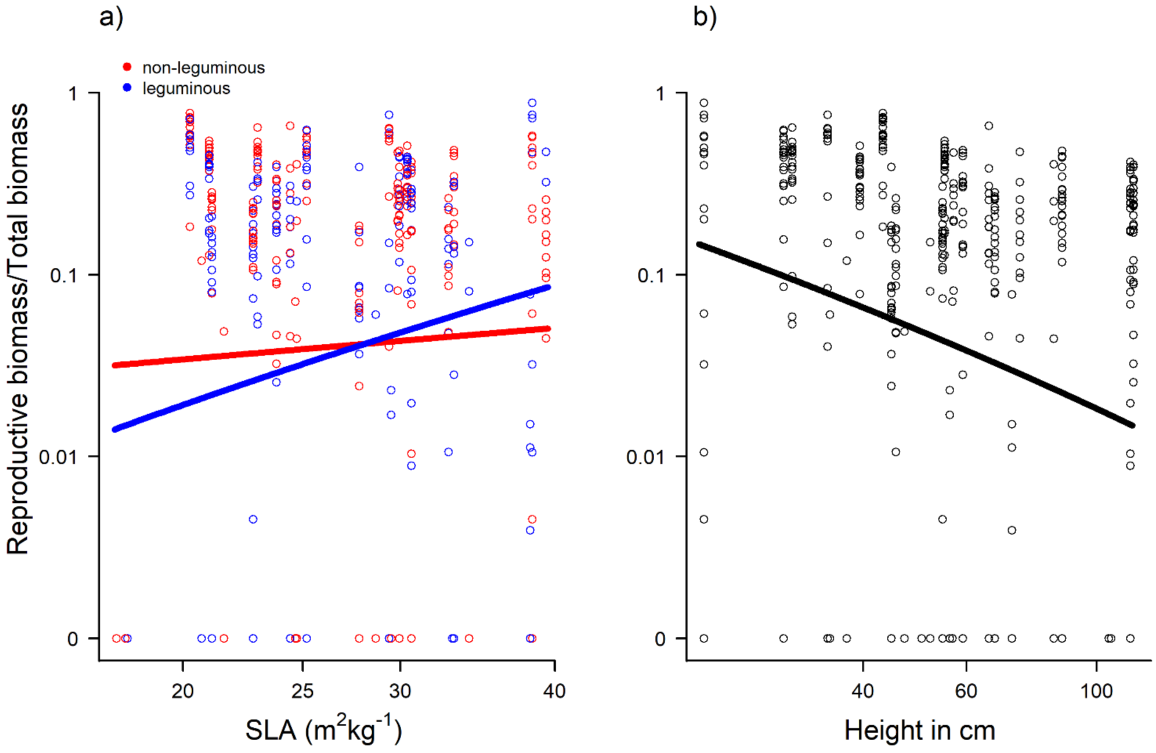

***Figure A3** Predictions of the relevant trait effects (a SLA; b height) based on the statistical model on the ratio between reproductive biomass to total biomass (as measure of resource allocation) at focal individual level. Zero indicates no seed production, the higher the value the more reproductive biomass in relation to total biomass was produced. Circles indicate raw data points. Axes are log-transformed. Different colours indicate different community types: non-leguminous (red) and leguminous (blue).*

Table A3 *Results of statistical tests, averaged across individuals within a pot, on how alien species fitness (response variables aboveground biomass and total seed mass, log-transformed) are affected by functional traits and their interaction with community type. All relevant terms after model simplification are listed with their respective* χ^2 *test statistic.* R^2 *values (calculated with the MuMIn package, Bartón, 2019) describe how much of the overall variation is explained by the explanatory variables (*R^2_m*) and the explanatory variables and random effects together (*R^2_c*).*

Response variable	Sample size	Explanatory variables	Test statistic	R^2_m	R^2_c
Log biomass	117	SLA*community	χ^2_{2df}=6.9; *P*<0.008	0.19	0.66
		Height	χ^2_{2df}=7.8; *P*=0.005		
Log (Total seed weight +1)	117	SLA*community	χ^2_{2df}=7.1; *P*=0.007	0.15	0.74
		Seed mass	χ^2_{2df}=2.8; *P*=0.094		

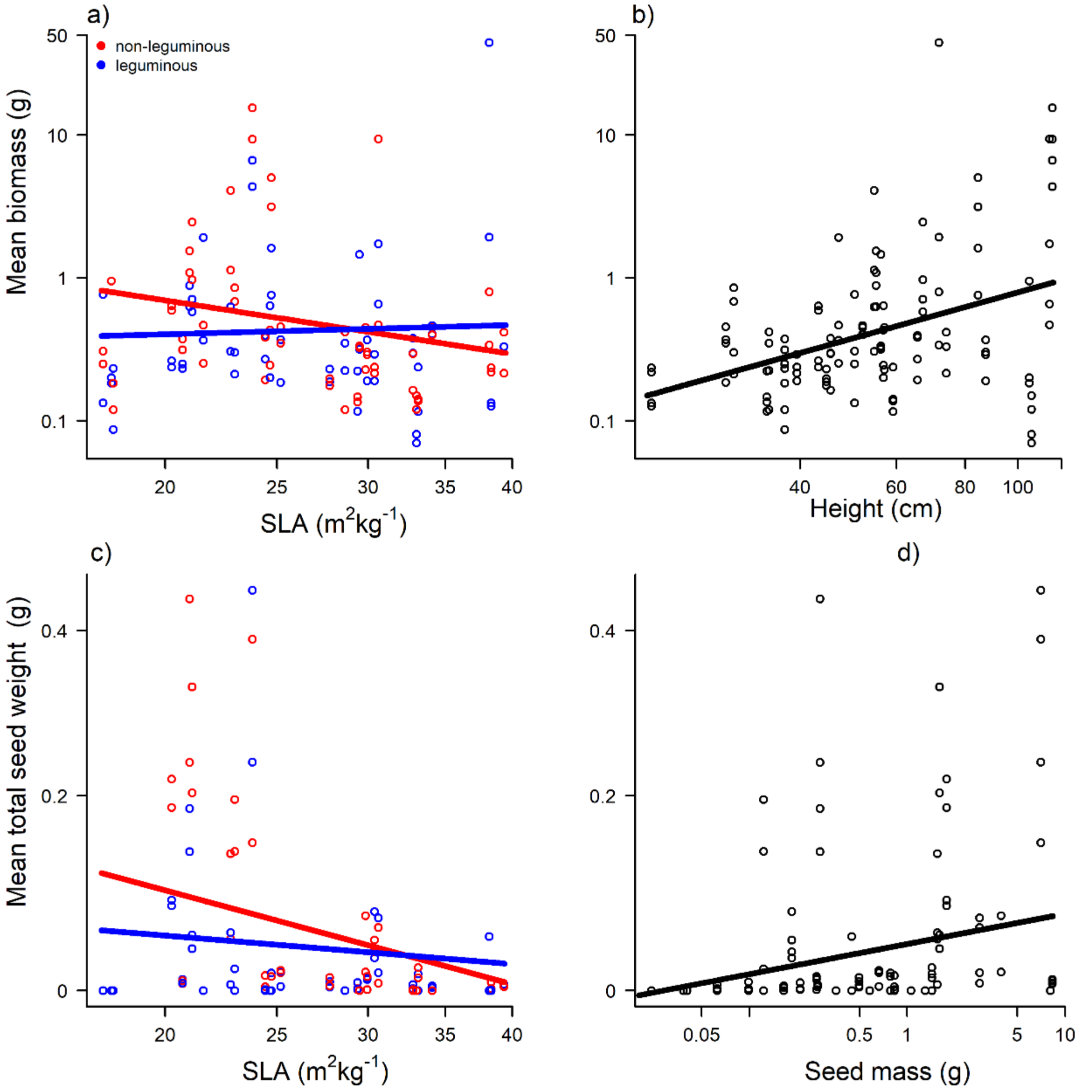

Figure A4 *Predictions of trait effects on the response variables biomass (upper panels) and total seed weight (lower panels), averaged across individuals within a pot (n= 117). Displayed are the predicted effects of the relevant traits for the respective response variable: SLA (a) and height (b) on biomass, and SLA (c) and seed mass (d) on total seed weight. All axes are log-transformed. Different colours indicate different community types: non-leguminous (red) and leguminous (blue).*

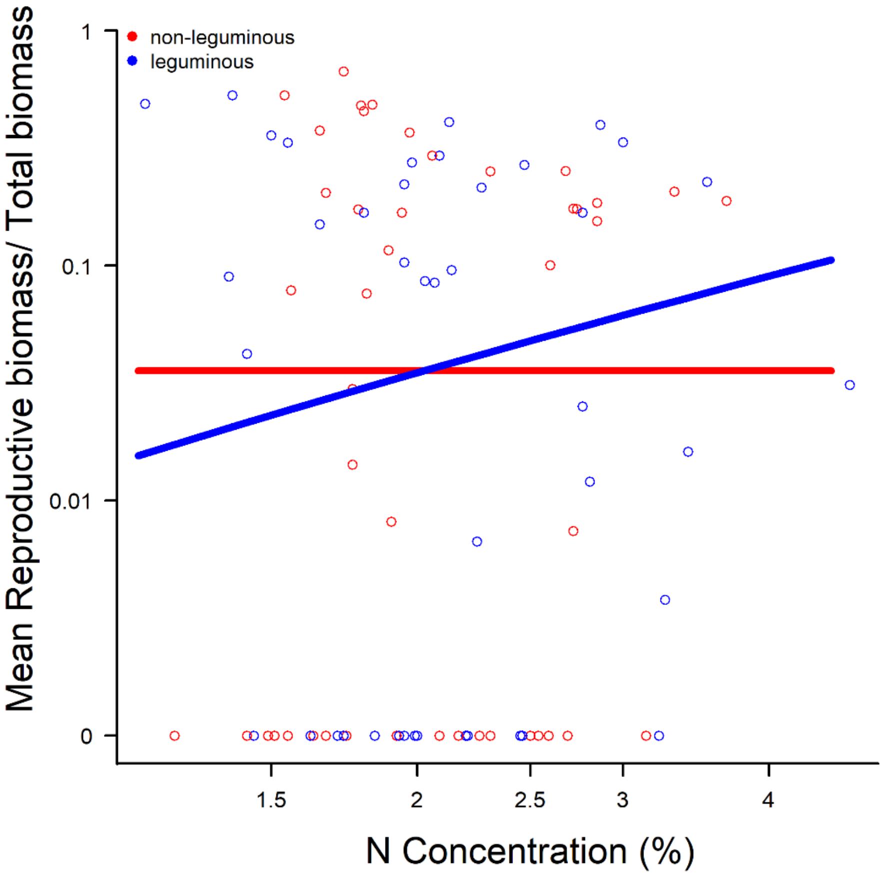

Figure A5 *Predictions of the effect of N concentration based on the statistical model on the ratio between reproductive biomass to total biomass (as measure of resource allocation) at pot level. Circles indicate raw data points. Axes are log-transformed. Different colours indicate different community types: non-leguminous (red) and leguminous (blue).*

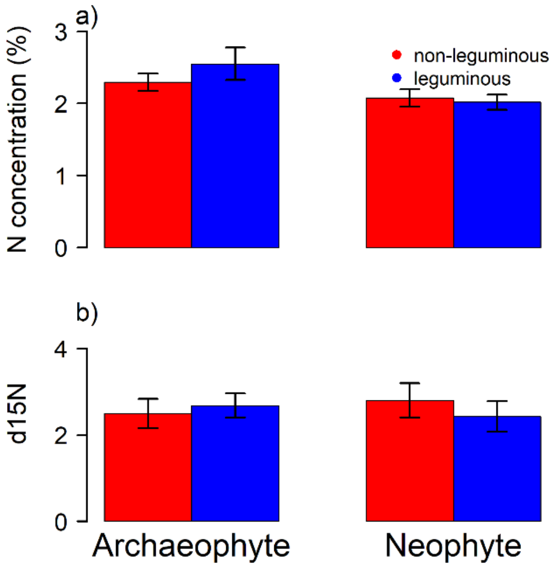

Figure A6 *Barplots comparing focal species isotope measures N concentration and δ^{15}N across status groups (archaeophyte and neophyte) growing in the two community types, without a legume (red) and with a legume (blue). Barplots show mean ± 1 SE.*

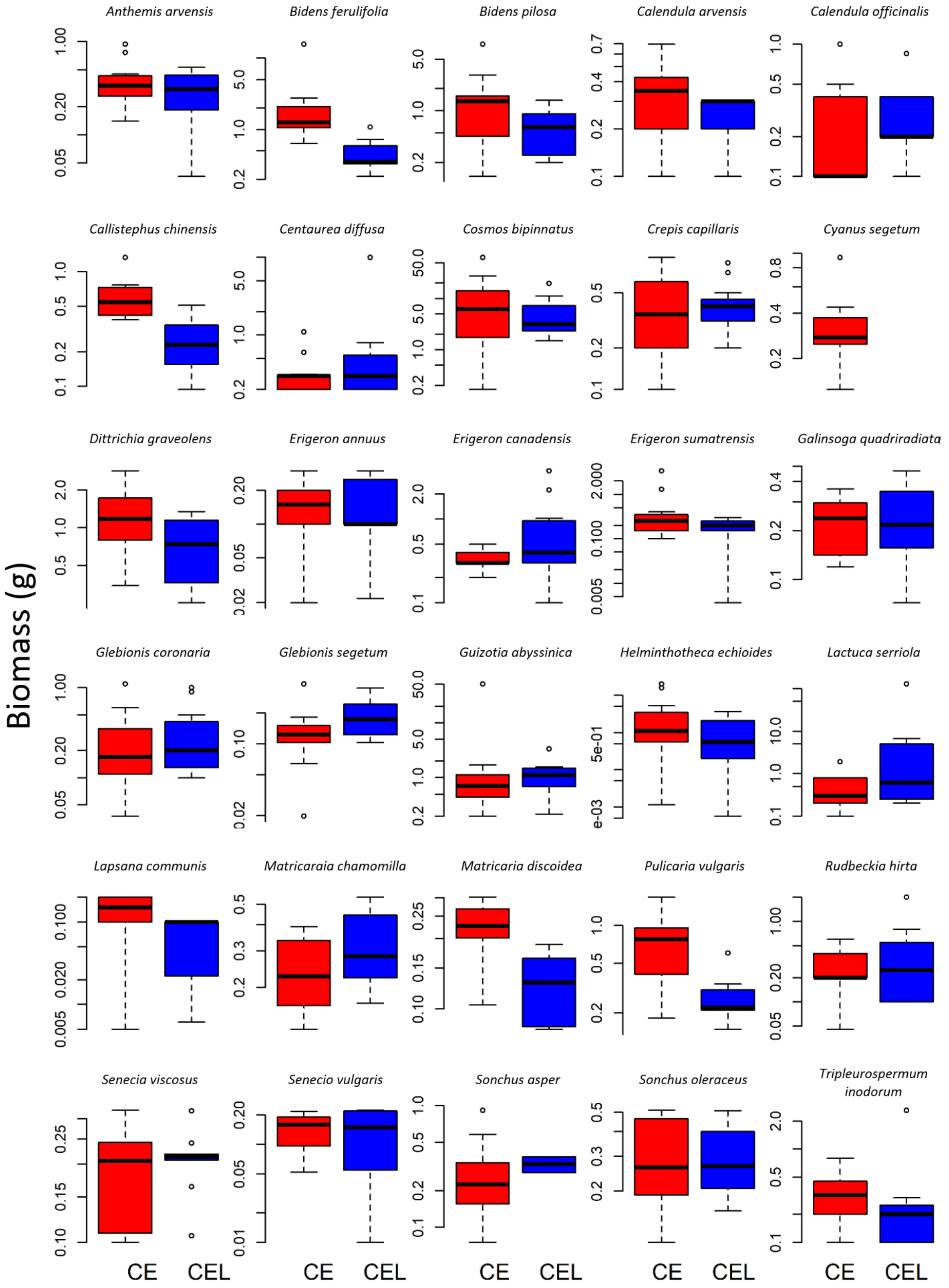

Figure A7 *Boxplots comparing aboveground biomass per community type for each Asteraceae species (usually across 12 individuals). Data for individuals growing without a legume (CE, red boxes) and with a legume (CEL, blue) is depicted. Note that for Cyanus segetum no individuals in legume presence survived. Y axis is on log-scale.*

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6.4. Supplementary material Discussion

Experimental attempts to investigate co-occurring alien species in a community context

6.4.1 Interactions among multiple co-occurring alien species in a native community and effects on the native community

Since I found interactions among two co-occurring alien species could be explained by hierarchical trait differences in chapter 2, I aimed at investigating whether this would also be found in a more natural setting including a native community. It is commonly assumed that more dissimilar species could invade a community more easily. In this context I conducted an experiment to test whether a set of more dissimilar alien species could invade a community better than more similar species. Further, whether this can be explained by phylogenetic or multi-trait distance to the community (community weighted means). I further wanted to test whether the number of co-occurring alien invader has an effect on competition outcome, so whether having one, two or three co-occurring alien species makes a difference.

As priority effects are an important part of community assembly research but this has rarely been investigated for invasive species within this experiment I set up a priority effect treatment investigating co-occurring alien species within a native community. I found strong effects of competitive ability explaining species performance in chapter 3. In this part of the experiment I wanted to investigate whether species with more similar niche requirements would pose stronger priority effects on each other or whether the magnitude of priority effects can be explained better by competitive ability.

To assess how competition outcome translated into the next generation (of the annual species) I aimed at quantifying intrinsic population growth of the species within the communities and investigate how the reproductive success in the first year would change community composition in the following year.

Experimental Set-Up

I set up a common garden pot experiment in February 2019. In total I set up 500 pots with 50L volume, filled with wood chips at the bottom to improve drainage and local soil. The native community was established first, in April I sowed the background community consisting of seven ruderal species that are native to Germany (*Achillea millefolium, Daucus carota, Festuca rupicola, Galium album, Poa angustifolia, Potentilla argentea, Silene vulgaris*). They were sown in the density usually used for restoration projects according to the seed supplier (Rieger-Hofmann, Blaufelden, Germany) with a density of 2 g/m^2. To assess the impact on the native community, I additionally planted one native phytometer individual (*Lapsana communis*) in each of the pots. Since these individuals would be subject to different communities but were planted at the same time and have the same origin, the effects can be compared.

After an establishment period of 8 weeks I sowed the 20 target species into the pot. I used 30 seeds in each community, for the one-alien treatment they consisted of one species, for the two-alien treatment of two different species with 15 seeds each and for the three-alien treatment we sowed three species with ten seeds each. Although we aimed at a full factorial comparison of a set of 20 different species due to logistical constraints I could not investigate all possible combinations of the three-alien treatment. I used two replicates of the one-species treatment, I used all possible two-alien combinations and randomly picked three-way combinations from all possible combinations but made sure to have even numbers of plant functional group combinations.

The two-alien treatment was additionally used as “simultaneous” for the second part of the experiment investigating priority-effects. To assess priority effects of one of the species to arrive earlier than the other we sowed the species combinations two more times with a delay between the species, to end up with each species being sown first, second and simultaneously (in three different pots).

I planned to assess species performance of all species after two seasons by harvesting biomass and count the reproductive output. Further I wanted to measure functional traits for each species in a control treatment to investigate how trait differences could explain the outcome of the species interactions.

Results

While the community grew very well (except for *Festuca rupicola*, see Figure A1), the focal species did not germinate to a large part (68% of the pots not a single target species seedling found no germination after 8 weeks). Since such a large share of focal species did not germinate, I tried to still perform the experiment by transplanting the focal individuals in the pots where nothing has germinated (6 in each pot, either one, two or three different species; all simultaneously and therefore dropping the priority part of the experiment). Although I could germinate most of the species in the greenhouse and could transplant them, it was already comparably late in the season (end of July) and the seedlings could not establish in the meanwhile very dense community (see Figure A2). By the end of September only 41 individuals flowered in total of which ca 20% were transplanted and the others sown. The 41 individuals belonged to only 4 different species (*Panicum capillare, Vicia villosa, Medicago polymorpha, Eragrostis minor*) of two families (Poaceae, Fabaceae). Of 1072 'target populations' (i.e. pots of the single-alien treatment contained one target population, pots of the three-alien treatment contained three target populations) only 196 populations (18%) had at least one individual present at the end of the season with an average target-population cover of 3%.

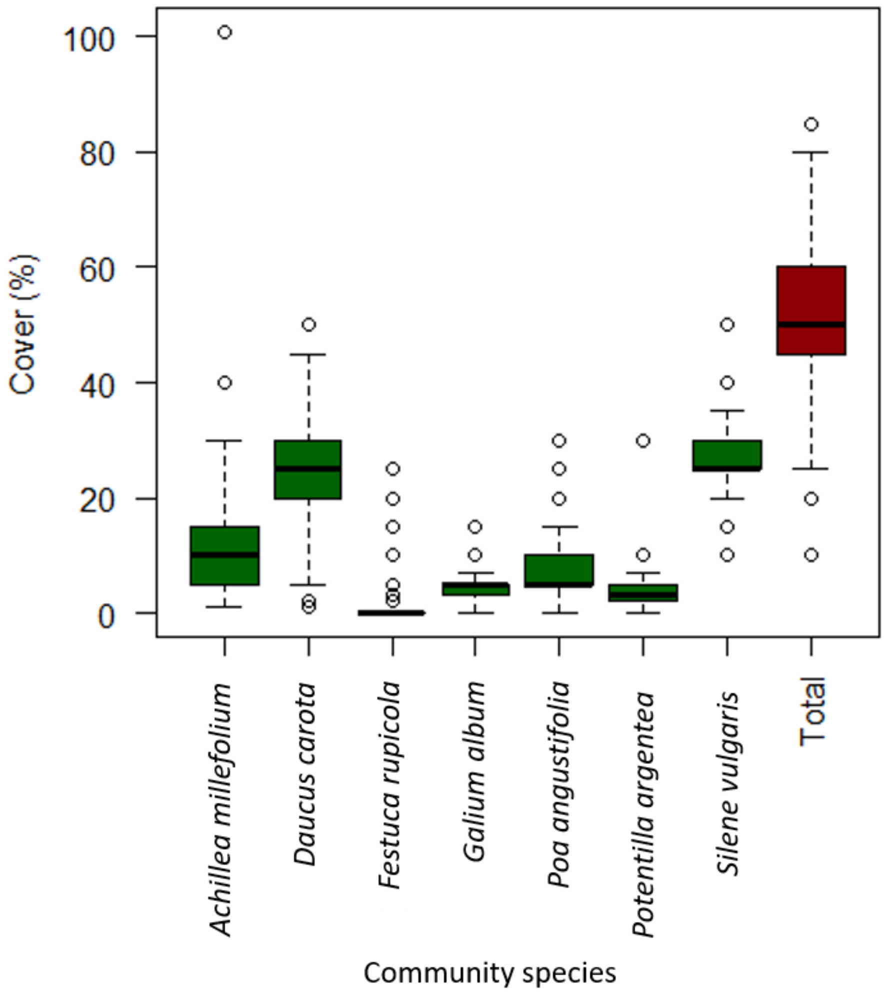

Figure 6.4.1 Boxplot of cover per pot of native community in June. Individual community species are depicted in green, total cover is shown in red.

Figure 6.4.2 Pictures of experimental community. Top picture shows established native community in 50L pots. The community is wrapped in organza mesh to prevent seeds to enter the community but also seeds to be dispersed out of the community (since the experiment was supposed to run for two years and measure population growth of the species). The bottom picture shows an *Amaranthus albus* seedling after transplanting in July. Both pictures illustrate the high community density that was presumably the reason why target individuals could not establish and produce seeds.

Discussion

Likely sowing the alien species into the native community was too late, and the community was already too competitive and most of the alien species could not establish (except for a few very fast-growing neophytes such as *Cosmos bipinnatus* or *Bidens pillosa*). Similarly, it was already too late for the transplanted seedlings, which even when establishing successfully perhaps would not have had time to finish their life cycle. Additionally, in June 2019 they experienced some days with high temperatures (more than 30 °C) and temperature drop and rain in mid-July, which could perhaps have influenced their establishment.

6.4.2 Establishment success of co-occurring alien species in a native community exposed to drought and disturbance

Due to climate change, plants are experiencing altered precipitation patterns such as drought. Drought has been shown to affect alien species establishment, however outcomes are variable and expected to depend on species and habitat types. Besides the impact on alien species, altered precipitation can also impact native communities and therefore result in reversed effects which might lead to more alien species establishment. Human-mediated disturbance is a commonly experienced factor and previous work shows alien species spread and establishment to benefit from disturbance. Especially, since alien species tend to have ruderal trait values which can be successful in habitats prone to disturbance. However only few studies up to date have investigated those factors simultaneously but studies that did hint at an interaction of climate change effects human-mediated (Lembrechts et al., 2016), but see more recent studies (Orbán et al., 2021; Yang et al., 2022) who identified interactive effects and hint at differing responses of native and alien species to these interactions. To further generalize these findings and assess the impact of combined factors in different environmental systems more studies are needed. Especially in the light of increasing alien species introductions more attention has to be paid to interactions among multiple co-occurring alien plant species. Therefore, studies should investigate different factors of climate change but also disturbance as well as a broader set of species and their interactions to identify whether effects are generalisable.

I aimed at investigating two research questions, a) can alien species establish better under more stressful conditions; b) are magnitude or direction of interspecific interactions among co-occurring alien species changing in different stress conditions.

To answer these questions, I used the communities from the (failed) common garden pot experiment of 2019 to simulate drought and soil disturbance. I only used pots in which no alien species was present anymore from the previous experiment, which resulted in 417 pots in total. Since the native community has produced much biomass I mowed all biomass down to 5cm above ground before attempting to establish focal species. The native community only consisted of six species, since the few individuals of *Festuca rupicola* that grew in 2019 (see 6.4.1, Figure 6.4.1) did not grow back in 2020. Since I used a drip-irrigation system for the experiment, I removed all drippers from pots assigned to drought-treatment from the beginning of the year to make sure the communities experience drought stress. I only started irrigation 12 weeks after starting the experiment. Disturbance treatment was applied by disturbing the soil surface and creating bare ground in a 10cm diameter around the designated position of the focal alien transplants.

I used 14 alien species from five different families. All species were sown in a greenhouse in the beginning of April to increase chances of high germination. I transplanted the seedlings in the last week of May. For the one-alien treatment I used three replicates of each species * treatment combination (drought + disturbance; no drought + disturbance; drought + no disturbance), with an additional control treatment (no drought + no disturbance) with a total of 168 pots. For the two-alien species I randomly picked 83 of all possible species combinations since only 249 pots were left (as the pots were re-used from the 2019 experiment, see 6.4.1).

Results

Out of 2502 transplanted individuals only 707 were present at harvest (28%) with a mean aboveground biomass of 1.4 g (standard deviation ± 0.24; highly impacted by *Cosmos bipinnatus*, see Figure 6.4.3). Across all species most biomass was produced in disturbance + drought treatment (1.7 ± 0.65 g); similar in the disturbance + no drought treatment (1.6 ± 0.37 g) and least biomass in no disturbance + no drought treatment (0.9 ± 0.17 g). Averaged across all pots the native community had a very high cover of 93.7 ± 0.45 %.

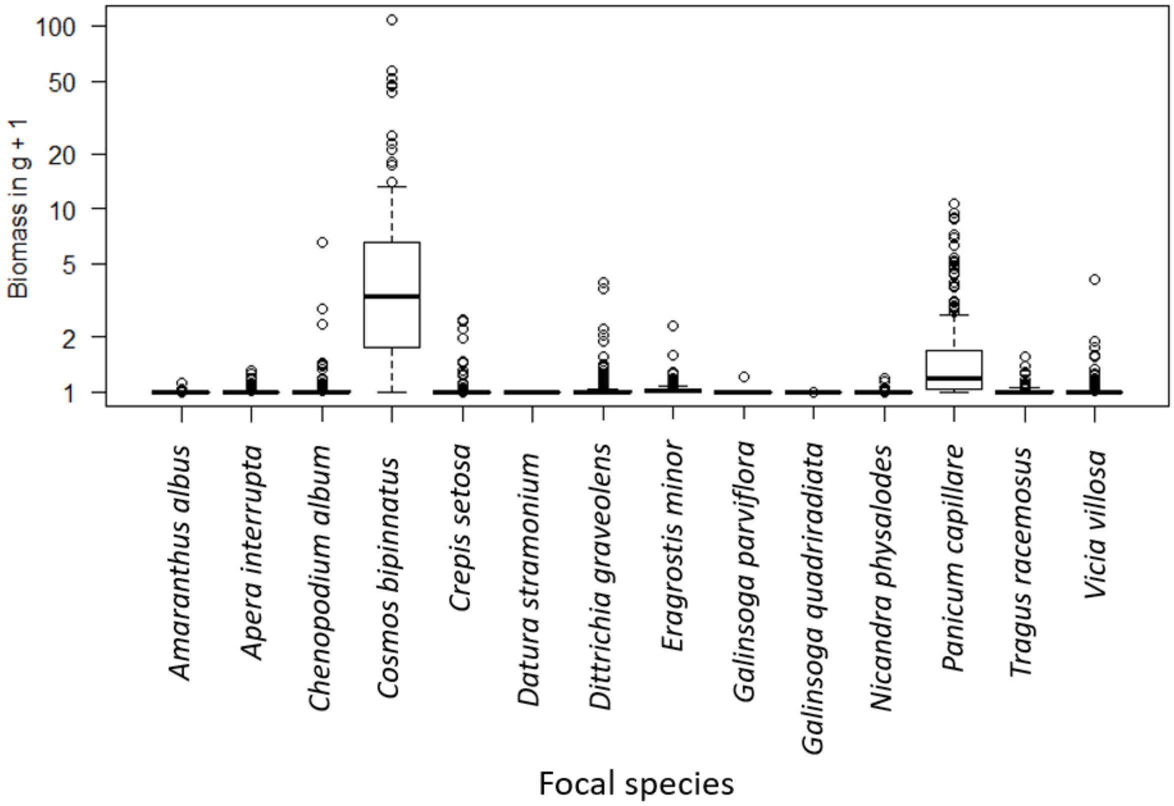

Figure 6.4.3 *Boxplot depicting biomass harvest of the 14 focal species. The y-axis is log-transformed.*

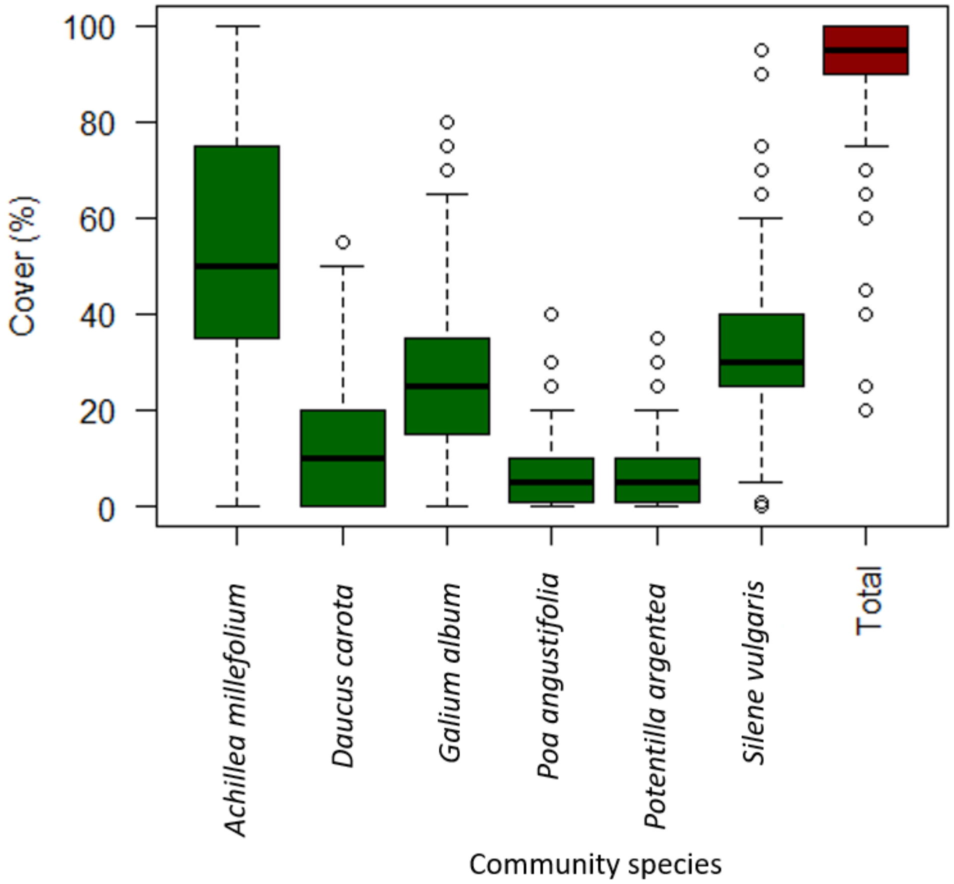

Figure 6.4.4 *Boxplot of cover per species of native community in September 2020. Individual community species are depicted in green, total cover is shown in red.*

Figure 6.4.5 *Pictures of experiment. The top picture shows an individual of Panicum capillare at the end of the season. Although it did flower and produce seeds it remained very small and had supposedly little impact on the native community, despite growing in a disturbance treatment. The bottom picture shows two native communities, on the left in no disturbance + drought treatment, on the right in disturbance + no drought treatment. Both communities grew very dense although in drought treatment I saw a tendency of lower community height.*

Discussion

The low establishment and growth of alien species likely is again due to a too strong native community. Although the community was cut down at the beginning of the season, and experienced drought and or disturbance, the impact was not severe enough to enable more alien species to establish. Another explanation could be low nutrient levels in the pots when transplanting the alien species, since in the previous year the native community has already been growing there. Perhaps due to their well-established root system they could still acquire enough resources while the alien seedlings could not compete.

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