

Phylogenetic and functional mechanisms of direct and indirect interactions among alien and native plants

Yanhao Feng* and Mark van Kleunen

Ecology, Department of Biology, University of Konstanz, Universitätsstrasse 10, Konstanz D-78464, Germany

Summary

1. Disentangling direct and indirect interactions among alien and native plants is essential to understanding the success of alien plants in multispecies communities, but studies have only focused on direct pairwise interactions. Moreover, it is also essential to explore phylogenetic and functional mechanisms driving these interactions.

2. In a greenhouse experiment, we selected nine groups of alien and native plant species from the herbaceous flora of Germany to disentangle their direct and indirect interactions. Each group had an alien (A) that is common or rare in Germany (i.e. non-native range), two natives that are phylogenetically closely related (N_{close}) and distantly related (N_{dist}) to A respectively, and a distantly related “target” native (T). We grew the four species of each group alone, and in two-species and three-species combinations. Specifically, we tested whether competition is greater between A and N_{close} than between A and N_{dist} , whether presence of N_{close} rather than N_{dist} indirectly alleviates competition of A on T, and whether these interaction patterns depend on commonness of A. Moreover, we tested how intensity of these interactions is explained by phylogenetic distance, functional traits (height, seed mass, SLA, leaf size, specific root length, leaf area ratio, root length ratio (root length/plant mass), shoot weight ratio) and traits-based functional distance.

3. We found A had stronger competition on N_{close} than on N_{dist} . In turn, A was more suppressed by N_{close} than by N_{dist} , but this was only true for rare rather than common A. The presence of N_{dist} rather than N_{close} indirectly reduced competition of A on T. The intensity of these interactions was not explained by phylogenetic or functional distance, but by some of the functional traits. Specifically, a plant experienced stronger competition when it was shorter and had smaller leaves and lower shoot weight ratio, and when its neighbours were taller, had greater SLA, leaf area ratio and shoot weight ratio, and had a lower root length ratio.

4. *Synthesis.* Functional traits can help explain competitive interactions. While direct competition tended to be stronger between more closely related alien and native plants, this did not indirectly facilitate other co-occurring native plants.

Key-words: competition, exotic plants, functional distance, functional traits, indirect interaction, invasiveness, niche, phylogenetic distance, plant-plant interactions, trait-based interactions

Introduction

The invasion by alien plants alters the structure and composition of recipient native communities in new ranges, and thereby causes ecological damage such as loss of biodiversity (Vilà *et al.* 2011). Once alien plants enter recipient communities, they will inevitably be involved in multispecies interactions with resident native plants. Success of alien plants in a recipient community is, to a certain degree, the ecological and evolutionary outcome of these interactions. Therefore, to

understand why some alien plants become successful in new ranges while others fail, it is essential to disentangle the complexity of interactions among alien and native plants (Mitchell *et al.* 2006; White, Wilson & Clarke 2006).

Darwin's naturalization hypothesis predicts that alien species that are phylogenetically more closely related to native species should be less successful in a recipient community (Darwin 1859). This is because more closely related species share ecological niches, and therefore should be less likely to stably coexist in a local community as a result of competitive exclusion (Gause 1934; MacArthur & Levins 1967; Silvertown 2004; Violle *et al.* 2011). However, studies testing for Darwin's naturalization hypothesis often found mixed results

*Correspondence author: E mail: yanhao.feng@uni-konstanz.de

for the relationship between phylogenetic relatedness and naturalization or invasion success (Duncan & Williams 2002; Strauss, Webb & Salamin 2006; Diez *et al.* 2008; Proches *et al.* 2008; Jiang, Tan & Pu 2010; Park & Potter 2013). It has also been frequently shown that the strength of competition between plant species is not correlated with phylogenetic relatedness (Cahill *et al.* 2008; Dostál 2011; Fritschie *et al.* 2013; Narwani *et al.* 2013). This indicates that how phylogenetic relatedness predicts coexistence and/or competitive exclusion among alien and native plants may be far more complex than posed by Darwin's hypothesis (MacDougall, Gilbert & Levine 2009; Godoy, Kraft & Levine 2014).

In nature, plants are usually entangled in multispecies interactive networks with not only direct but also indirect interactions (Strauss & Irwin 2004; Callaway & Howard 2007; Allesina & Levine 2011). For example, in a recipient community, alien plants are inevitably confronted with multispecies interactions with native plants. Therefore, the success and impact of alien plants in the community is determined not only by direct but also by indirect interactions (Miller 1994; White, Wilson & Clarke 2006; Metlen, Aschehoug & Callaway 2013). Studies have inspired by Darwin's naturalization hypothesis evaluated pairwise direct interactions between alien and native plants (Dostál 2011; Godoy, Kraft & Levine 2014). However, it has been acknowledged that the ecological and evolutionary outcome of multispecies interactions cannot be consistently predicted from pairwise interactions (Strauss 1991; Wootton 1994; Strauss & Irwin 2004). For example, addition of a new species into a two species competing system might alter the intensity and even direction of pairwise interactions via indirect pathways (Levine 1976, 1999; Strauss & Irwin 2004). In a recipient community, facilitative indirect interactions may emerge when an alien plant encounters two native plants with different phylogenetic relatedness to the alien. Specifically, if the presence of a closely related native plant directly suppresses the alien (due to their potential niche overlap), other co-occurring less related natives might be indirectly released from the strong competition exerted by the alien. However, how these direct and indirect interactions operate among alien and native plants is not yet clear.

Interaction and/or coexistence among alien and native plants in a recipient community is supposedly determined by the differences in ecological niches. Plant niche differences are thought to ultimately be governed by functional traits that influence plant performance (McGill *et al.* 2006; Violle *et al.* 2007; Violle & Jiang 2009). Examples of such traits are height, specific leaf area (SLA, leaf area per unit leaf mass), leaf size and seed mass, which are considered to be at the core of ecological strategies, form and function of plants (Westoby 1998; Díaz *et al.* 2016). However, the link between traits and ecological niche differences is likely to be complex or idiosyncratic (Cadotte 2013; Kraft, Godoy & Levine 2015). Phylogenetic distance and traits based functional distance are therefore proposed as complementary measures to capture the overall niche similarity of plants (Cadotte, Albert & Walker 2013; Godoy, Kraft & Levine 2014). Thus, it is

essential to understand how the outcome of direct and indirect interactions among alien and native plants is related to functional traits, phylogenetic distance and functional distance.

To disentangle how direct and indirect interactions operate among alien and native plants, and also to test how intensity of these interactions is explained by phylogenetic distance, functional traits and functional distance based on the traits, we selected nine groups of alien and native plant species from the herbaceous flora of Germany. Each group had an alien species that is common (widespread) or rare (less widespread) based on its occurrence frequency in Germany (i.e. non native range), two native species with close and distant phylogenetic distance to the alien respectively, and a "target" native species that is also distantly related to A (Table 1). We grew the four species of each group alone as single species, and in two species and three species combinations (Fig. 1), to test three specific questions: (i) is competition between alien species and closely related native species stronger than the one between alien species and distantly related native species? (ii) does the presence of a closely related rather than distantly related native species directly suppress the alien, and therefore indirectly release the "target" native species from competitive suppression by the alien? (iii) do the patterns in these interactions depend on commonness of the alien species in Germany? In addition, we tested how the intensity of these interactions was explained by phylogenetic distance, functional traits and traits based functional distance.

Materials and methods

STUDY SPECIES

To explore direct and indirect interactions among alien and native plants, we selected nine groups of one alien and three native plant species from the herbaceous flora of Germany, totalling 36 species representing different life histories (i.e. annual, biennial and perennial) (Table 1). Most of these species are naturally distributed in grasslands while a few of them occur mainly in ruderal habitats (Kuhn, Durka & Klotz 2004). In each group, we included an alien species (A), a native species that is (phylogenetically) closely related to A (N_{close}), a native species that is distantly related to A (N_{dist}), and a "target" native species that is also distantly related to A (T) (Table 1). The N_{close} species of each group was randomly drawn from two or three native species that are congeneric to the alien, while the N_{dist} species was randomly drawn from a pool of c. 30 native species that are non congeneric to the alien. The "target" species of each group was also randomly drawn from the c. 30 non congeneric native species pool. To test whether the interactions among alien and native species depend on whether the alien species is common or rare in Germany (i.e. non native range), five of the nine groups had common alien species, while the other four groups had rare alien species. We defined an alien species as common in Germany when the number of grid cells (c. 12×11 km) where the species is recorded exceeds 400 out of the total c. 3000 grid cells that cover all of Germany; otherwise as rare, based on a species distribution database of the German flora (FLOR KART, <http://www.floraweb.de/>, last accessed on the 1st of May 2015). This database originates from different regional plant mapping projects that follow a standardized methodology (Bergmeier 1992). The five common alien species and the four rare alien species were

Table 1. The nine groups of alien and native plant species in our study.

Group	Code*	Species	Family	Occurrence†	Life history	Seed source
1	A	<i>Cerastium tomentosum</i>	Caryophyllaceae	Common (1246)	perennial	Brno
1	N _{close}	<i>Cerastium holosteoides</i>	Caryophyllaceae	Common (2445)	perennial	Tallinn
1	N _{dist}	<i>Alyssum alyssoides</i>	Brassicaceae	Common (737)	annual	Konstanz
1	T	<i>Anthoxanthum odoratum</i>	Poaceae	Common (2702)	perennial	Jena
2	A	<i>Lepidium virginicum</i>	Brassicaceae	Common (633)	annual	Konstanz
2	N _{close}	<i>Lepidium campestre</i>	Brassicaceae	Common (1533)	annual	Munster
2	N _{dist}	<i>Senecio sylvaticus</i>	Asteraceae	Common (2120)	annual	Cean
2	T	<i>Silene nutans</i>	Caryophyllaceae	Common (1346)	perennial	Karlsruhe
3	A	<i>Galium mollugo</i>	Rubiaceae	Common (533)	perennial	Switzerland
3	N _{close}	<i>Galium verum</i>	Rubiaceae	Common (1985)	perennial	Rieger Hofmann
3	N _{dist}	<i>Persicaria maculosa</i>	Polygonaceae	Common (2046)	annual	Talence
3	T	<i>Veronica beccabunga</i>	Plantaginaceae	Common (2589)	perennial	Rieger Hofmann
4	A	<i>Veronica persica</i>	Plantaginaceae	Common (2344)	annual	Munster
4	N _{close}	<i>Veronica chamaedrys</i>	Plantaginaceae	Common (2594)	perennial	Rieger Hofmann
4	N _{dist}	<i>Cerastium arvense</i>	Caryophyllaceae	Common (2449)	perennial	Rieger Hofmann
4	T	<i>Silene vulgaris</i>	Caryophyllaceae	Common (2410)	perennial	Konstanz
5	A	<i>Senecio vernalis</i>	Asteraceae	Common (1702)	annual	Iasi
5	N _{close}	<i>Senecio vulgaris</i>	Asteraceae	Common (2570)	annual	Siena
5	N _{dist}	<i>Salvia pratensis</i>	Lamiaceae	Common (1428)	perennial	Rieger Hofmann
5	T	<i>Chenopodium polyspermum</i>	Amaranthaceae	Common (2233)	annual	Munster
6	A	<i>Campanula medium</i>	Campanulaceae	Rare (55)	biennial	Karlsruhe
6	N _{close}	<i>Campanula rapunculoides</i>	Campanulaceae	Common (2058)	perennial	Konstanz
6	N _{dist}	<i>Senecio jacobaea</i>	Asteraceae	Common (2406)	biennial	Konstanz
6	T	<i>Galium album</i>	Rubiaceae	Common (2358)	perennial	Rieger Hofmann
7	A	<i>Centaurea solstitialis</i>	Asteraceae	Rare (32)	annual	Konstanz
7	N _{close}	<i>Centaurea scabiosa</i>	Asteraceae	Common (1940)	perennial	Rieger Hofmann
7	N _{dist}	<i>Holosteum umbellatum</i>	Caryophyllaceae	Common (1141)	annual	Konstanz
7	T	<i>Veronica officinalis</i>	Plantaginaceae	Common (2491)	perennial	Rieger Hofmann
8	A	<i>Chenopodium vulvaria</i>	Amaranthaceae	Rare (150)	annual	TU Dresden
8	N _{close}	<i>Chenopodium album</i>	Amaranthaceae	Common (2248)	annual	Konstanz
8	N _{dist}	<i>Alopecurus pratensis</i>	Poaceae	Common (2735)	perennial	Rieger Hofmann
8	T	<i>Lactuca serriola</i>	Asteraceae	Common (2342)	annual	Munster
9	A	<i>Potentilla intermedia</i>	Rosaceae	Rare (369)	biennial	Stuttgart
9	N _{close}	<i>Potentilla argentea</i>	Rosaceae	Common (518)	perennial	Rieger Hofmann
9	N _{dist}	<i>Trifolium dubium</i>	Fabaceae	Common (2581)	annual	Konstanz
9	T	<i>Centaurea jacea</i>	Asteraceae	Common (2075)	perennial	Rieger Hofmann

*We have four species in each of the nine groups: A, an alien species that is common or rare in Germany (i.e. non native range); N_{close}, a native species that is closely related (i.e. congeneric) to A; N_{dist}, a native species that is distantly related (i.e. non congeneric) to A; T, a “target” native species that is also distantly related (i.e. non congeneric) to A.

†Based on the distribution database of the German flora (FLORKART, <http://www.floraweb.de/>, last accessed on the 1st of May in 2015). If the number (inside the bracket) of grid cells (c. 12 × 11 km) in which a species is recorded exceeds 400 out of the total c. 3000 grid cells that cover all of Germany, we defined it as common, otherwise as rare.

introduced to Germany at similar times ($t = 0.287$, d.f. 4.621, $P = 0.787$) (Table S1 in Appendix S1, see the Supporting Information). Moreover, the common and rare alien species do not differ in 1000 seed mass ($t = 0.041$, d.f. 3.847, $P = 0.969$), and most of them do not have specific dispersal mechanisms (Table S1). Therefore, the difference in commonness of these species in Germany is unlikely due to the difference in residence time and dispersal ability.

EXPERIMENTAL SETUP

On the 21st of May 2013, we sowed 100 seeds of each of the 36 species into four trays (i.e. 25 seeds per tray; size of the tray: 12 cm × 14 cm × 5 cm) filled with a standard potting soil (Einheitserde; Gebr. Patzer GmbH & Co. KG, Sinntal, Germany). We placed the trays in a growth chamber with a temperature of 21 °C/17 °C (day/night) and a photoperiod of 12 h. We obtained seeds of most of the 36 species from a seed company (Rieger Hofmann GmbH,

Blaufelden, Germany) and the botanic garden at the University of Konstanz, while seeds of the rest were mostly obtained from other botanic gardens in Germany (Table 1). About 1 month after sowing (17th of June 2013), we selected similarly sized seedlings of each of the 36 species and transplanted them into 3 L pots (height: 15 cm, diameter: 16 cm), following the design illustrated in Fig. 1. Specifically, we grew the four species in each of the nine groups alone as single species, and in two species and three species combinations. Pots were filled with a 1:1 mixture of vermiculite and sand, and 10 g slow release fertilizer (Osmocote Pro; Everris GmbH, Nordhorn, Germany) was mixed into the soil of each pot. We had six replicates for each of the 11 treatments per group (i.e. four single species, and five two species and two three species combinations, see Fig. 1), totalling 594 pots and 1080 seedlings.

Directly after transplanting, we moved all the pots to two green house compartments with a temperature of 24 °C/21 °C (day/night), each with three tables. We distributed each of the six replicates of 11

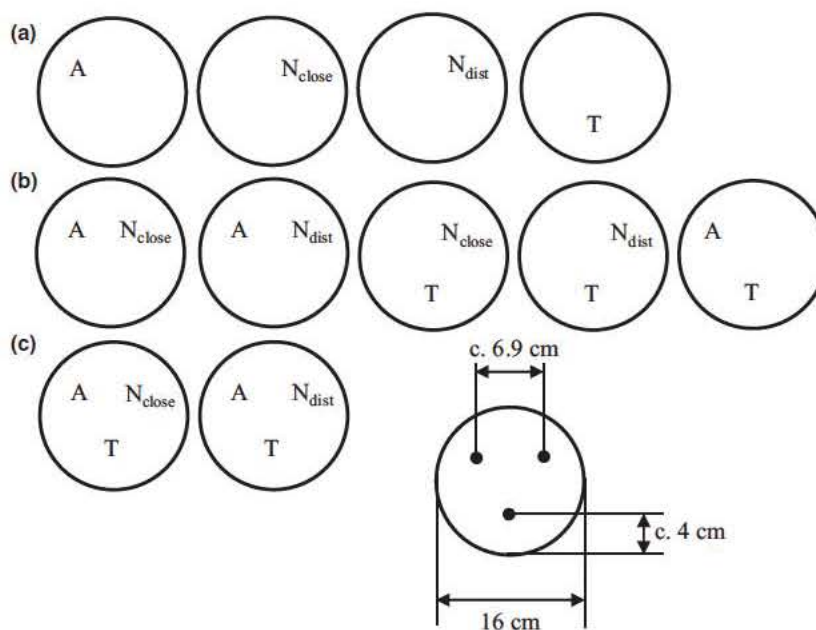


Fig. 1. The diagram illustrating the treatments within each of the nine species groups. We have four species in each of the nine groups: A, an alien species that is common or rare in Germany (i.e. non native range); N_{close} , a native species that is closely related (i.e. congeneric) to A; N_{dist} , a native species that is distantly related (i.e. non confamilial) to A; T, a “target” native species that is also distantly related (i.e. non confamilial) to A. The four species in each group were grown in 3 L pots (height: 15 cm, diameter: 16 cm), (a) alone as single species, (b) in two species combinations, and (c) in three species combinations.

treatments of the nine groups on each of the six tables (11 treatments \times nine groups = 99 pots per table), and randomly assigned the pots to the 99 positions on each table. So, we had a randomized block design with six blocks (tables), and we re-randomized the positions of the 99 pots on each table every 3 weeks. To keep the pot substrate constantly moist, we placed pots on a fleece and watered plants regularly from the bottom by filling tables with water. About 7 weeks after the start of the experiment (5th of August 2013), many plants had grown quite big. In order to avoid interference between plants of neighbouring pots, we spaced out pots by redistributing them from the two greenhouse compartments to three compartments. In other words, we maintained six blocks, but the 99 pots per table were now redistributed over 1.5 tables.

MEASUREMENTS AND HARVESTING

Estimate of initial biomass

After plants had recovered from transplanting (24–28th of June 2013), we took initial size measurements. For each plant, we counted the number of leaves and measured the length and width of the largest leaf to estimate total leaf area. We then estimated initial biomass of each plant based on the estimated total leaf area and a total biomass vs. total leaf area equation that we established for each species in a separate harvest of seedlings that were left over in the germination trays after transplanting (18–21st of June 2013) (for details on estimate of initial biomass, see Appendix S2).

Final harvesting

About 2 months after transplanting (14th of August 2013), we started to harvest plants group by group. As the whole harvest requires a number of weeks (it took about 7 weeks), we alternately harvested a group with a common alien species and one with a rare alien species, and for each group we harvested the plants block by block, to avoid introducing biases. For each plant, we harvested leaves, stems and roots separately. Plant height (i.e. natural height, stretched stem length or tallest leaf length, depending on species) was measured

before each plant was harvested. To determine leaf size (area of an individual leaf) of each plant, we picked the largest leaf on the plant, digitized it with a scanner (Expression 10000XL, Epson; Suwa, Japan), and determined its area using the software ImageJ (Abramoff, Magalhaes & Ram 2004). We then calculated specific leaf area (SLA) from the area and dried mass of the largest leaf. To calculate leaf area ratio (leaf area per unit plant biomass), we estimated total leaf area of each plant by multiplying SLA with total leaf mass. We washed roots from soil, and carefully separated roots of different plants in each pot. As the root washing and analysis is very time-consuming, we assessed root morphology only for plants in half of all the pots. Specifically, we took root subsamples from three randomly chosen replicates out of the six of each treatment in each group. To have a representative subsample of the entire root system, we first cut roots in 1.5–2.0 cm pieces and mixed them in a beaker filled with water. We then blindly took a pinch of c. 20–30 root pieces from the mixture as the root subsample for a plant. Root subsamples were stored in a 50% ethanol solution for < 2 weeks before they were stained with neutral red for more than 24 h. The stained roots were first digitized with the scanner, and were then analysed using the root system analysis software WinRHIZO (Regent Instruments Inc., Quebec, Canada). Specific root length was calculated as root length divided by dried mass of a root subsample. To calculate root length ratio (root length per unit plant biomass), we estimated total root length of a plant by multiplying specific root length with total root mass. All the biomass was dried at 70 °C for 72 h prior to weighing.

PHYLOGENETIC DISTANCE, FUNCTIONAL TRAITS AND FUNCTIONAL DISTANCE

To explore phylogenetic and functional mechanisms of direct and indirect interactions among alien and native plants, we calculated phylogenetic distance, collected functional traits and calculated functional distance based on the traits. We calculated phylogenetic distances between the four species in each of the nine groups based on a phylogenetic tree of the 36 study species (Fig. S1 in Appendix S3). This phylogenetic tree was pruned from the DaPhnE, a dated phylogeny of

the European flora (Durka & Michalski 2012), using the R package *phangorn* (Schliep 2011). In three species treatments, we calculated both average and nearest phylogenetic distance between a species and its two neighbours.

Eight functional traits were included in the analysis: plant height, 1000 seed mass, SLA, leaf size, specific root length, shoot weight ratio (shoot mass/total plant mass), leaf area ratio and root length ratio. These traits are frequently claimed to be strongly associated with performance of plants and reflect plant ecological strategies (Westoby 1998; Pérez Harguindeguy *et al.* 2013). Values of each trait except seed mass were taken from measurements on each plant in the two species and three species mixture treatments, as the values should be more relevant to actual interactions in a pot than trait values from plants in the single species treatment. In three species treatments, the values for each trait of two neighbours of a species were averaged. The 1000 seed mass of each species was determined by weighing a sample of 100 randomly chosen seeds from the same sources as those used to germinate the seedlings for the experiment. We then calculated Euclidian functional distances between plants in a pot in the two species and three species mixture treatments in each of the nine groups, based on the collected functional traits except shoot weight ratio. Shoot weight ratio was not included because it can be mathematically derived from specific root length and root length ratio. In three species treatments, both average and nearest functional distance between a plant and its two neighbours were calculated.

DATA ANALYSIS

To disentangle how direct and indirect interactions operate among alien and native plant species in each of the nine groups (i.e. A: alien species; N_{close} : native species that is closely related to A; N_{dist} : native species that is distantly related to A; T: “target” native species that is also distantly related to A), and to explore how intensity of the interactions is explained by phylogenetic distance, functional traits and functional distance, we did two major sets of analyses. First, we used linear mixed effects models to disentangle how direct and indirect interactions affected biomass production of the “target” (T), alien (A) and native (N_{close} and N_{dist}) species. Second, we used a linear mixed effects model to test how intensity of the interactions among the species (irrespective of whether it is A, N_{close} , N_{dist} or T) was explained by phylogenetic distance, functional traits and functional distance of the interacting species.

Analysis of direct and indirect interactions among alien and native plants

In the first set of analyses, we used linear mixed effects models to disentangle how direct and indirect interactions operate among alien and native species. Total biomass of T, A or N_{close} and N_{dist} species was the response variable. For all the models in this set of analyses, we included species group and block (i.e. table) as random factors. We applied a natural logarithm transformation to the total biomass of the species (i.e. response variable) to improve the normality and homogeneity of residuals.

First, we assessed how total biomass of “target” species (T) was affected by the presence of other species. As explanatory variables for the full model, we included whether species group has a common or rare alien species (*Status*), presence/absence of alien species (A), presence/absence of a native species irrespective of whether it is N_{close} or N_{dist} (N), whether native species if present is N_{close} or N_{dist} ($N_{\text{close}}N_{\text{dist}}$) and all the possible two way and three way interactions

of these variables (see Table 2 for an overview of the main and interaction terms). In addition, to correct for the variation in initial size of the “target” species, we included their initial biomass as a covariate. We then used the log likelihood ratio tests to assess the significance of each of the main and interaction terms by removing them one by one from the full model following the removal order as shown in Table 2.

Second, we assessed how total biomass of alien species (A) was affected by the presence of other species. In the full model, we used the same explanatory variables as presented for the previous model, except that presence/absence of T was replaced by presence/absence of A (see Table 3 for an overview). Similarly, we included initial biomass of A as a covariate and assessed the significance of each term following the removal order as shown in Table 3.

Third, we assessed how total biomass of native species (N_{close} and N_{dist}) was affected by the presence of other species. As explanatory variables for the full model, we included whether species group has a common or rare alien species (*Status*), presence/absence of alien species (A), presence/absence of “target” species (T), whether the native is N_{close} or N_{dist} ($N_{\text{close}}N_{\text{dist}}$) and all the possible two way, three way and four way interactions of these variables (see Table 4 for an overview). Similarly, we included initial biomass of native species

Table 2. Results of the linear mixed effects model disentangling direct and indirect interactions by analysing total biomass of “target” native species (T).

Questions*	Fixed [†]	Order [‡]	d.f.	χ^2	P
	Initial biomass	7	1	18.80	< 0.001
	Status	6	1	0.00	0.967
	A	6	1	6.37	0.012
	N	6	1	16.19	< 0.001
	$N_{\text{close}}N_{\text{dist}}$	5	1	0.00	0.999
	Status:A	4	1	0.19	0.660
	Status:N	4	1	0.59	0.443
	Status: $N_{\text{close}}N_{\text{dist}}$	3	1	2.22	0.136
	A:N	4	1	3.02	<i>0.082</i>
Question 2	A: $N_{\text{close}}N_{\text{dist}}$	3	1	4.13	0.042
	Status:A:N	2	1	5.16	0.023
Question 3	Status:A: $N_{\text{close}}N_{\text{dist}}$	1	1	0.65	0.419
Random					SD
Group					1.34
Block					0.11
Residual					0.73

**Question 2*: does the presence of a closely related native (N_{close}) rather than a distantly related native (N_{dist}) directly suppress the alien (A), and therefore indirectly release the distantly related “target” native (T) from competitive suppression by A? *Question 3*: do the patterns in these interactions depend on commonness of A in Germany (i.e. non native range)?

[†]*Initial biomass*: initial biomass of T; *Status*: whether species group has a common or rare A; A: presence/absence of A; N: presence/absence of a native irrespective of whether it is N_{close} or N_{dist} ; $N_{\text{close}}N_{\text{dist}}$: whether the native if present is N_{close} or N_{dist} . P values of significant effects ($\alpha = 0.05$) are printed in bold and those of marginally significant effects ($\alpha = 0.10$) are printed in italic.

[‡]The order of removing terms one by one to test the significance of each term in the linear mixed effects model. When terms have the same order number, they are removed one at a time while keeping all other terms with same order in the model.

Table 3. Results of the linear mixed effects model disentangling direct and indirect interactions by analysing total biomass of alien species (A).

Questions*	Fixed [†]	Order [‡]	d.f.	χ^2	<i>P</i>
Question 1	Initial biomass	7	1	16.88	< 0.001
	Status	6	1	0.04	0.838
	T	6	1	13.85	< 0.001
	N	6	1	5.40	0.020
	N _{close} N _{dist}	5	1	0.05	0.821
	Status:T	4	1	0.74	0.389
	Status:N	4	1	0.69	0.407
	Status:N _{close} N _{dist}	3	1	7.59	0.006
	T:N	4	1	5.20	0.023
	T:N _{close} N _{dist}	3	1	0.52	0.469
Status:T:N	2	1	0.37	0.542	
Status:T:N _{close} N _{dist}	1	1	0.50	0.480	
Random					SD
Group					0.61
Block					0.22
Residual					0.65

**Question 1*: is competition between alien species (A) and closely related native species (N_{close}) stronger than the one between A and distantly related native species (N_{dist})? *Question 3*: do the patterns in these interactions depend on commonness of A in Germany (i.e. non native range)?

[†]*Initial biomass*: initial biomass of A; *Status*: whether species group has a common or rare A; *T*: presence/absence of distantly related “target” native species (T); *N*: presence/absence of a native irrespective of whether it is N_{close} or N_{dist}; *N_{close}N_{dist}*: whether the native if present is N_{close} or N_{dist}. *P* values of significant effects ($\alpha = 0.05$) are printed in bold.

[‡]The order of removing terms one by one to test the significance of each term in the linear mixed effects model. When terms have the same order number, they are removed one at a time while keeping all other terms with same order in the model.

(N_{close} and N_{dist}) as a covariate and assessed the significance of each term following the removal order as shown in Table 4.

Effects of phylogenetic distance, functional traits and functional distance on relative interaction intensity

In this set of analyses, we explored how intensity of the direct and indirect interactions among alien and native plants across species (irrespective of whether the species is A, N_{close}, N_{dist} or T) was explained by phylogenetic distance, functional traits and functional distance. The intensity of the interactions was quantified by a relative interaction index (RII) that was proposed to measure the relative interaction intensity in plants (Armas, Ordiales & Pugnaire 2004). We calculated the RII for a plant as $(B_{mix} - B_{alone}) / (B_{mix} + B_{alone})$, where B_{alone} refers to total biomass of a plant when growing alone, i.e. in a single species treatment, while B_{mix} refers to total biomass of a plant when growing in each of the respective mixtures, i.e. all the two species and three species treatments, if applicable (Fig. 1). The negative RII values indicate competitive effects, while the positive ones indicate facilitative effects, on a plant.

To test whether more closely related species are more ecologically similar in functional traits than species drawn at random from the same tree (i.e. whether there are a phylogenetic signals in traits)

Table 4. Results of the linear mixed effects model disentangling direct and indirect interactions by analysing total biomass of phylogenetically closely related (N_{close}) and distantly related (N_{dist}) native species.

Questions*	Fixed [†]	Order [‡]	d.f.	χ^2	<i>P</i>	
Question 1	Initial biomass	5	1	38.71	< 0.001	
	Status	4	1	0.03	0.865	
	A	4	1	9.25	0.002	
	T	4	1	3.17	0.075	
	N _{close} N _{dist}	4	1	2.83	0.093	
	Status:A	3	1	0.26	0.611	
	Status:T	3	1	3.40	0.065	
	Status:N _{close} N _{dist}	3	1	35.40	< 0.001	
	A:T	3	1	2.45	0.117	
	A:N _{close} N _{dist}	3	1	7.09	0.008	
Question 3	T:N _{close} N _{dist}	3	1	0.00	0.962	
	Status:A:T	2	1	0.43	0.511	
	Status:A:N _{close} N _{dist}	2	1	0.41	0.522	
	Status:T:N _{close} N _{dist}	2	1	0.62	0.430	
	A:T:N _{close} N _{dist}	2	1	0.01	0.919	
	Status:A:T:N _{close} N _{dist}	1	1	0.91	0.341	
	Random					SD
	Group					0.82
	Block					0.11
	Residual					1.15

**Question 1*: is competition between alien species (A) and closely related native species (N_{close}) stronger than the one between A and distantly related native species (N_{dist})? *Question 3*: do the patterns in these interactions depend on commonness of A in Germany (i.e. non native range)?

[†]*Initial biomass*: initial biomass of N_{close} and N_{dist}; *Status*: whether species group has a common or rare A; *A*: presence/absence of A; *T*: presence/absence of distantly related “target” native species (T); *N_{close}N_{dist}*: whether the native species is N_{close} or N_{dist}. *P* values of significant effects ($\alpha = 0.05$) are printed in bold and those of marginally significant effects ($\alpha = 0.10$) are printed in italic.

[‡]The order of removing terms one by one to test the significance of each term in the linear mixed effects model. When terms have the same order number, they are removed one at a time while keeping all other terms with same order in the model.

(Munkemuller *et al.* 2012), as Darwin’s naturalization hypothesis assumes, we calculated the phylogenetic signal for each trait. First, for each species, we calculated mean of each trait over all the measurements in the two species and three species mixture treatments. We then used the mean of each trait of the species to calculate the phylogenetic signal for the trait, using four commonly used indices, Moran’s I, Abouheif’s C_{mean}, Pagel’s λ and Blomberg’s K, each measuring different aspects of phylogenetic signal (Munkemuller *et al.* 2012).

To disentangle how the intensity of the direct and indirect interactions was explained by phylogenetic distance, functional traits and functional distance, we used a linear mixed effects model in which the relative interaction intensity (measured as RII) on a plant (irrespective of whether it is A, N_{close}, N_{dist} or T species) was the response variable. As explanatory variables in the model, we included average phylogenetic and traits based functional distance between a plant and its neighbour(s), and seven functional traits except shoot weight ratio of a plant and its neighbour(s). To account for variation in initial size of plants and accordingly in values of initial RII, we included initial RII as a covariate in the model. The initial RII was

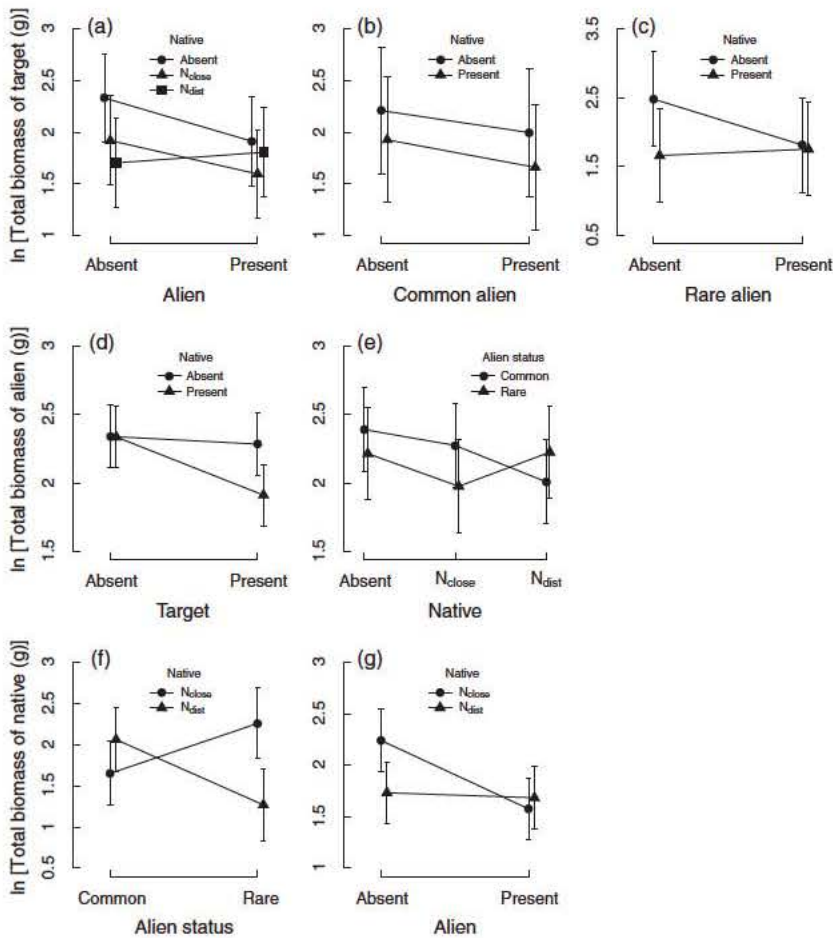


Fig. 2. Total biomass of “target” species (T) as a function of the interaction between (a) presence/absence of alien species (A) and whether a native species if present is N_{close} or N_{dist} , and (b and c) presence/absence of common alien species or rare alien species and presence/absence of a native species irrespective of whether it is N_{close} or N_{dist} ; Total biomass of A as a function of the interaction between (d) presence/absence of T and presence/absence of a native species irrespective of whether it is N_{close} or N_{dist} , and (e) whether species group has a common or rare A and whether a native species if present is N_{close} and N_{dist} ; Total biomass of phylogenetically closely related (N_{close}) and distantly related (N_{dist}) native species as a function of the interaction between (f) whether species group has a common or rare A and whether the native species is N_{close} or N_{dist} , and (g) presence/absence of A and presence/absence of a native species irrespective of whether it is N_{close} or N_{dist} . Means (symbols) and standard errors (error bars) are predicted from the models.

also calculated as $(B_{mix} - B_{alone}) / (B_{mix} + B_{alone})$, but based on initial biomass of plants in the grown alone and mixture treatments. As random factors in the model, we included group, family, species (nested within family) and block (table). To ensure that interpretation of the effect of explanatory variables is not influenced by multicollinearity between the variables, we calculated the variance inflation factor (VIF) for each variable (Zuur *et al.* 2009). The VIF values of all the variables were much lower than 4 (the rule of thumb), indicating negligible multicollinearity (Zuur *et al.* 2009). The significance of each explanatory variable was assessed by comparing the full model to the model without the variable of interest, using the log likelihood ratio test. We also analysed the data by including nearest instead of average phylogenetic distance and functional distance as the explanatory variables in the model, but it gave very similar results (not shown). In addition, we ran a separate model with shoot weight ratio as the only explanatory variable to test its importance in explaining the intensity of the interactions.

All of the analyses were performed in R version 3.0.3 (R Core Team 2014).

Results

DIRECT AND INDIRECT INTERACTIONS AMONG ALIEN AND NATIVE PLANT SPECIES

The presence of alien species (A), and native species (N) irrespective of whether it is closely related (N_{close}) or distantly related (N_{dist}) to A, significantly suppressed total biomass pro-

duction of “target” species (T) by 19.3% and 30.9% respectively (Table 2). However, the total biomass production of T was less suppressed by the presence of A when N_{dist} rather than N_{close} was present (significant A: $N_{close}N_{dist}$ interaction in Table 2; Fig. 2a). In other words, contrary to our expectation, the presence of N_{dist} rather than N_{close} tended to indirectly reduce direct competitive effects of A on T. Moreover, the presence of N also overall indirectly reduced direct competitive effects of A on T, but this only occurred when A is a rare rather than common species in Germany (i.e. non native range) (significant Status:A:N interaction in Table 2; Fig. 2b and c).

The presence of T and N significantly suppressed total biomass production of A by 25.5% and by 17.2% respectively (Table 3). Competitive effects of T on A tended to be absent when N was absent (significant T:N interaction in Table 3; Fig. 2d). Moreover, rare alien species were more suppressed by N_{close} than by N_{dist} while the opposite was true for common alien species (significant Status: $N_{close}N_{dist}$ in Table 3; Fig. 2e).

Total biomass production of N, was significantly suppressed by the presence of A (−30.0%), but the suppression by the presence of T was marginally significant (−20.6%) (Table 4). In groups in which A is a rare species, total biomass of N_{close} was greater than the one of N_{dist} , while the opposite was true in groups in which A is a common species

(significant Status: N_{close} N_{dist} interaction in Table 4; Fig. 2f). Moreover, the presence of A suppressed total biomass production of N_{close} more strongly than the one of N_{dist} (significant A: N_{close} N_{dist} interaction in Table 4; Fig. 2g).

EFFECTS OF PHYLOGENETIC AND FUNCTIONAL DISTANCE AND FUNCTIONAL TRAITS ON RII

There were significant phylogenetic signals in five of the eight functional traits, i.e. plant height, leaf size, specific root length, shoot weight ratio and root length ratio (for more details, see Table S3 in Appendix S4). The interaction intensity measured as relative interaction index (RII) on a focal plant (irrespective of whether it is A, N_{close} , N_{dist} or T species) was explained neither by phylogenetic distance nor by functional distance (Table 5). However, variation in RII of focal plants was explained by some of the functional traits of a focal plant and its neighbour(s), i.e. height and leaf size of a focal plant, and height, SLA, leaf area ratio and root length ratio of its neighbour(s) (Table 5). Specifically, RII of a focal plant significantly increased (i.e. competitive effects on the focal plant decreased) with height and leaf size of the focal plant, and with SLA and root length ratio of its neighbour(s)

Table 5. Results of the linear mixed effects model testing how intensity of the interactions among alien and native plants (assessed by the relative interaction index, RII) is explained by phylogenetic and functional distance (average phylogenetic and functional distance if in three species treatments) between a focal plant and its neighbours, and each of the seven functional traits of a focal and its neighbours. *P* values of significant effects ($\alpha = 0.05$) are printed in bold.

Fixed	d.f.	χ^2	<i>P</i>
Initial RII	1	53.54	< 0.001
Phylogenetic distance	1	0.29	0.592
Functional distance	1	1.38	0.241
Functional traits of focal plant			
Height	1	18.40	< 0.001
Leaf size	1	4.44	0.035
Specific leaf area (SLA)	1	1.64	0.201
Specific root length (SRL)	1	0.06	0.803
Leaf area ratio	1	0.07	0.793
Root length ratio	1	0.53	0.465
Seed mass	1	0.00	0.947
Functional traits of neighbour plants			
Height	1	4.40	0.036
Leaf size	1	1.28	0.258
Specific leaf area (SLA)	1	5.04	0.025
Specific root length (SRL)	1	2.19	0.139
Leaf area ratio	1	7.36	0.007
Root length ratio	1	7.33	0.007
Seed mass	1	0.37	0.541
Random			SD
Group			0.029
Species			0.140
Family			<0.001
Table			0.021
Residual			0.307

(Fig. 3a,c,d,f), while it decreased (i.e. competitive effects on the focal plant increased) with height and leaf area ratio of its neighbour(s) (Fig. 3b,e). Moreover, RII of a focal plant increased with shoot weight ratio of the focal plant but decreased with shoot weight ratio of its neighbour(s) (for more details, see Table S4 and Fig. S2 in Appendix S5).

Discussion

Some of our results tended to support Darwin's naturalization hypothesis, since alien species had stronger competitive effects on closely related natives than on distantly related natives. In turn, alien species was more competitively suppressed by closely related natives than by distantly related natives, although this was only true when the alien is a rare instead of common species in Germany (i.e. non native range). However, contrary to our expectation, the presence of distantly related rather than closely related native species indirectly reduced competitive effects of alien species on distantly related "target" native species. Moreover, although there were significant phylogenetic signals in most of the collected functional traits, the intensity of the interactions among alien and native plants was not explained by phylogenetic and functional distance, but by some of the functional traits of the interacting plants.

DIRECT AND INDIRECT INTERACTIONS AMONG ALIEN AND NATIVE PLANTS

Darwin (1859) proposes that the struggle for existence is more severe between more closely related species because they are likely to be more ecologically similar. This idea was further developed using ecological theories (e.g. Gause's law, limiting similarity theory) which pose that similarity in ecological niches intensifies species competition (Gause 1934; Hardin 1960; MacArthur & Levins 1967). Darwin's idea and the limiting similarity theory have invoked many empirical studies testing whether phylogenetic relatedness predicts competitive outcome and/or coexistence between native species (Cahill *et al.* 2008; Fritschie *et al.* 2013; Narwani *et al.* 2013), and between alien and native species (Jiang, Tan & Pu 2010; Dostál 2011; Godoy, Kraft & Levine 2014; Tan *et al.* 2015). However, the findings of these studies are rather mixed.

In our study, an alien species, irrespective of whether it is common or rare in Germany (i.e. non native range), had stronger competitive effects on closely related natives than on distantly related natives (Fig. 2g). In turn, the alien was also more competitively suppressed by closely related natives than by distantly related natives, but this was only true when the alien is a rare species in Germany (Fig. 2e). This result might have arisen because closely related natives grew larger than distantly related ones in the "rare" groups (i.e. the species groups with the rare alien), whereas the opposite was true for the "common" groups (Fig. 2f). The common alien species in our study have much the wider occurrence (more widespread) in Germany than the rare alien species (see Table 1), although

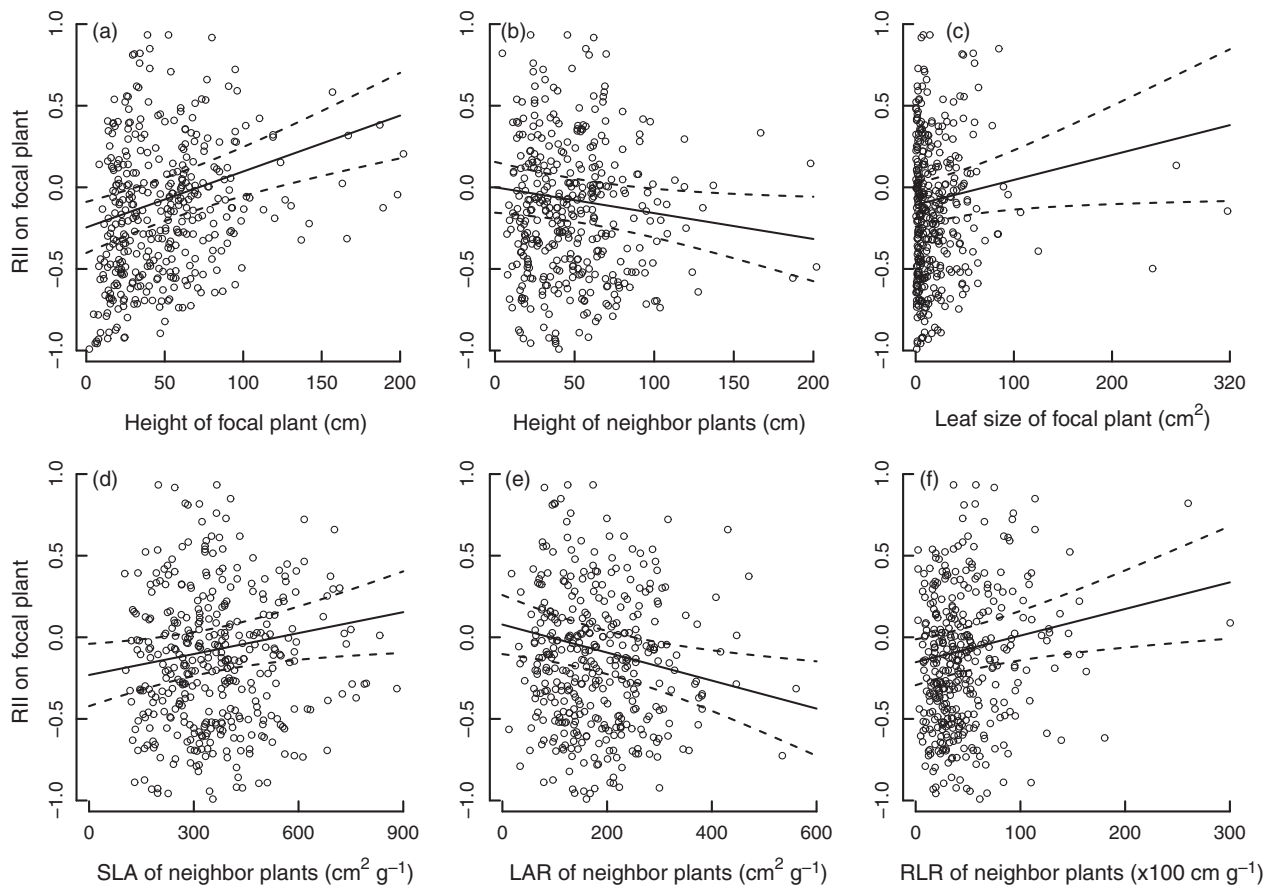


Fig. 3. Importance of height and leaf size of a focal plant, and height, specific leaf area (SLA), leaf area ratio (LAR) and root length ratio (RLR) of its neighbour plants in explaining the intensity of interactions among alien and native plants (assessed by the relative interaction index, RII).

they have similar residence time and dispersal ability (see Appendix S1). This suggests that other biological processes such as species interactions may have influenced spread of these alien species. Indeed, our findings indicate that common and rare alien species differed in the ability to overcome competitive constraints posed by their closely related natives. This also suggests that commonness of species may have confounded the results of previous studies testing for Darwin's naturalization hypothesis or the limiting similarity theory because, with a few exceptions (e.g. Diez *et al.* 2008), most of these studies did not explicitly account for (alien) species' commonness or rarity. For example, Dostál (2011) did not find supportive evidence for Darwin's hypothesis, but this might be because only common (widespread) alien species were used in his study.

The relationship between phylogenetic relatedness and competitive outcome and/or coexistence of multiple species is even more complex due to the existence of indirect interactions. A body of evidence has shown that indirect facilitation arises when a third species is added into a two species competing system (e.g. Miller 1994; Levine 1999; Cuesta *et al.* 2010; Le Bagousse Pinguet *et al.* 2012; Metlen, Aschehoug & Callaway 2013; Aschehoug & Callaway 2015). However, it is poorly understood how the emergence of this indirect facilitation is dependent on ecological similarity of interacting

species. In our study, while direct competition tended to be stronger between more closely related alien and native plants, competitive effects of alien species on distantly related "target" species were indirectly reduced by the presence of distantly related rather than closely related natives, which is contrary to our expectation. This suggests that the outcome of multispecies interactions is difficult to be predicted from the direct interactions (Wootton 1994; Strauss & Irwin 2004). Moreover, for rare rather than common alien species, the presence of the natives (irrespective of whether it is closely or distantly related to the alien) indirectly reduced competitive effects of alien species on distantly related "target" species. This result suggests that, compared to common alien species, rare ones are more likely to be directly constrained by native species, which may indirectly benefit other co-occurring natives in recipient communities.

In our study, the emergence of the interaction patterns might have also depended on variation in life histories (annual, biennial or perennial) of interacting species. Annuals are presumably better competitors than perennials (or biennials) in early establishment and environments with high resource availability, as was the case in our pots. This seems to be supported by a PCA analysis of the eight functional traits on life history (see Fig. S4 in Appendix S6; also see more detailed discussion below). Therefore, we did a separate anal

ysis to test whether the presence of annuals rather than perennials indirectly reduced competitive effects of alien species on “target” species. However, the results did not suggest that variation in life histories has driven the observed patterns (for details, see Appendix S7).

NICHE AND PHYLOGENETIC AND FUNCTIONAL MECHANISMS OF THE INTERACTIONS AMONG ALIEN AND NATIVE PLANTS

In line with several other studies (Cahill *et al.* 2008; Dostál 2011; Fritschie *et al.* 2013; Narwani *et al.* 2013; Godoy, Kraft & Levine 2014), our results showed that the intensity of the interactions among alien and native plants (measured as the relative interaction index, RII) was not correlated with phylogenetic distance. This is probably because phylogenetic distance as an integrated measure failed to effectively capture stabilizing niche differences favouring species coexistence and average fitness differences favouring competitive exclusion (Chesson 2000; Narwani *et al.* 2013; Godoy, Kraft & Levine 2014). Stabilizing niche differences are those species differences that cause intraspecific competition to exceed interspecific competition, e.g. resource partitioning, host specific natural enemies or storage effects (HilleRisLambers *et al.* 2012). By contrast, average fitness differences are those that give one competitor frequency independent advantages (i.e. higher competitive ability) over others, e.g. the difference in plant height in a light limited system (Mayfield & Levine 2010). Compared to phylogenetic distance, functional traits should better reflect stabilizing niche and average fitness differences, even if there are strong phylogenetic signals in traits. Therefore, variation in functional traits has the potential to better explain competitive outcomes of plants.

Indeed, the intensity of the interactions among alien and native plants was significantly associated with most of the functional traits (plant height, leaf size, SLA, leaf area ratio, root length ratio and shoot weight ratio) (Table 5; for shoot weight ratio, see Appendix S5). Plant height is indicative of the ability to pre-empt light resources and thereby competitive ability (Westoby 1998; Kunstler *et al.* 2016). In line with this, our study showed that a plant experienced more intense competition when it was shorter and when its neighbour plants were taller (Fig. 3a,b). Leaf size is usually linked to adaptation of environmental stresses, e.g. drought or nutrient shortage (leaves tend to be relatively smaller in stressful environments) (Pérez Harguindeguy *et al.* 2013). In our study, plants with larger leaves experienced lower competitive effects of their neighbours, which may indicate that variation in leaf size is also involved in a trade off between stress tolerance and competitive ability. SLA reflects a trade off in leaves between carbon gain and longevity (Evans & Poorter 2001). Our results showed that a plant experienced less competition when SLA of its neighbour plants increased, which is in line with other studies showing that a greater SLA does not necessarily lead to greater competitive advantage (Kraft *et al.* 2014; Kraft, Godoy & Levine 2015; Kunstler *et al.* 2016). Alternatively, this result may suggest that competitive

superiority is positively correlated with a more resource conservative foraging strategy (lower SLA) (Kraft, Godoy & Levine 2015). Moreover, in our study, a plant suffered more competition when its neighbours had a greater leaf area ratio. This may imply that leaf area ratio is an effective measure in reflecting the efficiency of light capture and competitive ability (Medek, Ball & Schortemeyer 2007).

As a below ground analogue of SLA and leaf area ratio, specific root length and root length ratio are associated with the uptake efficiency of water and nutrients (Jackson, Mooney & Schulze 1997). However, in our study, specific root length was not significantly associated with the intensity of the interactions, and a plant experienced less competition when its neighbours had a greater root length ratio. Possibly, the limited rooting volume of our pots may have hindered plants from exhibiting effects of the root traits. Or, our plants were grown in pots with relatively high nutrient and water supplies, and consequently competition for below ground resources may have been lower than that for above ground resources. This seems to be true, since a separate analysis showed that a plant experienced less competitive effects when it allocated more biomass to shoots (for above ground competition) and when its neighbours did the opposite (see Table S4 and Fig. S2 in the Appendix S5).

It is likely that variation in a trait differentially contributes to niche differences conferring coexistence and fitness differences conferring competitive ability (Adler *et al.* 2013; Godoy & Levine 2014; Kraft, Godoy & Levine 2015). In our study, we did not quantify the two differences and their relative relevance to functional traits. However, we speculate that some of the traits (e.g. height, leaf area ratio) might have had more influences on fitness differences while others (e.g. SLA) might have had more influences on niche differences. Unlike most of the functional traits, functional distance based on these traits did not hold significant explanatory power for the outcome of the interactions. This is probably because, just like phylogenetic distance, functional distance as an integrated measure is not informative in capturing niche and fitness differences. The multiple functional traits collected in our experiment seemed to have captured species differences in life histories, i.e. annual, biennial or perennial (see a PCA analysis in Fig. S4 in Appendix S6). For example, height tended to be greater for annuals than for perennials or biennials (Fig. S4). Given that greater height conferred greater competitive ability as shown (Fig. 3), this seems to be in accordance with the expectation that annuals are better competitors than perennials in our system. Indeed, a separate analysis showed that a plant experienced more competition when it is a perennial or biennial rather than an annual, and when its neighbour plants are annuals rather than perennials or biennials (Table S5 and Fig. S3 in the Appendix S5).

Conclusion

An unresolved puzzle in invasion ecology is how multiple alien and native plants interact and coexist in a recipient community. Following the idea of Darwin (1859), many stud

ies have evaluated whether phylogenetic relatedness predicts pairwise direct interactions between alien and native plants. However, in natural ecosystems, alien and native plants are usually involved in multispecies interactions in which indirect effects are also abundant. Using multiple species groups, our study for the first time tested Darwin's naturalization hypothesis in the context of both direct and indirect interactions, and explored underlying niche based phylogenetic and functional mechanisms thereof. While direct competition overall tended to be more intense between more closely related alien and native plants, this did not lead to the indirect facilitation on other co occurring natives as we had predicted. Functional traits, potentially because of their more mechanistic relations to stabilizing niche and average fitness differences, rather than phylogenetic or functional distance, explained the intensity of interactions among alien and native plants. However, to gain a greater mechanistic understanding of the multispecies interactions and coexistence among alien and native plants in recipient communities, future studies are needed to explicitly assess how functional traits translate into stabilizing niche and average fitness differences that ultimately drive coexistence, competitive exclusion and community patterns.

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Supporting Information

Appendix S1. Residence time, seed mass and dispersal mechanisms of alien species.

Table S1. Residence time, 1000 seed mass and dispersal mechanisms of alien species.

Appendix S2. Estimate of initial biomass.

Table S2. Relationship between area of the largest leaf and its length and width (Area = Length * Width * Coef), and linear regression between total leaf area and total biomass (Total biomass = Coef * Total leaf area + Intercept), for each species in the initial harvest.

Appendix S3. Phylogenetic tree of 36 study species.

Figure S1. Phylogenetic tree of 36 study species.

Appendix S4. Phylogenetic signal of functional traits.

Table S3. Phylogenetic signal of eight functional traits using the mean of each trait averaged over all the measurements in the two species and three species mixture treatments, using four commonly used indices, Moran's I, Abouheif's Cmean, Pagel's λ and Blomberg's K, each measuring different aspects of phylogenetic signal (Munkemüller *et al.* 2012).

Appendix S5. Importance of shoot weight ratio and life history in explaining the intensity of the interactions.

Table S4. Results of the linear mixed effects model testing how intensity of the direct and indirect interactions among alien and native plants (assessed by the relative interaction index, RII) is explained by shoot weight ratio of a focal plant and its neighbor plants.

Figure S2. Importance of shoot weight ratio (SWR) of a focal plant and its neighbor plants in explaining the intensity of interactions among alien and native plants (assessed by the relative interaction index, RII).

Table S5. Results of the linear mixed effects model testing how intensity of the direct and indirect interactions among alien and native plants (assessed by the relative interaction index, RII) is explained by life history (annual, biennial or perennial) of a focal plant and its neighbor plants.

Figure S3. Importance of life histories (annual, biennial or perennial) of a focal plant and its neighbor plants in explaining the intensity of interactions among alien and native plants (assessed by the relative interaction index, RII).

Appendix S6. Principal component analysis of eight functional traits on life history.

Figure S4. Principal component analysis of eight functional traits, i.e. plant height, seed mass, SLA, leaf size, specific root length (SRL), leaf area ratio, root length ratio (total root length/plant mass) and

shoot weight ratio, on life histories (i.e. annual, biennial and perennial).

Appendix S7. Dependence of direct and indirect interactions on life history.

Table S6. Results of the linear mixed effects model disentangling direct and indirect interactions by analyzing total biomass of “target” native species (T).

Table S7. Results of the linear mixed effects model disentangling direct and indirect interactions by analyzing total biomass of the alien (A).

Table S8. Results of the linear mixed effects model disentangling direct and indirect interactions by analyzing total biomass of annual native species (N_{ann}) and perennial native species (N_{per}).