

Contrasting effects of phylogenetic relatedness on plant invader success in experimental grassland communities

Shao-peng Li^{1,2}, Tao Guo¹, Marc W. Cadotte², Yong-jian Chen¹, Jia-liang Kuang¹, Zheng-shuang Hua¹, Yi Zeng¹, Ying Song¹, Zheng Liu³, Wen-sheng Shu¹ and Jin-tian Li^{1*}

¹State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resource, College of Ecology and Evolution, Sun Yat-sen University, Guangzhou 510275, China; ²Biological Sciences, University of Toronto-Scarborough & Ecology and Evolutionary Biology, University of Toronto, 1265 Military Trail, Toronto, ON M1C 1A4, Canada; and ³Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, USA

Summary

1. Identifying the factors determining the success of invasive species is critical for management of biological invasions. Darwin's naturalization conundrum states that exotic species closely related to natives should be successful because of a shared affinity for local environmental conditions, but at the same time close relatives often compete more intensively, limiting 'niche' opportunities for the invaders. Previous studies have generally considered these two 'opposing' hypotheses as mutually exclusive, yet evidence for both of them abounds, indicating a practical dilemma for management of biological invasions.

2. In this study, we sowed the seeds of the invasive exotic *Ambrosia artemisiifolia* L. into 369 experimental plant communities to mimic an introduction of the exotics into a series of new habitats. We further linked the establishment and growth performance of the invader in these experimental communities to the phylogenetic relatedness between the invader and the community residents where it was introduced.

3. We found that the probability of invader establishment declined with increasing phylogenetic distance between the invader and residents, whereas the average size of surviving invader individuals increased with the phylogenetic distance.

4. These results can be at least partly explained by the observations that close relatives tend to create similar soil microhabitat through harbouring similar soil enzymes (e.g. alkaline and acid phosphatases) benefiting invader establishment and that intense competitive interactions between the invader and its close relatives suppressed exotic growth.

5. *Synthesis and applications.* This study presents the first experimental evidence that phylogenetic relatedness has contrasting effects on different aspects of invader success, thus shedding light on the long-standing Darwin's naturalization conundrum. Moreover, our findings also have important implications for management of plant invasions: for controlling invasive species characterized by high establishment probability, native species distantly related to the invasive species can be planted in sites surrounding the invasion foci, whereas the opposite seems to be true for controlling those characterized by large individual size.

Key-words: biological invasion, Darwin's naturalization hypothesis, interspecific competition, phylogenetic niche conservatism, pre-adaptation hypothesis

Introduction

What determines the success of exotic species in introduced range has long been a major topic in ecology and of considerable applied relevance. Darwin (1859) proposed that

*Correspondence author. E-mail: lijtian@mail.sysu.edu.cn

exotic species without close relatives in target regions were more likely to become naturalized because they might exploit unfilled 'niches', experiencing reduced competition, which has been termed 'Darwin's naturalization hypothesis' (Daehler 2001). However, Darwin (1859) himself also recognized an alternative possibility, now referred to as the 'pre-adaptation hypothesis' (Ricciardi & Mottiar 2006) or 'environmental filtering hypothesis' (Maitner *et al.* 2012), positing that exotic species were favoured in new ranges occupied by their close relatives because they were likely to share key adaptations allowing them to survive and reproduce there. This apparent paradox is referred to as Darwin's naturalization conundrum (Diez *et al.* 2008).

In the two past decades, Darwin's ideas on naturalization of exotic species have received renewed attention, fuelled largely by concerns over the negative ecological and economic impacts of exotic species invasions (Thuiller *et al.* 2010). However, previous studies examining associations between naturalized or invasive exotic species and natives often produce mixed results, providing evidence for either of the 'opposing' hypotheses in Darwin's naturalization conundrum (Rejmanek 1996; Daehler 2001; Duncan & Williams 2002; Ricciardi & Atkinson 2004; Strauss, Webb & Salamin 2006; Diez *et al.* 2008; Cadotte, Hamilton & Murray 2009; Jiang, Tan & Pu 2010). This not only highlights a need to update the theoretical framework for dealing with the conundrum, but also indicates a practical dilemma for management of biological invasions.

Only recently, however, has there been recognition that the 'opposing' hypotheses in Darwin's naturalization conundrum may not be mutually exclusive (Diez *et al.* 2008; Procheş *et al.* 2008). Yet empirical tests examining the interactive effects of the mechanisms underlying the two hypotheses on the success of exotics have been lacking. One notable exception is a recent observational study by Diez *et al.* (2008). In their pioneering work, the authors found that the probability of exotic plant naturalization in New Zealand was positively correlated with the number of their close relatives belonging to the same genus, but negatively with the abundance of those close relatives. This intriguing finding provided preliminary evidence that both pre-adaptation and competitive interactions may have affected the naturalization of the exotic species. However, like most other observational studies, their results are not immune to the influences of other confounding factors, such as introduction frequency, which may allow exotics to overcome some environmental or competitive barriers (e.g. Bucharova & van Kleunen 2009). Further, the ability to detect competitive interactions may be greater at smaller spatial scales, where many individual species do interact directly with each other (Procheş *et al.* 2008; Thuiller *et al.* 2010). Manipulative, fine-scale experiments with explicit biological context are therefore needed to examine how Darwin's naturalization conundrum can be further reconciled.

In this study, we introduced the seeds of an invasive exotic plant species *Ambrosia artemisiifolia* L. to a set of

experimental grassland communities, which consisted of either one or several resident species (i.e. monocultures or polycultures) and varied in phylogenetic relatedness to the invader. We then determined the effects of phylogenetic relatedness on the invader establishment and growth performance in these experimental grassland communities. We focused on invader establishment (indicated by 'the proportion of successfully establishing invader seeds') and growth performance (indicated by 'the average size of surviving invader individuals'), since they are two distinctly different aspects of fitness (Primack & Kang 1989). While invader establishment is largely dependent on seed germination and seedling survival, both of which are expected to be affected substantially by the local environment of new habitats (Donohue *et al.* 2010); growth performance is always considered as a reflection of the competition intensity experienced by the species (Primack & Kang 1989).

Local environment consists of two important components (Dethier & Hacker 2005): abiotic environmental conditions (e.g. temperature, precipitation and soil texture) and biotic habitat features (e.g. soil nutrient profile and enzyme activity, which may be altered by resident plant species and the associated soil microbes; Glenn-Lewin, Peet & Veblen 1992; Orwin *et al.* 2010; de Vries *et al.* 2012; Grigulis *et al.* 2013). However, the previous studies dealing with Darwin's naturalization conundrum have largely assessed the effects of abiotic environmental conditions on the success of exotics (i.e. traditional 'pre-adaptation effect': e.g. Diez *et al.* 2008; Cadotte, Hamilton & Murray 2009), since these works were based on data from large-scale observational studies with substantial abiotic heterogeneity present. In this study, we aimed to determine the effects of biotic habitat alterations (i.e. another kind of 'pre-adaptation effect', which has been largely neglected in the previous studies), considering that our experimental grassland communities were set up over an area of roughly one hectare with the abiotic environmental conditions showing little variation. We attempted to link potential biotic habitat alterations to differences in invader establishment through exploring the phylogenetic signals in soil nutrients and enzyme activities for three reasons. First, it has been found that the presence of different plant species can lead to significant divergence in soil properties, and many of these alterations are linked to variation in plant traits (Orwin *et al.* 2010; de Vries *et al.* 2012). Given that closely related species tend to share similar traits (Cavender-Bares *et al.* 2009; Kembel & Cahill 2011), it is likely that they can result in similar alterations in soil properties (i.e. phylogenetic signal is present). Secondly, there is evidence that closely related plant species share similar germination and early survival niches, which are largely attributed to phylogenetically conserved aspects of soil properties (Burns & Strauss 2011). Thirdly, soil nutrients and enzyme activities are very important components of soil properties and are found to be highly correlated to a wide range

of organisms including plant species, soil bacteria and fungi (Burke *et al.* 2011).

Specifically, the present study was aimed to test the following two hypotheses: (1) a higher proportion of invader propagules should establish in a community occupied by closely related resident species compared to one occupied by a distantly related resident (Fig. 1a), if a closely related resident species is more likely to yield biotic habitat alterations (e.g. similar soil enzyme properties) that are beneficial to the invader (i.e. a pre-adaptation effect based on biotic habitat alterations); (2) the surviving invader individuals will show reduced growth performance in the presence of close relatives compared to those surrounded by distantly related species (Fig. 1b), assuming that closely related species do occupy similar niches and thus compete with each other most intensely (Darwin 1859; Burns & Strauss 2011).

Materials and methods

STUDY SITE

Our study site is located in an arable field (111°48'E, 23°31'N) near Hei-shi-ding Forest Natural Reserve, Guangdong Province, China. The area has a subtropical humid monsoon climate, with an average annual temperature of 19.6 °C and precipitation of 1743.8 mm. This study site is a suitable system for studying exotic species invasion in a disturbed habitat at a local scale, since it represents a typical abandoned agricultural field that has been continuously colonized by diverse plant species.

EXPERIMENTAL DESIGN

In January 2009, the field was thoroughly ploughed, levelled and then divided into nine similar blocks, where 387 experimental

plots (each 4 × 1 m) were established. Before sowing, each plot was divided into three subplots, each 1 × 1 m, separated from each other by 0.5-m walkways. Seeds were sown in April 2009. Our species pool contained 16 non-invasive resident plant species belonging to nine families and five functional groups (Table 1). These resident species were chosen because they are common and abundant natives or well-naturalized species that frequently co-occur nearby, and their seeds can be easily collected. A large proportion of our experimental plots (i.e. 369) were randomly assigned to one of the following four species richness treatments: one (144 plots, with nine replicate plots for each of the 16 species), four (81 plots, with three replicate plots for each of the unique 27 combinations of four species drawn randomly from the species pool), nine (81 plots, with three replicate plots for each of the unique 27 combinations of nine species drawn randomly from the species pool) or 16 species (63 plots). The species richness gradient used in this study was based on a field survey that found that nearby grassland species richness was 1–10 per square metre, which is comparable to those values reported for grasslands in tropical/subtropical regions (Faber-Langendoen & Josse 2010). Of the remaining 18 plots, half was left unsown (control plots) and the other half was planted with *A. artemisiifolia* L. (Asteraceae) at the same time as the resident species. *Ambrosia artemisiifolia* is a competitive annual weed native to North America and has been identified as one of the most dangerous invasive exotic species in many countries throughout the world (Genton, Shykoff & Giraud 2005). In our study region, *A. artemisiifolia* is one of the most problematic invasive species in arable fields and strongly reduces crop yield. This plant also poses a health risk to people living near because of its strongly allergenic pollen, which is one of the main causes of hay fever.

Each subplot received a total of 2000 seeds divided equally among all the species planted. The number of seeds per square metre used was well within the range of values reported by Tilman (1997) who determined how the invasibility of grassland communities depended on community characteristics such as species richness. We maintained the species richness treatments by weeding three times during the first year. Weeds were removed

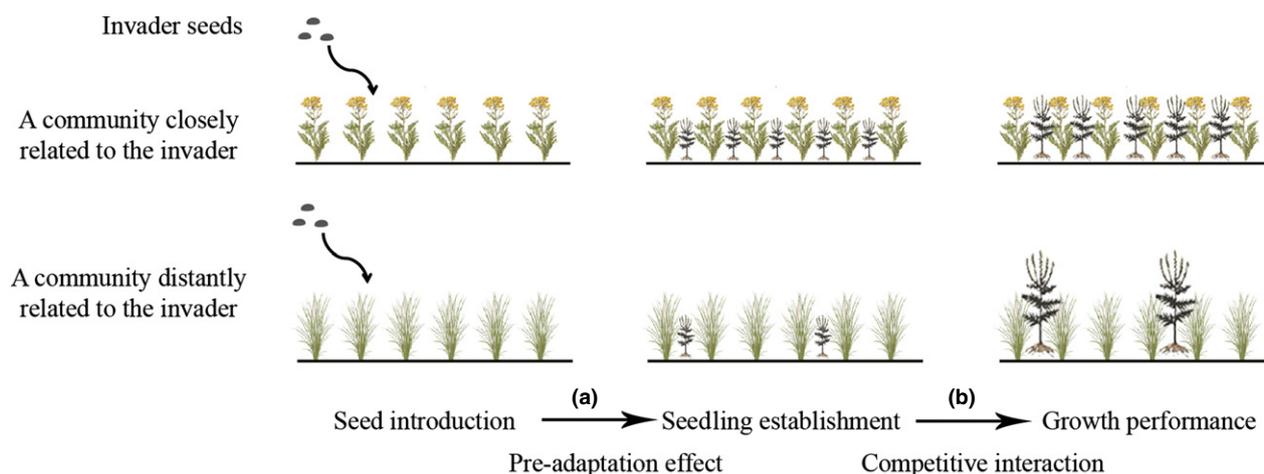


Fig. 1. A schematic diagram showing the potential effects of phylogenetic relatedness on different aspects of a potential invasion in the introduction stage. The important ecological processes are seed introduction, seedling establishment and growth performance. During seedling establishment, the invader tends to have a higher proportion established (the aggregate rate of seed germination and seedling survival) in a community dominated by its close relatives, because of a pre-adaptation effect (a). In growth performance phase, however, the average individual growth performance (indicated by average individual size) of the surviving invaders in a community dominated by its close relatives may be reduced, due to strong competition with close relatives (b).

Table 1. Summary of the plant species used in this study

Species	Family	Functional group
Resident species		
<i>Celosia argentea</i> L.	Amaranthaceae	Annual forb
<i>Ageratum conyzoides</i> L.	Asteraceae	Annual forb
<i>Bidens pilosa</i> L.	Asteraceae	Annual forb
<i>Phyllanthus urinaria</i> L.	Euphorbiaceae	Annual forb
<i>Mosla dianthera</i> (Buch.-Ham. Ex Roxb.) Maxim.	Lamiaceae	Annual forb
<i>Corchorus capsularis</i> L.	Tiliaceae	Shrub
<i>Sida mysorensis</i> Wight & Arn.	Malvaceae	Shrub
<i>Urena lobata</i> L.	Malvaceae	Shrub
<i>Dactyloctenium aegyptium</i> (L.) Willd.	Poaceae	Grass
<i>Paspalum notatum</i> Flügge	Poaceae	Grass
<i>Pennisetum alopecuroides</i> (L.) Spreng.	Poaceae	Grass
<i>Lespedeza cuneata</i> (Dum. Cours.) G. Don	Fabaceae	Legume (woody)
<i>Senna occidentalis</i> (L.) Link	Fabaceae	Legume (woody)
<i>Medicago sativa</i> L.	Fabaceae	Legume (herbaceous)
<i>Senna tora</i> (L.) Roxb.	Fabaceae	Legume (herbaceous)
<i>Dichondra micrantha</i> J.R. Forst & G. Forst	Convolvulaceae	Perennial forb
Invasive exotic species		
<i>Ambrosia artemisiifolia</i> L.	Asteraceae	Annual forb

manually while taking care to minimize any disturbance. In all monoculture plots, the target species established successfully. Very few species had been lost from only a small portion of polycultures during the first growth season, and these species were reseeded at the beginning of the second growth season. In September 2009, the nine monocultures of *A. artemisiifolia* were harvested before they produced mature seeds and remained unplanted thereafter, whereas the remaining 369 experimental plant communities were left to continue growing. In March 2010, 200 seeds of *A. artemisiifolia* were planted in one randomly selected subplot from each of the 369 plots that had been left to continue growing in September 2009. The other two subplots of each plot were reserved for other experiments. A diagram showing the experimental design is provided in Fig. S1 (Supporting information).

PLANT HARVEST AND SOIL SAMPLING

In October 2010, a timing at which the vegetation reached peak standing biomass, we counted the number of *A. artemisiifolia* individuals in each of the selected subplots (144 monocultures and 225 polycultures) and then harvested these individuals in the subplots to determine the above-ground living plant biomass. Plant materials were dried at 70 °C for at least 48 h and weighed. One soil sample was collected from each subplot of all the monocultures at each of the following two time points, respectively: April 2009 (immediately before sowing) and September 2009 (after the first growth season). Each soil sample was a composite of three soil cores (2.5 cm in diameter, 15 cm deep). After collection,

a portion of each soil sample was frozen immediately and used to assess soil enzyme activity; the remaining portion was air-dried for determination of soil nutrient profile.

SOIL CHEMICAL ANALYSIS

Sixteen metrics of nutrient profile of the soil samples collected from all the monocultures (before sowing and after the first growth season) were measured according to Schinner *et al.* (1996), including: pH, total organic carbon (TOC), readily oxidizable organic carbon (ROC), dissolved organic carbon (DOC), microbial biomass carbon (MBC), total nitrogen (TN), inorganic nitrogen (IN), total phosphorus (TP), available phosphorus (AP), Ca, Cu, Fe, K, Mg, Mn and Zn. Meanwhile the activities of eight widely investigated soil enzymes of these samples, including alkaline phosphatase, acid phosphatase, polyphenoloxidase, peroxidase, urease, protease, β -glucosidase and carboxymethyl-cellulase, were also determined as described by Schinner *et al.* (1996).

PHYLOGENETIC ANALYSIS

We constructed phylogenies of the 16 resident species and *A. artemisiifolia* using three commonly sequenced genes from the GenBank: ITS, *rbcL* and *matK* (Table S1, Supporting information). Of the 17 species, 15 had at least one gene represented in GenBank. For *M. dianthera* and *S. mysorensis*, whose sequence data on the genes were not available in GenBank, we used the gene sequences from congeneric relatives (i.e. *Mosla chinensis* Maxim. and *Sida rhombifolia* L., respectively) as a proxy. We also used the gene sequences of two additional species that diverged early in angiosperm evolution (i.e. *Amborella trichopoda* Baill. and *Magnolia grandiflora* L.) to serve as out-group species (Cadotte *et al.* 2008). Sequences were aligned for each region independently using MUSCLE (Edgar 2004) and combined into a single supermatrix. We then selected best-fit models of nucleotide substitution for each gene by jModeltest (Posada 2008).

We then constructed a rate-smoothed Bayesian phylogeny, using BEAST version 1.7.5 (Drummond *et al.* 2012), assuming a relaxed uncorrelated lognormal clock and a Yule speciation tree prior. We used five fossils (i.e. Eudicots, Rosids, Asterids, Poaceae and Asteraceae) as node age priors according to the Angiosperm Phylogeny Group tree (Apg III 2009). Parameters were estimated by using a Markov chain Monte Carlo (MCMC) simulation for 30 million generations and sampled every 1000 generations. The best-fit model (GTR + G + I) selected by jModelTest and relaxed molecular clock model were used for the three gene regions. Stationarity and effective sample sizes (ESS > 200) were examined using Tracer version 1.5 (Rambaut & Drummond 2004). We sampled the runs every 1000 generations, and the first 3000 trees were discarded as burn-in. Tree files were combined using TreeAnnotator version 1.7.5 (Drummond *et al.* 2012). Among the posterior distribution trees, the maximum clade credibility tree was used to quantify community phylogenetic patterns (Fig. S2, Supporting information).

We used the phylogenetic distance between the invader and its nearest relative resident species in a recipient community (hereafter nearest phylogenetic distance, NPD) and average phylogenetic distance between the invader and all resident species in a recipient community (hereafter mean phylogenetic distance, MPD) as metrics to represent the phylogenetic relatedness between the invader

and a recipient community (Webb *et al.* 2002). Both metrics were calculated using the R package *picante* (Kembel *et al.* 2010).

DATA ANALYSIS

We calculated the proportion of successfully establishing invader seeds in each invaded subplot as the number of surviving invader individuals in that subplot in October 2010 divided by 200 (i.e. the number of invader seeds sown in March 2010). The average size of surviving invader individuals, calculated by dividing the total above-ground biomass of the invader in an invaded subplot in October 2010 by the number of surviving invader individuals in that subplot in October 2010, was used as a metric of the invader's growth performance. We also considered the total above-ground biomass of surviving invader individuals in an invaded subplot in October 2010 as an additional metric of invader success.

Data on the proportion established were square root-transformed, while those on the average size and biomass were log-transformed ($\log_{10}(x + 1)$), in order to improve the normality of the error distribution. To assess the potential effects of phylogenetic relatedness, species richness and resident species biomass on different aspects of the invader success (i.e. proportion established, average size and biomass of the invader in an invaded community), we used generalized linear models (GLM) including NPD, MPD, species richness, biomass of the nearest relative resident species of the invader (if two nearest relative species are equally related to the invader, we consider the biomass of the more abundant one) and total community biomass (i.e. biomass of all residents) as potential predictors and then used Akaike Information Criterion (AIC) and Akaike weights (AW) to infer the best regression models. Specifically, the proportion established was fitted with a binomial distribution, while the log-transformed invader size and biomass data were fitted with a Gaussian distribution. Note that the proportion established was also included as a potential predictor of the invader size in the

multiple regressions to determine the effect of intraspecific competition.

To determine whether the closely related species of the invader were associated with biotic habitat alterations similar to those of the invader (thereby the closely related species may promote the adaptation of the invader propagules), we tested for phylogenetic signals in the 16 soil nutrients and the activities of the eight soil enzymes of the monocultures after the first growth season, using the *K* statistic (Blomberg, Garland & Ives 2003) implemented via the *multiPhylosignal* function in *Picante* (Kembel *et al.* 2010). We tested whether the observed *K* values for the monocultures were significantly different from those expected at random by comparing them to a null model constructed by shuffling taxa labels across the tips of the phylogeny. The significant *K* values indicate that closely related species tended to be associated with biotic habitat alterations similar to those of the invader. Similar analyses were done to test for phylogenetic signals of these monocultures before sowing. One-way analysis of variance (ANOVA) was also used to test whether there were significant differences between the monocultures in the soil nutrient profile and enzyme activity before sowing.

Non-metric multidimensional scaling (NMDS) was used to ordinate the 16 resident species based on the 16 metrics of soil nutrient profile of their monocultures after the first growth season. We then employed a simple Mantel test to test the correlation of the matrix of pairwise distances in the NMDS ordination with the matrix of pairwise phylogenetic distances of these species. A similar analysis was done with the data on the eight soil enzymes of the monocultures after the first growth season. In addition, we used Spearman's rank correlation test to relate the differences between the monocultures of each resident species and the invader monocultures in the soil nutrients or enzyme activities to the proportion established of the invader in monocultures of the corresponding resident species. A negative correlation indicates that the invader tended to exhibit greater proportion established in the monocultures of a resident species having a higher

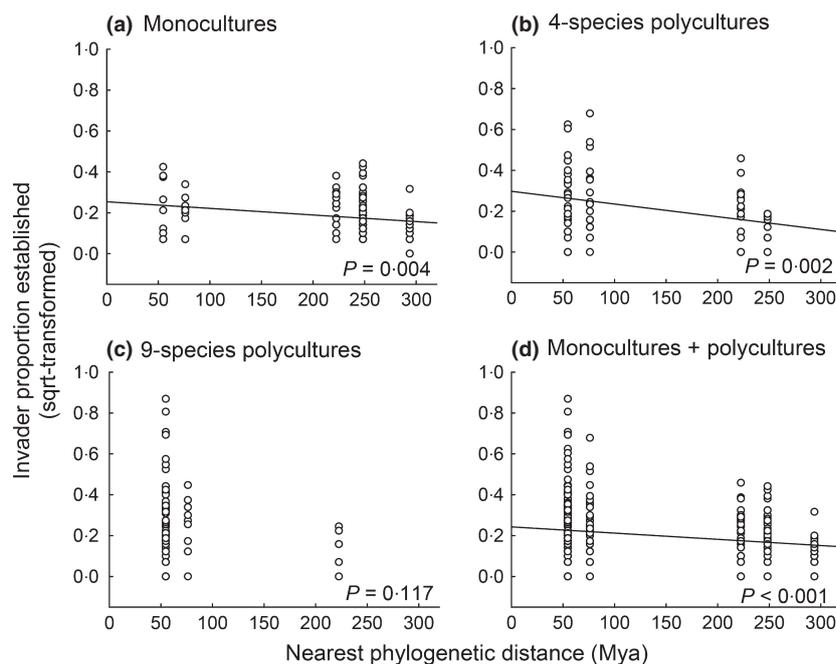


Fig. 2. Relationships between nearest phylogenetic distance (NPD) and proportion of the invader that established in the (a) monocultures, (b) 4-species polycultures, (c) 9-species polycultures and (d) all plots together. Linear regression lines are shown if significant.

similarity (i.e. a lower difference) to the invader monocultures in a given soil property.

Results

EFFECT OF PHYLOGENETIC RELATEDNESS ON THE INVADER ESTABLISHMENT

The invader successfully established in 345 of the 369 subplots and failed in 24 of the four or nine resident species, which were also included in our analysis. NPD and MPD showed similar results for the relationships between phylogenetic relatedness and different aspects of invader success. Yet, we report NPD here, since NPD was identified as a single best predictor of not only the invader establishment but also the invader size (Table S2, Supporting information). Those results based on MPD can be found in the Supporting Information (Figs S3–S5).

In the monocultures and four-species polycultures, the proportion established was negatively correlated to the phylogenetic distance ($P < 0.01$, Fig. 2a,b). A similar tendency was found in nine-species polycultures, yet it was not statistically significant ($P = 0.117$, Fig. 2c). One possible reason for this may be that NPD values from the nine-species polycultures had low variance, making it difficult to obtain a significant relationship between the phylogenetic distance and the proportion established (Cadotte & Strauss 2011). However, when the monocultures and polycultures were taken into account simultaneously, we again found a significant negative relationship between the proportion established and the phylogenetic distance ($P < 0.001$, Fig. 2d). In addition, the negative relationship between the proportion established and the phylogenetic distance observed in the monocultures was still robust

after removing a particular functional group (e.g. legume, $P < 0.05$; Fig. S6a, Supporting information), while NPD was a better predictor of proportion established than biomass of the nearest relative resident and biomass of all residents (Table S2, Supporting information). These results indicated that the observed effect of phylogenetic relatedness on invader establishment was unlikely driven by certain functional groups, the nearest relative resident or biomass of all residents.

EFFECT OF PHYLOGENETIC RELATEDNESS ON AVERAGE SIZE OF THE INVADER

In the monocultures and four-species polycultures, there was a significant (or marginally significant) positive relationship between the average invader size and the phylogenetic distance (Fig. 3a,b). Yet, the average invader size in the nine-species polycultures seemed to be unaffected by phylogenetic relatedness ($P = 0.117$, Fig. 3c), which may be caused also by the low variation in NPD from the polycultures. Nonetheless, a significant positive relationship between the average invader size and phylogenetic distance was found, when the monocultures and polycultures were included in the analysis simultaneously ($P < 0.001$, Fig. 3d). Consistent with our results on invader establishment, we found that the observed effect of phylogenetic relatedness on the average invader size did not appear to be driven by particular functional groups, the nearest relative resident or biomass of all residents. We observed that the positive relationship between the average invader size and phylogenetic distance observed in the monocultures was still robust after removing a particular functional group (e.g. grass, $P < 0.05$; Fig. S6b, Supporting information). Further, NPD was a better

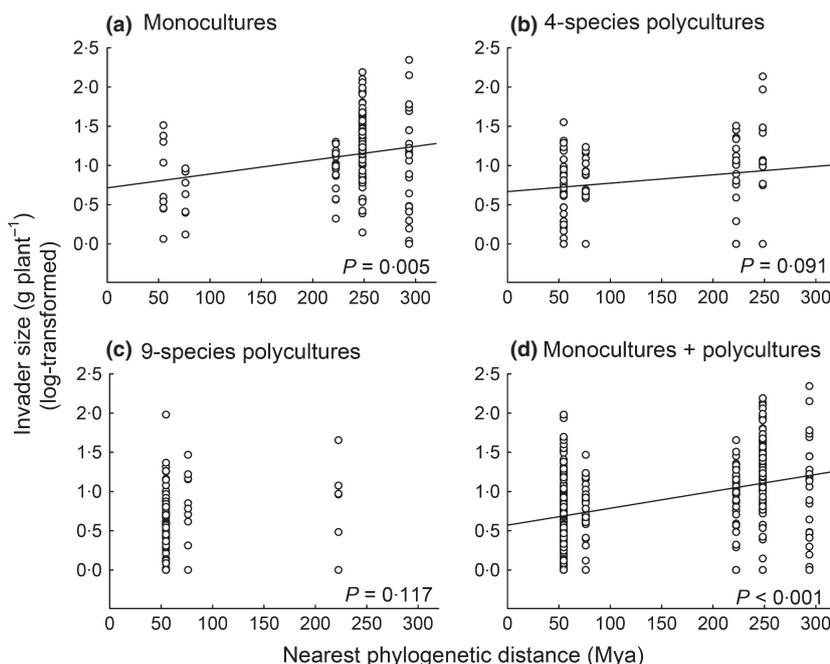


Fig. 3. Relationships between nearest phylogenetic distance (NPD) and average individual size of the invader in the (a) monocultures, (b) 4-species polycultures, (c) 9-species polycultures and (d) all plots together. Linear regression lines are shown, if significant.

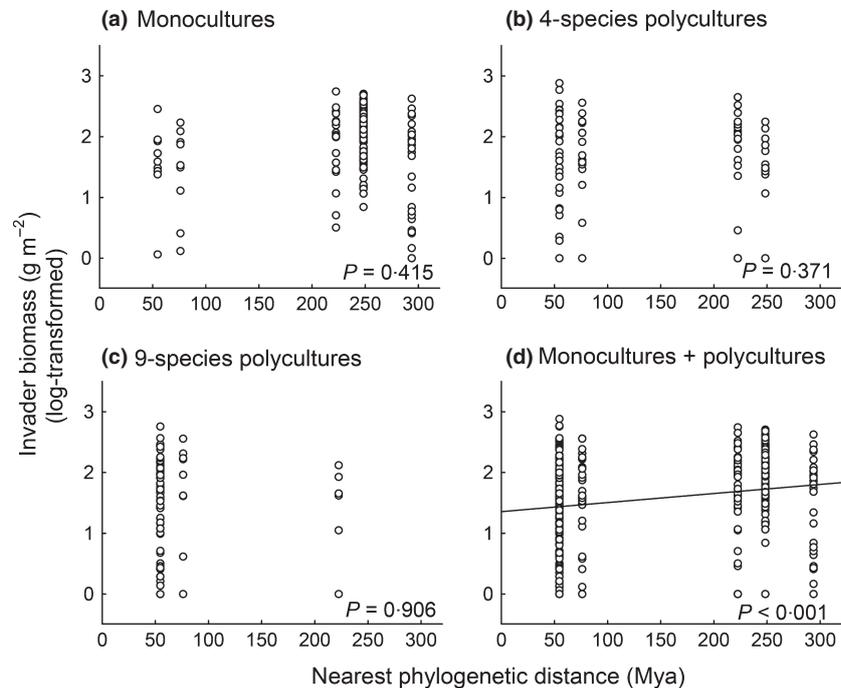


Fig. 4. Relationships between nearest phylogenetic distance (NPD) and above-ground biomass of the invader in the (a) monocultures, (b) 4-species polycultures, (c) 9-species polycultures and (d) all plots together. Linear regression lines are shown, if significant.

predictor of the average invader size than biomass of the nearest relative resident and biomass of all residents (Table S2, Supporting information).

Compared with the best predictor of the average invader size – NPD, proportion established of the invader provided a poor explanation of the average invader size (Akaike weight ≈ 0 , Table S2, Supporting information). Indeed, we found no significant relationship ($r^2 < 0.01$, $P = 0.592$) between the average individual size (log-transformed) and the proportion of the invader individuals successfully established (square root-transformed). These results indicated that the observed positive relationships between invader individual size and phylogenetic distance are not likely to be driven by invader intraspecific competition that may result in reduced per capita biomass with larger invader populations.

PHYLOGENETIC RELATEDNESS, SPECIES RICHNESS AND BIOMASS OF THE INVADER

In contrast to the results for the proportion established and average invader size, multiple regression analyses showed that a model with species richness and the biomass of all residents was a better predictor of invader biomass in the plant communities than measures of phylogenetic relatedness (NPD or MPD; Table S2, Supporting information). Indeed, invader biomass in the monocultures, four- or nine-species polycultures seemed to be unaffected by phylogenetic relatedness ($P > 0.30$, Fig. 4a–c). However, when considering all treatment levels simultaneously, invader biomass was positively related to the phylogenetic distance ($P < 0.001$, Fig. 4d). In addition, we found a significant negative relationship between species richness and the invader biomass across all the

treatments as well ($P < 0.001$; Fig. S7c, Supporting information).

PHYLOGENETIC RELATEDNESS, SOIL NUTRIENT PROFILE, ENZYME ACTIVITY AND INVADER SUCCESS

Before sowing, there were no significant difference for the monocultures in the 16 soil nutrients (except inorganic nitrogen) and the activities of the eight soil enzymes (Tables S3 and S4, Supporting information). Concomitantly, there was no significant phylogenetic signal for all these soil properties (except peroxidase; Tables S3 and S4, Supporting information), suggesting the biotically mediated soil microhabitats of the plots for these monocultures showed little variation at the beginning of this study. After the first growing season, however, a significant phylogenetic signal was detected for four of the eight soil enzymes (i.e. alkaline phosphatase, acid phosphatase, phenoloxidase and peroxidase; Table 2), despite the observation that there was no significant phylogenetic signal for the 16 soil nutrients (i.e. the summed observed K values of them showing no significant difference to the null model; Table S5, Supporting information). When either the 16 metrics of soil nutrient profile or the eight soil enzymes were considered as a whole, however, there were no significant correlations between the phylogenetic relatedness and the soil nutrient profile (Mantel test, $P = 0.536$) or the soil enzymes (Mantel test, $P = 0.407$).

On the other hand, we found that the differences between the monocultures of each resident species and the invader monocultures in the activities of two soil enzymes (i.e. alkaline and acid phosphatases) were significantly negatively correlated to the proportion established of the invader in monocultures of the corresponding resident

Table 2. Phylogenetic signals of the eight soil enzyme activities in the monocultures after the first growth season and the Spearman's rank correlation coefficients for the differences between the monocultures of each resident species and the invader monocultures in soil enzyme activities and proportion of the invader that established in the corresponding monocultures

Soil enzyme	Phylogenetic signal		Spearman's rank correlation coefficient	
	<i>K</i> value	<i>P</i> value	<i>r</i>	<i>P</i> value
Alkaline phosphatase	1.02*	0.026	-0.65	0.006
Acid phosphatase	1.00	0.008	-0.51	0.041
Polyphenoloxidase	0.96	0.024	-0.15	0.582
Peroxidase	0.62	0.449	-0.26	0.334
Protease	0.88	0.038	-0.29	0.283
Urease	0.77	0.144	-0.30	0.262
β-Glucosidase	0.71	0.216	-0.06	0.811
Carboxymethyl-cellulase	0.87	0.067	0.12	0.655

*Significant values are highlighted in bold.

species (Spearman's rank correlation: for alkaline phosphatase, $r = -0.65$, $P = 0.006$; for acid phosphatase, $r = -0.51$, $P = 0.041$; Table 2). Yet, there was no similar correlation for the other six soil enzymes and all the 16 soil nutrients ($P > 0.08$ for all, Tables 2 and S5, Supporting information).

Discussion

Exotic plant invasion can be roughly divided into three stages: introduction, colonization and naturalization (Radosovich 2007). Introduction is the shortest stage, occurring in a brief window of time. Yet, there is evidence that most exotic species fail in the introduction stage and thus only a small percentage of introduced exotics transition to the colonization or naturalization stage (Williamson 1996). Despite this, previous studies dealing with Darwin's naturalization conundrum (e.g. Duncan & Williams 2002; Strauss, Webb & Salamin 2006) focused simply on either the colonization or naturalization stage, leaving open the question of how relatedness between resident species and invaders influences the performances of invaders in the introduction stage. In this study, we looked at how phylogenetic relatedness affects invader success in the introduction stage.

On the one hand, we found that the invader establishment decreased significantly with increasing phylogenetic distance between the invader and its recipient communities (Fig. 2). Consistent with our finding, previous studies have provided some evidence for a positive effect of species relatedness on invader establishment. For example, Lambrinos (2002) reported that an invasive grass *Cortaderia selloana* tended to have higher seed germination and seedling survival in the habitat dominated by a close relative than in those dominated by its distantly related species. However, previous studies have relied on taxo-

nomic classification rather than molecular phylogeny to infer the effect of species relatedness on invader establishment. The one exception is the work of Jiang, Tan & Pu (2010) who tested Darwin's naturalization hypothesis using laboratory bacterial communities. Note that the invading bacterial species was allowed to undergo many generations in resident bacterial communities before it was sampled, and thus, the 'establishment' defined by Jiang, Tan & Pu (2010) is quite different from that in this study.

Despite a growing literature documenting the effects of species relatedness on invader success (Thuiller *et al.* 2010), the previous studies have rarely examined specific mechanisms responsible for the observed effects. As an attempt to reveal the potential mechanisms underlying the positive effect of phylogenetic relatedness on invader establishment recorded in this study, we found that four enzymes had significant phylogenetic signals (Table 2). Moreover, we showed that the invader was more likely to exhibit higher proportion established in the resident species monocultures having higher similarity to the invader monocultures in the activities of alkaline and acid phosphatases (Table 2). Given that the two soil enzymes are responsible for the hydrolysis of organic phosphorus compounds to plant-available inorganic phosphorus (Burke *et al.* 2011), these results strongly suggest the possibility that the invader establishment may have been benefiting from the similar microhabitat niches related to inorganic phosphorus in the communities of its closely related species. This mechanism seems to be important, since the concentrations of soil-available phosphorus recorded in this study were relatively low ($<10 \text{ mg kg}^{-1}$). Indeed, *A. artemisiifolia* is a mycorrhizal plant, and its invasion could be facilitated by arbuscular mycorrhizal fungi, which can enhance phosphorus uptake of the invader (Fumanal *et al.* 2006). Yet it should be noted that phylogenetic relatedness *per se* accounted for more variation in the invader proportion established than the soil enzymes, indicating that the closely related species may still have other phylogenetically conserved traits that can directly or indirectly benefit the establishment of the invader. Nevertheless, our results suggest that the biotic habitat alterations associated with the close relatives of *A. artemisiifolia*, which were at least partly mediated by the two soil enzymes, can facilitate the establishment of the invader, demonstrating a non-traditional pre-adaptation effect occurred at a relatively small spatial scale.

On the other hand, it was observed that the average size of surviving invader individuals increased significantly with increasing phylogenetic distance between the invader and its recipient communities (Fig. 3). Following recent observations highlighting the importance of facilitation among distantly related species in structuring plant communities (Valiente-Banuet & Verdú 2013), our results could be driven by not only the strong competitive interactions between the invader and its close relatives, but also facilitation between the invader and its distant rela-

tives, or a combination of both. Yet, the most distantly related species of the invader (i.e. three grass species, Table 1) have traits similar to those of the invader (e.g. high growth rate and large biomass), indicating that facilitation is unlikely to be responsible for the positive relationships (Fig. 3). Despite the evidence that competitive interactions drive the positive relationship, further studies are needed to uncover the resources for which the closely related species competed more intensively with each other. Indeed, with very few exceptions (e.g. Jiang, Tan & Pu 2010), previous studies supporting Darwin's naturalization hypothesis failed to identify specific resources that are involved in the more intensive competition among close relatives (e.g. Burns & Strauss 2011).

No attempt has been made in the literature to explicitly link the total biomass of an exotic invader in a community to the phylogenetic distance between the invader and the recipient community, although the total biomass has been often considered as a measure of invader success (Fridley *et al.* 2007). Here, no significant invader biomass–phylogenetic distance relationship was recorded in either monocultures or polycultures (Fig. 4a–c), although the opposite seemed to be true when monocultures and polycultures were taken into account simultaneously (Fig. 4d). One possible explanation for these results is that the invader biomass is a product of invader establishment and the average size of surviving invader individuals, which showed inverse relationships with phylogenetic relatedness. It should also be noted that when monocultures and polycultures were taken into account simultaneously the effects of phylogenetic relatedness are likely compounded by those of species richness due to the covariation between the two measures. Indeed, species richness was found to be a better predictor of the invader biomass than phylogenetic relatedness (Table S2, Supporting information). This is consistent with the finding that the model containing species richness and biomass of all residents was the best predictor of the invader biomass (Table S2, Supporting information), given that species richness is generally positively correlated with productivity (Hooper *et al.* 2005). Moreover, we found a negative invader biomass–resident species richness relationship (Fig. S7c, Supporting information), supporting Elton's biotic resistance hypothesis (Elton 1958). This result can be also explained in terms of phylogenetic relatedness: in more diverse communities, there is a higher probability that both distantly and closely related resident species are included because of sampling effect (Huston 1997), which leads to the reduced establishment and growth performance of the invader, thereby the reduced invader biomass.

Taken together, our results revealed that phylogenetic relatedness has contrasting effects on different aspects of invader success in introduction stage via distinctive ecological mechanisms consistent with the two 'opposing' hypotheses in Darwin's naturalization conundrum. Yet, further studies are required to evaluate the generality of our findings. An important next step needs to explore whether the patterns observed here are applicable to

more mature plant communities, because plant communities of different successional ages may vary greatly in their invasibility by exotic plant invasion, and the present study as well as most previous studies (e.g. Levine 2000; Crutsinger, Souza & Sanders 2008; Hulvey & Zavaleta 2012) focused on new establishment plant communities. It will also be crucial to determine the effects of phylogenetic relatedness on long-term dynamics of plant invasion, considering that in the present study and most previous studies (e.g. Levine 2000; Crutsinger, Souza & Sanders 2008; Hulvey & Zavaleta 2012) the success of invaders were measured after one growing season. Further work should also strive to test whether the patterns observed here apply to other invasive plant species. Despite these concerns, our findings have significant implications for management of plant invasions: to reduce the success of invasive species characterized by high establishment probability, native species distantly related to the invasive species can be planted in sites surrounding the invasion foci, given that this approach is efficient to decrease the establishment probability of invasive species, whereas the opposite seems to be true to reduce the success of those characterized by large individual size. For example, *Hesperantha falcata* and *Hypochaeris glabra* are two invasive plant species in Australia, which exhibited an high establishment probability (van Kleunen & Johnson 2007) and a large individual size (Milberg, Lamont & Pérez-Fernández 1999), respectively; therefore, for controlling *H. falcata*, its distantly related species native to Australia should be a prime consideration, whereas, the opposite should be true for controlling *H. glabra*.

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Data accessibility

The data set used in this paper is available on DRYAD entry <http://dx.doi.org/10.5061/dryad.27c5b> (Li *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. A diagram showing the experimental design of this study.

Fig. S2. Bayesian phylogeny of the plant species used in this study.

Fig. S3. Relationships between mean phylogenetic distance and proportion of the invader that established.

Fig. S4. Relationships between mean phylogenetic distance and average individual size of the invader.

Fig. S5. Relationships between mean phylogenetic distance and the invader biomass.

Fig. S6. Relationships between phylogenetic relatedness and the establishment, average size and biomass of the invader in the monocultures, with different functional groups indicated by different colors.

Fig. S7. Effects of species richness on proportion of the invader that established, average individual size and biomass of the invader.

Table S1. Summary of the sequences used to establish the phylogenetic trees.

Table S2. Results of generalized linear models (GLM) fitting proportion of the invader that established, average individual size and biomass of the invader with a set of potential predictors.

Table S3. Results of one-way ANOVA of the 16 soil nutrients in the monocultures before sowing and tests for phylogenetic signals.

Table S4. Results of one-way ANOVA of the activities of the eight soil enzymes in the monocultures before sowing and tests for phylogenetic signals.

Table S5. Phylogenetic signals of the 16 soil nutrients in the monocultures after the first growth season and correlations between soil nutrient differences and proportion of the invader that established.