



## Original article

## Two invasive acacia species secure generalist pollinators in invaded communities



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## ARTICLE INFO

## Article history:

Received 12 February 2016

Received in revised form

26 April 2016

Accepted 6 June 2016

## Keywords:

Pollination networks

Pollination services

Mutualistic interactions

Biodiversity

Plant-animal interactions

## ABSTRACT

Exotic entomophilous plants need to establish effective pollinator interactions in order to succeed after being introduced into a new community, particularly if they are obligatory outbreeders. By establishing these novel interactions in the new non-native range, invasive plants are hypothesised to drive changes in the composition and functioning of the native pollinator community, with potential impacts on the pollination biology of native co-flowering plants. We used two different sites in Portugal, each invaded by a different acacia species, to assess whether two native Australian trees, *Acacia dealbata* and *Acacia longifolia*, were able to recruit pollinators in Portugal, and whether the pollinator community visiting acacia trees differed from the pollinator communities interacting with native co-flowering plants. Our results indicate that in the invaded range of Portugal both acacia species were able to establish novel mutualistic interactions, predominantly with generalist pollinators. For each of the two studied sites, only two other co-occurring native plant species presented partially overlapping phenologies. We observed significant differences in pollinator richness and visitation rates among native and non-native plant species, although the study of  $\beta$  diversity indicated that only the native plant *Lithodora fruticosa* presented a differentiated set of pollinator species. Acacias experienced a large number of visits by numerous pollinator species, but massive acacia flowering resulted in flower visitation rates frequently lower than those of the native co-flowering species. We conclude that the establishment of mutualisms in Portugal likely contributes to the effective and profuse production of acacia seeds in Portugal. Despite the massive flowering of *A. dealbata* and *A. longifolia*, native plant species attained similar or higher visitation rates than acacias.

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## 1. Introduction

Once an exotic plant is introduced into a new community, it typically needs to engage in a number of mutualistic interactions in order to successfully establish into the new area (Richardson et al., 2000; Traveset and Richardson, 2006). For entomophilous plants, one key mutualism for the effective production of offspring is the interaction with pollinators, which is of particular importance for obligatory outbreeders (Gibson et al., 2011). Invasive plants tend to be pollinated by generalist insects both in their native and non-native ranges (Chrobock et al., 2013; Gibson et al., 2011; Gotlieb et al., 2011; Lopezaraiza-Mikel et al., 2007; Padrón et al., 2009), and often offer large floral displays and floral rewards which can influence pollinator behaviour and preferences and thus affect the

surrounding plant community either positively, by attracting shared pollinators to the site, neutrally, or negatively, by competing for pollination services (Moragues and Traveset, 2005; Larson et al., 2006; Bartomeus et al., 2008). Such effects are often species-specific, and invasive plants can benefit some native species while harming others in the same community (Ferrero et al., 2013; Moragues and Traveset, 2005). In any case, the presence of invasive species has the potential to influence pollinator species composition and relative abundance, influencing pollination efficiency and reproductive success of native plants (Ferrero et al., 2013; Gibson et al., 2012; Gotlieb et al., 2011; Lopezaraiza-Mikel et al., 2007).

Nevertheless, the potential impact of invasive species on the reproductive success of natives should be strongly related to their phenological synchrony. Because flowering phenology is an essential factor of pollination success, the degree of synchrony between the flowering period of the exotic species and the native flora has important implications both for the success of exotic species and for the native plant species (Traveset and Richardson,

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2006). Invasive species tend to present high phenological plasticity (Munguía-Rosas et al., 2013) and because flowering phenology has important fitness implications, phenology is usually under strong selection (Munguía-Rosas et al., 2011). Phenological asynchrony between native and invasive plants is frequent at invaded communities (Godoy et al., 2009a, b) and although it has been scarcely studied, the available literature suggests that asynchrony with native plant species is mostly beneficial to invasives, which have the opportunity to benefit from an at least partially empty niche (Godoy et al., 2009a, b; Wandrag, 2012). Pollinators tend to favour early flowering, particularly if it involves extending the pollination season (Elzinga et al., 2007; Munguía-Rosas et al., 2011; Rafferty and Ives, 2011). Unsurprisingly, the exploitation of new temporal niches with reduced competition for biotic and abiotic resources has been found to benefit invasive species (Godoy et al., 2009a, b; Lediuk et al., 2014) and is believed to benefit invasive acacias (Gibson et al., 2011) although experimental data is not yet available for this group of plants.

*Acacia dealbata* Link and *Acacia longifolia* (Andrews) Willd. [*Acacia* subgenus *Phyllodineae* (DC.) Ser. = *Acacia* s.s.], are two native Australian trees which have become invasive in several parts of the world, including Portugal where they are considered the most problematic and widespread invasive species in the country (Almeida and Freitas, 2012; 2006). Although both acacia species have the ability to successfully self-fertilize, the efficiency of self-pollination is low (below 0.5% of seed:ovule ratio), thus both species require pollination vectors to produce significant seed yields, and both are considered predominantly outcrossing species (Correia et al., 2014). In their native range both species sustain mutualistic interactions essential for the successful maintenance of populations and for the colonization of new areas (Berg, 1975; Gibson et al., 2011; O'Dowd and Gill, 1986; Rodríguez-Echeverría et al., 2011). In Australia they are predominantly pollinated by bees, beetles and flies, and are occasionally visited also by birds, butterflies and bee flies (Bombyliidae) (Lorenzo et al., 2010; Stone et al., 2003). Previous studies found that acacias introduced into New Zealand and South Africa successfully established mutualistic interactions with pollinators, where *Apis mellifera* played an important role (Rodger, 2012; Wandrag, 2012). However, pollination mutualisms for these two invasive species are poorly known in other invaded ranges. Previous studies on the reproductive biology of *A. dealbata* and *A. longifolia* in Portugal showed that supplementary pollination significantly increased fruit set, suggesting pollen limitation (Correia et al., 2014). *Acacia* species produce massive and long-lasting floral displays but flowering occurs during the winter, when pollinators are less active and have to face floral resource scarcity (Gibson et al., 2011; Godoy et al., 2009a, b). In the present study, we studied *A. dealbata* and *A. longifolia*, pollination ecology and networks in Portugal, as well as that of the co-occurring native species with overlapping flowering phenologies. Specifically, we aimed to assess if the two invasive acacias were able to (1) effectively guarantee pollination services in their invaded range of Portugal, and (2) whether the pollinator community visiting acacia trees differed from the pollinator communities interacting with native co-flowering plants.

## 2. Materials and methods

### 2.1. Study species

*Acacia dealbata* and *A. longifolia* are perennial trees native to Australia with a lifespan of 20–50 and 30 years, respectively (Richardson et al., 2011). Both species were introduced in Portugal in the beginning of the 20th century as forestry species, as ornamental trees, and for soil stabilization (Almeida and Freitas, 2006;

Lorenzo et al., 2010). They have expanded out of their introduction sites, particularly during the last ten years, and nowadays are dominant in many areas, being among the most aggressive invasive species in Portugal (Gibson et al., 2011; Lorenzo et al., 2010; Marchante et al., 2010). Each species has different habitat requirements and consequently thrives in different regions of Portugal, similarly to what is observed in the native area in Australia. *Acacia dealbata* inhabits Portuguese mountain ranges and road sides of continental regions, whereas *A. longifolia* inhabits coastal sand dunes across most of the Portuguese coast (Almeida and Freitas, 2006; Buscardo et al., 2010; Lorenzo et al., 2010; Rodríguez-Echeverría et al., 2009). *Acacia dealbata* and *A. longifolia* produce many small flowers that are organized in, respectively, spherical or elongated cylindrical flower heads (Correia et al., 2014). The flower heads are considered the unit with which pollinators interact, and thus, all interactions were recorded as visits per flower head and we will refer to them as “flowers” hereafter. Both acacia species present winter flowering both in their native Australia (July–September) and in the invaded Portugal (January–March), while the native co-occurring plant species start flowering in January and extend their flowering period until April (*Ulex europaeus*) or as far as June (*Erica australis*; *Lithodora fruticosa*) (Castroviejo, 2012, Vols. 4,7,11; [www.flora-on.pt](http://www.flora-on.pt) [last accessed 2016-01-04]).

### 2.2. Study sites

The pollination network was studied in the secondary dunes of Tocha for *A. longifolia* (40.328420, -8.807237, datum WSG84); and in the mountainous area of Lousã for *A. dealbata* (40.102568, -8.233698). Both sites have a Mediterranean climate with Atlantic influence. Tocha is a coastal dune system in central Portugal; the mean annual precipitation is 948 mm and mean monthly temperature ranges from 10.2 °C in January to 20.2 °C in June, with the annual mean being 16.2 °C. It consists on a well-preserved dune system with foredunes, primary dunes, inter-dune slacks and secondary dunes. The study was performed on the secondary dunes area. Vegetation is composed by trees (*Pinus pinaster* and *A. longifolia*) and characteristic sand dune species of herbs and small shrub such as *Corema album*, *Halimium halimifolium*, *Cistus salviifolius*, *Cytisus grandifolius*, *Cytisus striatus*, *Euphorbia paralias*, *Ulex europaeus*, and *Erica australis*. Lousã mountains are located 70 km inland from Tocha; the mean annual precipitation is 752 mm and mean monthly temperatures range from 3.0 °C in January to 17.6 °C in August, with an annual mean of 9.2 °C. Vegetation is composed by a mixed oak forest dominated by *Quercus canariensis*, *Castanea sativa*, *P. pinaster*, and *A. dealbata*; with an understory dominated by *E. australis*, *Erica arborea*, *Pterospartum tridentatum* and *L. fruticosa*.

### 2.3. Pollinator observations

Within each site, we selected and marked several observation plots, at least 20 m from each other, for each of the species flowering simultaneously with acacia trees. We only considered visits to flowers occupying a volume of up to one cubic meter even when the individual plant to be observed was larger (typically acacias) to guarantee the correct monitoring of all visits. For *A. dealbata* (in Lousã), we selected 13 acacia plots and seven native species observation plots: four for *E. australis* and three for *L. fruticosa*; the only two co-occurring species flowering at the time of the study. For *A. longifolia* (in Tocha), we selected 14 acacia plots and 20 native species plots: ten for *E. australis*, and ten *U. europaeus*; the only co-occurring flowering species at the time. Since we were only at the beginning of the native plant species flowering time, it was

extremely difficult to find more native individuals flowering simultaneously with acacias at the study sites. At each observation plot we counted the number of open flowers per plant at each observation time. On both sites, observations were carried out during the entire acacias flowering period, which occurred during the months of February and March 2012. During that period of time, a total of 25 and 23 h of direct observations were made in Lousã and Tocha, respectively (divided in periods of 15 min evenly distributed per plot). Observations were made during dawn and dusk (from 09:00 to 17:00 h, GMT), strictly alternating among plant species to avoid potential time-species biases. Any insect visiting any flowers in the observation plot were visually identified and collected, and the number of flowers visited recorded for each plant species. To test for visitor sampling completeness of our sampling we used eEstimateS 9.1.0 (Colwell, 2013), using bias-corrected formula for Chao1 and Chao2, running 500 randomizations with replacement to obtain rarefaction curves for each plant species on each site (see Supplementary Figs. 1 and 2).

#### 2.4. Statistical analyses

We analyzed different variables deriving from our observational data in order to characterize and describe the community of pollinators of each species and its relative importance. Information derived from these variables allowed us to compare the effective integration of acacia invaders into native pollination networks, and to compare it to those of native plant species. Variables studied included: pollinator species richness, Shannon-Wiener diversity index, Pielou's equitability index, and several analyses of  $\alpha$  and  $\beta$  diversity. After testing the data for homoscedasticity, we run separate statistical tests with SPSS 19 (IBM, 2010) for each acacia species, using plot as a replicate for each plant species. We tested each variable for differences in the pollinator community among the plant species present in each site. Pollinator species richness was estimated by simple counts. Additionally, flower visitation rates from each plot and species (number of pollinator visits per flower present per time unit in each individual plant) were analyzed with R 3.0.2 (R Development Core Team, 2010) with visitation rates as the study variable, plant species as the fixed factor, and plot as a random factor; we tested for data overdispersion and homogeneity of residuals and used Generalized Linear Mixed Models with Poisson distribution of errors fitted by maximum likelihood by using the procedure *glmmPQL* available in the library *MASS*.

Shannon-Wiener diversity index was used to estimate the pollinator diversity associated to each plant species. It was estimated for each plot as:  $H' = \sum p_i (\ln p_i)$  in which  $p_i$  represents the abundance of each pollinator species. It was then analyzed with a General Linear Model in SPSS with  $H'$  as the study variable and plant species as the fixed factor on each site. We calculated Pielou's evenness index  $J' = H'/H'\max$ , based on the average  $H'$  for all pollinators of each of the plant species, with  $H'\max$  being the maximum value of  $H'$  for a given community, calculated as the natural logarithm of the total number of pollinator species present in that site. Pielou's  $J'$  ranges from zero to one, with one indicating the highest possible evenness and zero the lowest. Since only one index could be obtained from each plant species, no further statistical testing was done for this index.

$\beta$  diversity was used to assess the variation in pollinator species composition among sites, and provides an interesting link between pollinator diversity for visitors of each plant species ( $\alpha$  diversity) and the total species pool on a site ( $\gamma$  diversity) (Anderson et al., 2011). We calculated the commonly used Whittaker's  $\beta$  diversity index  $B_w$  (Whittaker, 1972) in which  $\beta_w = ((\gamma - \alpha) / \alpha) = (\gamma / \alpha - 1)$ , being  $\gamma$  the total number of pollinator species at the site and  $\alpha$  the

number of pollinator species observed for each plant species. This is the most commonly used index that measures variation among micro-environments; however, the classic  $\beta_w$  statistic does not consider the relative importance of the studied interactions. Jost (2007) recently defined a measure for  $\beta$  diversity,  $\beta_{\text{Shannon}}$ , that includes information not only on species richness but also relative abundance information and that is calculated as follows:  $\beta_{\text{Shannon}} = H_\gamma / H_\alpha$ ; where  $H_\gamma = \exp(H'_{\text{pooled}})$  is an exponentiated Shannon-Wiener index in which the  $H'_{\text{pooled}}$  is calculated by pooling all data prior to the calculation of the  $H'$  (as opposed to an average of several  $H'$ ), and  $H_\alpha = \sum \exp(H'_i) / N$  is the average of each individual  $H'$  index exponentiated ( $H'_i$ ) for each of the sampling units ( $N$ ). Both  $\beta_w$  and  $\beta_{\text{Shannon}}$  were calculated in this study. Finally, we wanted to test for variation in community structure among the study species. Thus, we tested the homogeneity of multivariate dispersions by using functions "vegdist" and "betadisper" from package "vegan" for R (Oksanen et al., 2013). This allows to compare variation among *a priori* categorical groups (Anderson et al., 2011, 2006).

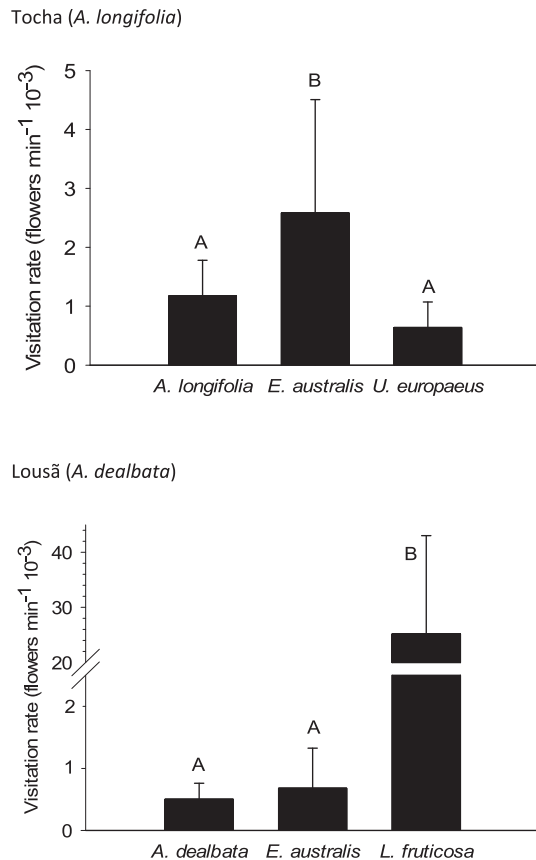
For each site, two different interaction networks were plotted: one bipartite network based on *qualitative* data, defined for this particular case as the raw number of interactions between each receptive flower and each insect visitor species; and a second bipartite network based on *quantitative* data, i.e. the number of interactions observed per receptive flower present at the moment of observation, per unit of observation time. The use of both network plots reflected different aspects of the studied interactions and allowed for a more comprehensive analyses of the interactions. For the qualitative networks, we calculated the following indexes: *connectance*, indicating the realized proportion of possible links, obtained by the total sum of links divided by the number of cells in the interaction matrix (Dunne et al., 2002); *nestedness* (weighted NODF) indicating how the system is organized, with values closest to zero indicating high nestedness and values nearing 100 low nestedness (Rodríguez-Girones and Santamaría, 2006); *network specialization index H2'*, describing the level of specialization of the entire bipartite network, and ranging between zero (no specialization) and 1 (complete specialization) (Blüthgen et al., 2006); *species specialization index d'*, describing the level of specialization of each an species in the network ( $d'$ ), and also ranging between zero (no specialization) and 1 (complete specialization) (Blüthgen et al., 2006); *animal robustness*, measuring the sensitivity of the system to the loss of plant species; and *plant robustness*, measuring the sensitivity of the system to the loss of animal species (Burgos et al., 2007; Memmott et al., 2004). For detailed descriptions of the different indexes used, see Dormann et al. (2009) and references therein. Interaction networks were obtained by using the statistical package "bipartite" on R (Dormann et al., 2009).

### 3. Results

#### 3.1. Pollinator visitation rates

In Tocha all three plant species presented significantly different pollinator visitation rates ( $t_{2,220} = -2.053$ ;  $p = 0.041$ ). *Erica australis*, experienced the highest pollinator visitation rate (mean  $\pm$  SD;  $2.581 \cdot 10^{-3} \pm 5.453 \cdot 10^{-3}$  visits per minute;  $p \leq 0.05$ ), *U. europaeus*, showed the lowest ( $0.638 \cdot 10^{-3} \pm 1.508 \cdot 10^{-3}$ ;  $p \leq 0.05$ ), whereas *A. longifolia* presented an intermediate value ( $1.117 \cdot 10^{-3} \pm 2.273 \cdot 10^{-3}$ ;  $p \leq 0.05$ ) (Fig. 1; see list of pollinator species in Table 1).

The invasive *A. dealbata* presented pollinator visitation rates (mean  $\pm$  SD;  $0.503 \cdot 10^{-3} \pm 0.930 \cdot 10^{-3}$ ) similar to those of the native *E. australis* ( $0.651 \cdot 10^{-3} \pm 1.118 \cdot 10^{-3}$ ;  $t_{2,118} = -0.401$ ;  $p = 0.689$ ). Both species visitation rates were significantly lower than those of the



**Fig. 1.** Pollinator visitation rates (pollinator visits per thousand flowers per minute) for species co-occurring on sites colonized by *A. longifolia* (top) or *A. dealbata* (bottom). Bars indicate mean  $\pm$  SE. Different letters indicate statistically significant differences.

native species *L. fruticosa* ( $25.167 \cdot 10^{-3} \pm 30.932 \cdot 10^{-3}$ ;  $t_{2,118} = -8.031$ ;  $p = 0.001$ ) (Fig. 1).

Rarefaction curves show that our flower visitor sampling completeness was correct, only two of the plant species in Tocha were not fully saturated, but were nearly so (see Supplementary Figs. 1 and 2).

### 3.2. Pollinator species richness

A total of 22 floral visitor species were identified for the three plant species in the dunes of Tocha, invaded by *A. longifolia* (Table 1). *Acacia longifolia* flowers were visited by 16 different pollinator species, nine of them exclusive to this species; *E. australis* received visits from nine different pollinator species, none exclusive to this species; and *U. europaeus* was visited by 12 different species, two of them exclusive to this species (see merged native  $\bar{\alpha}$  and  $\gamma$  values on Table 2). The diversity of pollinators for each plant species showed low values for the native species *U. europaeus* and *E. australis*, and slightly higher values for the invasive *A. longifolia*, but no statistically significant differences were found among species (mean  $H'$ :  $0.999 \pm 0.396$ ;  $F_{1,2} = 0.980$ ;  $p = 0.388$ ; Fig. 2).

A total of 20 floral visitor species were identified for the three plant species in the mountains of Lousã, invaded by *A. dealbata* (Table 1). *Acacia dealbata* flowers were visited by up to 14 different pollinator species, six of them exclusive to the species; *E. australis* was visited by 13 different species, four of them exclusive to this plant species; and *L. fruticosum* was visited by only four pollinator species, one of them exclusive to this plant species (see merged native  $\bar{\alpha}$  and  $\gamma$  values in Table 2). The three plant species showed

**Table 1**

Specialization index  $d'$  for each studied plant or animal species in decreasing  $d'$  order. Index  $d'$  indicates specialization of each species based on its discrimination from random selection of partners; it ranges from 0 to 1 to indicate extreme generalization or specialization, respectively (Blüthgen et al., 2006).

Tocha ( <i>A. longifolia</i> )		Lousã ( <i>A. dealbata</i> )	
Plant species	$d'$	Plant species	$d'$
<i>Ulex europaeus</i>	0.193	<i>Lithodora fruticosa</i>	0.961
<i>Acacia longifolia</i>	0.159	<i>Erica australis</i>	0.361
<i>Erica australis</i>	0.147	<i>Acacia dealbata</i>	0.291
Animal species	$d'$	Animal species	$d'$
<i>Oxythyrea funesta</i>	0.455	<i>Bombylus major</i>	0.986
Formicidae 1	0.339	Formicidae 2	0.332
<i>Vespa</i> sp. 2	0.339	<i>Oxythyrea funesta</i>	0.266
<i>Bombylus major</i>	0.287	<i>Tabanus</i> sp.	0.237
<i>Tabanus</i> sp.	0.150	<i>Apidae</i> sp. 3	0.227
<i>Apidae</i> sp. 3	0.129	Coleoptera	0.223
Silphidae	0.128	<i>Coccinella septempunctata</i>	0.166
<i>Vespa</i> sp. 3	0.083	<i>Bombus</i> sp. 2	0.147
<i>Bombus terrestris</i>	0.077	<i>Gonepteris</i> sp.	0.119
<i>Pieris</i> sp.	0.076	<i>Bombus terrestris</i>	0.096
<i>Vespa communis</i>	0.073	<i>Melicta athalia</i>	0.090
<i>Musca</i> sp. 3	0.068	Culicidae	0.090
Culicidae	0.066	<i>Pieris</i> sp.	0.085
<i>Musca</i> sp. 2	0.066	<i>Apis mellifera</i>	0.081
Curculionidae	0.061	<i>Vespa</i> sp. 2	0.077
Silphidae 2	0.061	<i>Vespa communis</i>	0.064
<i>Vespa</i> sp. 4	0.055	<i>Apidae</i> sp. 2	0.063
<i>Apidae</i> sp. 2	0.043	<i>Vespa</i> sp. 4	0.062
<i>Musca domestica</i>	0.042	<i>Vespa</i> sp. 3	0.012
<i>Apis mellifera</i>	0.022	<i>Musca domestica</i>	0.000
<i>Gonepteris</i> sp.	0.010		
Formicidae 2	0.000		

significantly different  $H'$  pollinator diversity values ( $F_{1,2} = 2.368$ ;  $p = 0.019$ ), with *E. australis* showing the highest pollinator diversity value, *L. fruticosa* the lowest, and *A. dealbata* an intermediate value (Fig. 2).

### 3.3. Pollinator evenness

On the dunes invaded by *A. longifolia*, the invasive species presented the highest evenness value (Pielou's  $J'$ ) while the native *E. australis* and *U. europaeus* had lower and similar values (Table 3). On the mountains of Lousã, invaded by *A. dealbata*, Pielou's evenness index was highest for the native *E. australis*, intermediate for *A. dealbata*, and lowest for *L. fruticosa*, indicating the specialist nature of the pollinator network of the latter native species, which experienced visits from only a very limited sub-set of specialized pollinators (Tables 1 and 3; see further details within the subsection "Interaction networks").

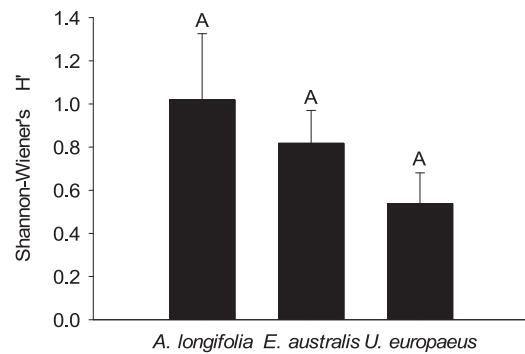
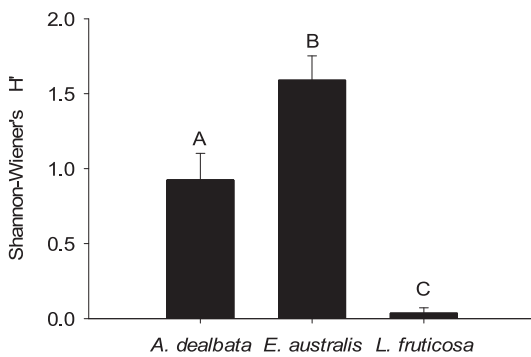
### 3.4. Pollinator $\beta$ diversity

For both the coastal Tocha (with *A. longifolia*) and the mountainous Lousã (with *A. dealbata*)  $\beta_W$  was higher for acacia trees and lower for other native plants, and  $\beta_{Shannon}$  indices showed a similar pattern (Table 2), indicating that the composition of the pollinator community is more stable across native plant species plots, whereas acacias seem to present a high variation in the pollinator community among plots within each of the sites. The study of  $\beta$  diversity through the homogeneity of multivariate dispersions (Fig. 3) showed that, in *A. longifolia*'s site (Tocha), average distances from each plot to each plant species centroid are very similar for the three plant species, indicating that pollinator species turnover is very similar among them. However, for *A. dealbata*'s site (Lousã), plots of the native *L. fruticosa* presented a species turnover clearly

**Table 2**

Pollinator diversity indexes for acacia and native species within each site. See the methods for an explanation of each index. See  $H'$  values and errors on Fig. 2.

		$J'$	$\bar{\alpha}$	$\gamma$	$\beta_w$	$\beta_{Shannon}$
Tocha	<i>A. longifolia</i>	0.340	5.375	16	1.977	0.933
	Natives	0.300	4.555	12	1.195	0.757
Lousã	<i>A. dealbata</i>	0.380	3.375	14	1.667	0.890
	Natives	0.414	6.250	14	1.240	0.677

Tocha (*A. longifolia*)Lousã (*A. dealbata*)

**Fig. 2.** Shannon-Wiener  $H'$  indexes for each plant species present on sites colonized by either *A. longifolia* (top) or *A. dealbata* (bottom). Bars indicate mean  $\pm$  SE. Different letters indicate statistically significant differences.

differentiated from that of the other two plant species, as indicated both by a higher distance to centroid for this plant species and by the scatter plot of principal component analyses of beta values.

#### 4. Interaction networks

The studied networks were small in size, thus bipartite analyses showed high connectance, and low specialization and nestedness in both qualitative networks (Table 4; Fig. 4A and C). The highly

specialized guild of pollinators associated with *L. fruticosa* made the network of the mountain site invaded by *A. dealbata* to have a nestedness value more than twice as high as that of the dunes network invaded by *A. longifolia*. The  $H2'$  index for specialization at the whole network level was much higher for the mountainous Lousã network than for the coastal Tocha. This pattern was also evident in the quantitative plots (Fig. 4B and D). The network in Lousã was dominated by the interactions between *L. fruticosa* and two pollinator species. This plant species presented not only the highest rate of visits per flower but a high specialization being visited only by four pollinators. The other two plant species had more visitor species but much lower pollinator visitation rates.

Pollinators had medium to high robustness to the loss of plant species, while plants presented notably high robustness values to the loss of pollinator species, which again indicates the overall low dependence from specialized pollinators, with the remarkable exception of *L. fruticosa* (Table 4). At the plant species level, *L. fruticosa* presented the highest level of specialization of all plant species ( $d' = 0.96$ ), being almost three times higher than the closest specialized plant species in the system. The principal pollinator of *L. fruticosa*, *Bombylius major*, also obtained the highest value of specialization for insects ( $d' = 0.99$ ) in the Lousã network. This species is also present in Tocha, where it obtained the fourth highest  $d'$  value but much lower than in Lousã ( $d' = 0.29$ ). This indicates that despite the high specialization of *B. major* in *L. fruticosa*, this insect species explores other resources with a lower degree of specialization when *L. fruticosa* is not present. Overall, the most important acacia pollinators had very low specialization values (e.g., *A. mellifera* obtains  $d' = 0.08$  and  $d' = 0.02$  for each network) indicating that these invasive acacia species are mainly pollinated by generalists.

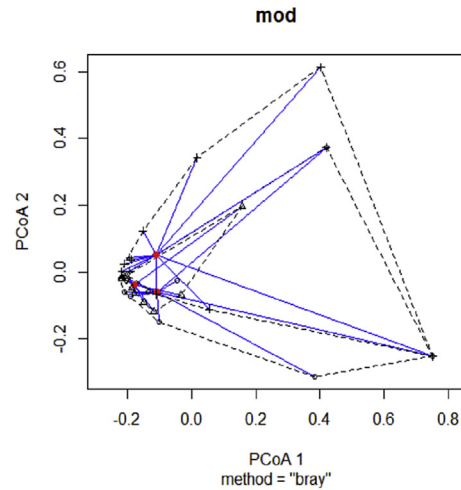
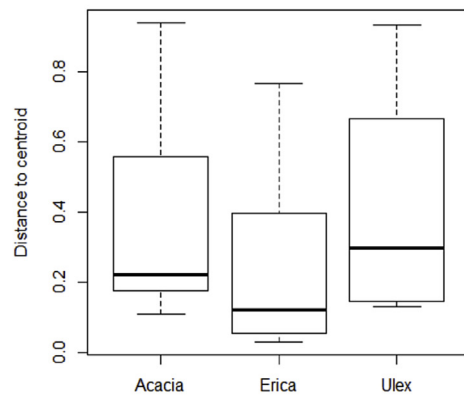
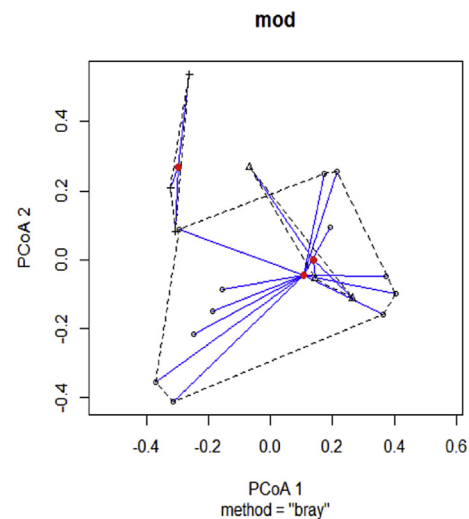
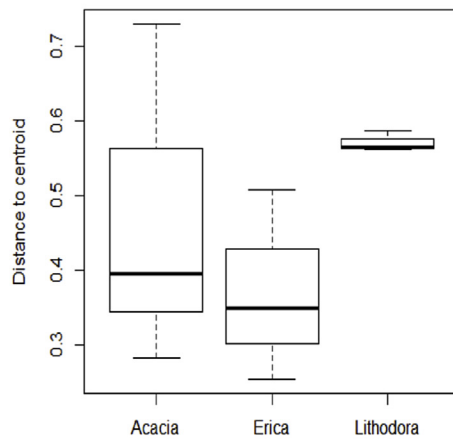
#### 5. Discussion

Both *Acacia dealbata* and *A. longifolia* successfully established new mutualistic interactions with pollinators in the invaded range of Portugal. Acacias tend to be pollinated by generalist insects in their native range (Gibson et al., 2012; Rodger, 2012; Wandrag, 2012). Our results show the same trend for the invaded range, which is the most general case for invasive plants (Bezemer et al., 2014; Ferrero et al., 2013; Lopezaraiza-Mikel et al., 2007; Richardson et al., 2000) (Table 1). Generalized pollination systems increase the likelihood of the introduced plant species to attract generalist pollinators in the novel range and to readily establish new

**Table 3**

Pollinator diversity indexes for each plant species blooming at the study time within each site, regardless of micro-environment. See the methods for an explanation of each index. See  $H'$  values and errors on Fig. 2.

		$J'$	$\bar{\alpha}$	$\gamma$	$\beta_w$	$\beta_{Shannon}$
Tocha	<i>A. longifolia</i>	0.330	4.429	19	3.290	1.128
	<i>Erica australis</i>	0.264	3.083	9	1.919	1.113
	<i>Ulex europaeus</i>	0.174	2.300	10	3.348	1.254
Lousã	<i>A. dealbata</i>	0.315	4.000	14	2.500	0.879
	<i>Erica australis</i>	0.540	7.333	13	0.774	0.564
	<i>Lithodora fruticosa</i>	0.012	1.333	3	1.251	1.436

Tocha (*A. longifolia*)Lousã (*A. dealbata*)

**Fig. 3.** Variation on *Beta* diversity ( $\beta$ ) based on homogeneity of multivariate dispersions for *A. longifolia* (top) and *A. dealbata* (bottom). Mean distance to centroid (left) for observations from each plot to each plant species; and scatter plot (right) of principal component analyses of beta values for each individual plot, and the calculated centroid for each of the three plant species (red dot) present in each site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 4**

Plant-pollinator network level indices for both study sites. See the methods for an explanation of each index.

	Tocha network ( <i>A. longifolia</i> )	Lousã network ( <i>A. dealbata</i> )
Connectance	0.576	0.517
Nestedness	14.220	31.860
$H2'$ (specialization)	0.285	0.464
Robustness of animals	0.625	0.621
Robustness of plants	0.941	0.902

mutualistic interactions (Gibson et al., 2011; Lopezaraiza-Mikel et al., 2007; Olesen et al., 2002). Previous works reported that acacias introduced into New Zealand and South Africa established mutualistic interactions with pollinators, where *Apis mellifera* was of particular importance (Rodger, 2012; Wandrag, 2012). Thus absence of suitable pollinators is unlikely to hinder invasion of acacia trees, particularly since *A. mellifera* occurs in most regions suitable to invasion by acacias (Rodger, 2012). In our study of acacia flower visitation the *net number of visits* to acacia flowers was very

high, although acacia's massive floral display resulted in only intermediate to low *visitation rates* when compared with native plants (Fig. 1). Low visitation rates are, in agreement with previous studies, detecting pollen limitation in Portugal (Correia et al., 2014). Although we did not directly evaluate the effect of pollinator visitation rates on acacia fitness, the available data indicates that, in spite of the low visitation rates detected in our study, seed production in Portugal is surprisingly high (Correia et al., 2014; Lorenzo et al., 2010; Marchante et al., 2010). In fact, final

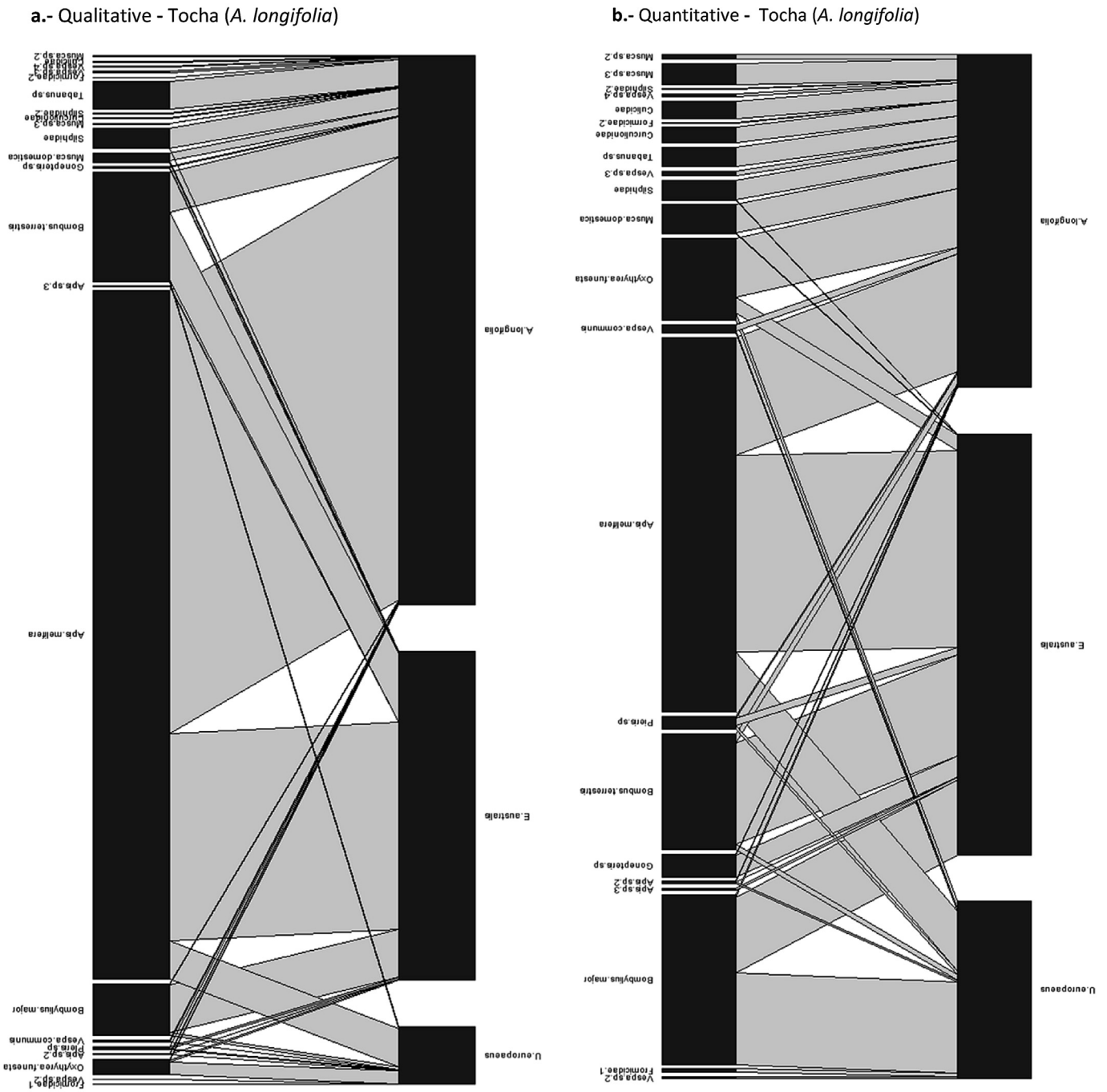


Fig. 4. Qualitative (a,c, based on total number of interactions) and quantitative (b,d, based on interactions per flower per minute) bipartite interaction networks for *A. longifolia* (a,b) and *A. dealbata* (c,d) at the whole site level.

reproductive outputs in Portugal (soil seed banks) seem to be similar or higher than in their native range (Gibson et al., 2011; Marchante et al., 2010). Altogether, the available data indicate that, irrespective of the low pollination efficiency found in the invaded range of Portugal, acacias are not limited in their reproductive outputs, a particularly relevant fact, since acacia's self-fertilization rate is extremely low (Correia et al., 2014). In our study, acacia's winter flowering resulted in little competition for pollinators with native plant species. On each site, only two other native species showed partially overlapping phenologies with acacia, and flowering of those native species

extended over longer periods (Castroviejo, 2012, Vols 4,7,11; www.flora-on.pt [last accessed 2016-01-04]). Acacias are known to be an important source of honey (Moncur et al., 1995) and their abundant flowering early in the season might be favoring certain types of generalist pollinators, including *A. mellifera*, which could be taking advantage of an extension on the pollination season. The domestic honeybee is known to compete with wild native pollinator species, like *Bombus* spp., relying on the same restricted suite of plant species (Kenis et al., 2008; Moritz et al., 2005). Still, habitat modification caused by high density acacia tree stands should also be considered when evaluating potential impacts of acacia invasions

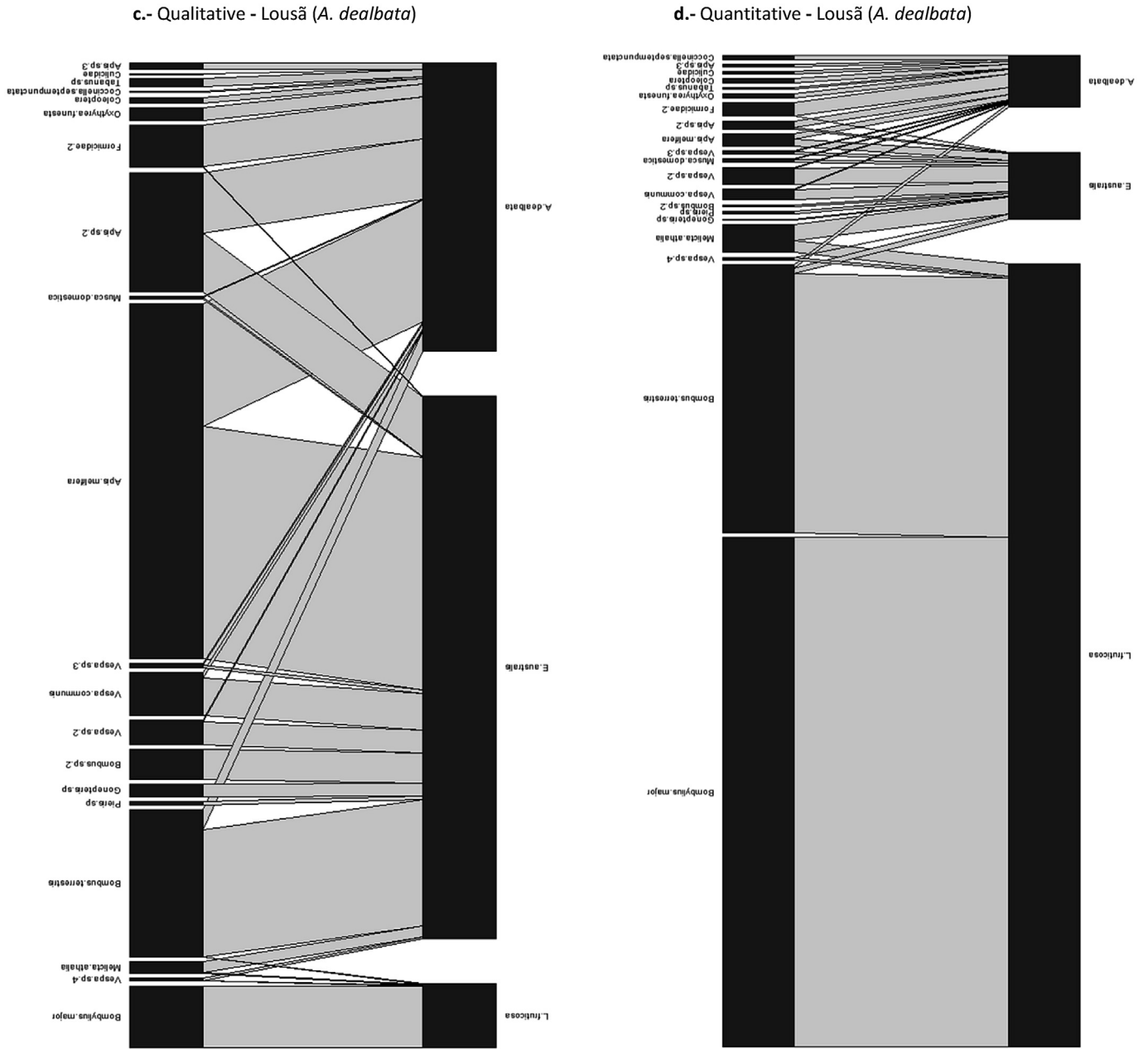


Fig. 4. (continued).

on the native pollinator communities, since pollinator communities are highly sensitive to changes in their nesting habitats, and invaded habitats could become increasingly dominated by generalist species able to take advantage of an extended pollination season (Pyšek et al., 2012; Steffan-Dewenter et al., 2002).

At the invaded location by *A. longifolia* (dunes of Tocha) the three plant species with overlapping phenologies were visited by a similar array of pollinator species (Tables 2 and 3, Figs. 2 and 3). In contrast, in the site with *A. dealbata* (mountains of Lousã) we observed two clearly distinct strategies: on the one side the invasive *A. dealbata* and the native *E. australis* produced large floral displays, attracting proportionally more generalist pollinators and achieving the highest net number of visits but the lowest visitation rates per flower; on the other side, the small native *L. fruticosa* produced very few flowers specialized in few pollinators, which resulted in a low number of interactions but the highest visitation

rates per flower. This translated into a high pollinator diversity index for *E. australis*, an intermediate value for the invasive *A. dealbata*, and a low value for *L. fruticosa*, which was visited by only four insect species. The study of  $\beta$  diversity among plant species confirms the existence of clearly separated sets of specialized pollinators for *L. fruticosa*, whereas the rest of the plant species, native or invasive, were pollinated by a similar set of insect species within each site. Other  $\beta$  diversity indexes showed a similar pattern, although some species (like *L. fruticosa*, *A. longifolia* or *U. europaeus*) showed higher  $\beta_W$  values than others; when equitability was taken into account ( $\beta_{Shannon}$ ) only the native *L. fruticosa* stood out with a clearly different set of very specialized pollinators, some of which rarely interacted with the other plant species (Tables 2 and 3, Figs. 2 and 3). This is in agreement with previous studies that described a specialized set of pollinators for this native species (Ferrero et al., 2010). Pielou's  $J'$  evenness reflects this



pattern: although the index was unaffected by acacia presence, it differed among plant species. *Lithodora fruticosa* and *U. europaeus* had the lowest values, representative of the uneven composition of their flower visitors, while *A. longifolia* and *E. australis* showed the highest values indicating that they are visited by a more homogeneous array of insects. Notably, both species of acacia were visited by the highest total number of insect species ( $\gamma$ ) in both sites, supporting the generalist pollination systems attributed to these species on their native Australia (Gibson et al., 2011).

The comparison of bipartite pollination networks based on net number of interactions (i.e. qualitative network) or on number of interactions corrected per number of flowers per unit of time (i.e. quantitative network) helped to explain the patterns shown by diversity indexes. In the qualitative networks acacias showed a high number of interactions, a logical consequence of a massive flowering display with a generalist syndrome (Table 4, Fig. 4). However, when the strength of those interactions is taken into account in the quantitative plots, the proportional number of interactions of acacia species in each global pollination network is considerably smaller. Remarkably, the small native plant *L. fruticosa* presents a dramatically high visitation to flower ratio, becoming the plant species experiencing the highest interaction rates in *A. dealbata*'s quantitative network. From the pollinator's perspective, the comparison of qualitative and quantitative networks also offers interesting information relative to pollinator's abundance and importance. The domestic *Apis mellifera* is the most frequent pollinator species in both qualitative networks (*A. dealbata* and *A. longifolia*). In the quantitative network for the region invaded by *A. longifolia* the generalist *A. mellifera* prevails as the most important pollinator. However, in the quantitative network from the region invaded by *A. dealbata*, *Bombylus major* and *Bombus terrestris* take over as the most important visitors as a consequence of their highly efficient interaction with *L. fruticosa*.

## 6. Conclusions

Overall, our data showed significant differences in pollinator richness and visitation rates among native and non-native plant species. The native species *E. australis*, in the secondary dunes, and *L. fruticosa*, in the mountain area, achieved the highest visitation rates. However, significant differences in the number of pollinator species interacting with each species were detected only for *A. dealbata*'s mountain area of Lousã. Our results indicate that both acacia species have established novel mutualisms in the invaded area in Portugal, which likely contributed to their invasiveness and the establishment of dynamic populations in the invaded area. However, invasive success is likely explained by a combination of traits (García et al., 2013), of which successful pollination would likely be a necessary but not sufficient component (e.g. Wandrag, 2012). The use of two different species and habitats allows for an easier generalization of our findings, however, pollination services need to be evaluated across time and space to extrapolate the results obtained. The relative importance of pollinator services over other factors contributing to invasiveness would only be fully determined after evaluating other factors such as seed predation and dispersal.

## Author's contributions

DM, SC and SRE conceived and designed the experimental work. DM developed the field work and analyzed the data. DM, SC and SRE wrote the manuscript.

## Acknowledgements

Thanks to Ruben Heleno, Marta Correia, Andreia Jorge, Pali Somogyi, and Álvaro Peris Mateo for their help. Research was funded by the Portuguese Fundação para a Ciência e a Tecnologia (FCT) via project Mutualnet (PCT/BIA-BEC/103507/2008). DM received funding from the FCT (SFRH/BPD/72595/2010), and the EU (FP7-PEOPLE-MC-CIG-321909). SC received funding from the FCT (Starting grant IF/01267/2013). SRE received funding from the FCT (Development Grant IF/00462/2013). FCT is partially funded by the EU via QREN, COMPETE and FEDER.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.actao.2016.06.002>.

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