

ESCOLA DE CIÊNCIAS E TECNOLOGIA

DEPARTAMENTO DE BIOLOGIA

BIODIVERSITY AND PLANT-POLLINATOR INTERACTIONS IN NATIVE FOREST AREAS OF TERCEIRA ISLAND (AZORES) |

Mariana Moreira Ferreira |

Orientação Doutor Mário Boieiro | Prof^a. Doutora Maria Paula Simões |

Mestrado em Biologia da Conservação

Dissertação

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Biodiversidade e interações planta-polinizador em áreas de floresta nativa da ilha Terceira (Açores)

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Biodiversidade e interações planta-polinizador em áreas de floresta nativa da ilha Terceira (Açores)

Resumo

Neste trabalho avaliámos o impacto que a perturbação humana das comunidades da floresta nativa da ilha Terceira (Açores) tem sobre a riqueza específica e nas interações ecológicas entre plantas e polinizadores. Para o efeito selecionámos dois locais emparelhados (um bem conservado e outro degradado), em duas áreas de estudo (Lomba e Pico Galhardo) e analisámos a integridade das redes ecológicas planta-polinizador. Constatámos que nas áreas bem conservadas as interações são dominadas por espécies nativas, enquanto que nas áreas perturbadas, apesar da prevalência de plantas introduzidas, os polinizadores nativos têm um papel relevante. Em cada área houve apenas uma planta que concentrou as visitas dos polinizadores, que na sua maioria pertenceram à ordem Diptera e tiveram hábitos generalistas.

<u>Palavras-chave</u>: biodiversidade, polinização, interações bióticas, floresta nativa, redes ecológicas

Biodiversity and plant-pollinator interactions in native forest areas of Terceira island (Azores)

Abstract

In this work we evaluate the effect of anthropogenic disturbance in native forest communities of Terceira island (Azores) on species richness and on plant-pollinator ecological interactions. We selected paired sampling sites (one well-preserved and one disturbed) in two study areas (Lomba and Pico Galhardo) and we analysed plant-pollinator networks integrity. We found that native species dominate in well-preserved sites whereas in disturbed sites, despite the prevalence of introduced plants, native pollinators play an important role. In each area there was a plant that received most of the insect visits, being these mainly from Diptera order and having generalist behaviour.

Keywords: biodiversity, pollination, biotic interactions, native forest, ecological networks

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1. Introduction

In "The Theory of Island Biogeography", MacArthur and Wilson (1967) say that there are more islands than continents and oceans. Islands come in many shapes and sizes, and their arrangement in space, geology, environments, and biotic characteristics are each extremely variable and diverse, which makes them ecologically and culturally unique but also fragile and vulnerable (Whittaker and Fernández-Palacios, 2007). This set of properties and key variables influence island diversity and the abundance and distribution of species on islands.

Given that the total number of living species on the planet is not yet fully known, it is difficult to be aware of the effective contribution of islands to global biodiversity. Yet, there are sufficient data to demonstrate that despite being species-poor for their size, in comparison to the mainland, islands are rich in endemisms and therefore known to highly contribute to global biodiversity, being biodiversity hotspots (Whittaker and Fernández-Palacios, 2007).

However, islands also harbour much of the world's threatened biodiversity (Kaiser-Bunbury et al., 2009). Impacts from natural or artificial disturbances, such as changes in environment and anthropogenic threats, are felt more intensely on island ecosystems, particularly oceanic islands (volcanic origin). In general, oceanic islands are fragile ecosystems mainly because of their small size and evolution in isolation from continental biotas (Whittaker and Fernández-Palacios, 2007).

Many of the islands' endemisms have been lost as a direct consequence of habitat destruction (loss and fragmentation), exploitation of natural resources and introduction of alien species (Reaser et al., 2007; Fernández-Palacios, 2010; Tritantis et al., 2010).

Biological invasions, the introduction and spread of organisms from one region to another by human activities, purposefully or accidentally, with detrimental effects on native biota, are one of the most important problems faced by island ecosystems (Hortal et al., 2009). Their susceptibility to invasion is due to the peculiarities of the native island fauna and flora, such as low species richness, existence of unsaturated communities and low competitive ability of many native species (Whittaker and Fernández-Palacios, 2007; Hulme et al., 2008; Hortal et al., 2009; Kaiser-Bunbury et al., 2009).

Invasive species affect ecosystems in many ways, replacing native species from topologically important roles (structure and composition), changing the abundance of species, altering and disrupting species interactions like those among plants and their pollinators (functioning), and even lead to extinctions of native species (Traveset and Richardson, 2006; Charles and Dukes, 2007; Reaser et al., 2007; Albrecht et al., 2014; Sugiura, 2016). Besides the introduction of alien plants and pollinators, agricultural practices such as natural habitat clearing, grazing, and the application of pesticides and herbicides are among the many threats to plant-pollinator interactions (Bjerknes et al., 2007). Since the original settlement of humans on the Azores a great number of arthropods and other poorly known taxa have already become extinct due to deforestation and most of the species, sensitive to disturbance, were never recorded (Triantis et al., 2010).

Species alone may continue to persist for a long time, continuing in an ecosystem's species inventory, whereas interactions and their strength within an ecosystem may respond faster to changes (Kaiser-Bunbury et al., 2009). Consequently, the susceptibility of pollinators to anthropogenic changes can be better assessed by monitoring changes in phenology, behavior, physiology, relative abundance or frequency of interactions with plant species (Kaiser-Bunbury et al., 2009; Burkle et al., 2013).

Pollination is an important ecosystem function since provides vital services such as plant sustainability and food production. It has a fundamental role in the regeneration of natural communities and is crucial for maintaining the structure, diversity and gene flow in ecosystems. The global decline in pollinators lowers the resistance of natural areas and agroenvironments to disturbances (Traveset and Richardson, 2006; Albrecht et al., 2014; Kaiser-Bunbury et al., 2017; Picanço et al., 2017a).

Pollination can be viewed at the level of an entire ecological community as a web, or network of mutualistic interactions between species. An impact on a given species can propagate through the network affecting other species (Carvalheiro et al., 2008). It is thus important to understand these ecological interactions, which are a major and often neglected component of biodiversity (Valiente-Banuet et al., 2015).

Studies of ecological networks mostly focus on interactions among species. Networks are constituted by nodes and their links, where nodes may stand for species, species populations, guilds, functional groups, etc., and links are defined in an interaction matrix. Through the links of networks, we can obtain information about populations, species, guilds,

functional groups, entire communities, or even entire networks. In a plant-pollinator network the links may represent the number of visitors to a plant, number of visits, number of pollen grains transferred to the stigma, seedlings or reproductive individuals (Hagen et al., 2012).

As stated by Lucas-Barbosa (2015), plants and insects are among the most abundant groups of organisms on Earth and ecological interaction networks between them are complex and the most species-rich of all mutualistic networks (globally involving about 88% of all angiosperm species and at least 1 million insect species belonging to several orders) (Fontaine et al., 2006; Hagen et al., 2012). However, insular ecosystems support less complex networks with lower numbers of pollinator species (mostly generalist species), usually lacking some important groups that are common in mainland (Picanço et al., 2017b).

Oceanic islands, with all their particularities, are ideal model systems and allow evaluating the impact of land use change on the diversity, distribution and abundance of pollinator species and their interaction networks (Picanço et al., 2017b). Studies on island biotas also provide information on the ecological roles of native versus introduced species (Olesen et al., 2002). Because of their distinct ecological characteristics, island pollinator systems are more prone to extinction and vulnerable to habitat disturbance and to invasive species than mainland ones (Valido and Olesen, 2010).

Azores is a remote archipelago, where significant changes were inflicted to native habitats and biodiversity (Borges et al., 2010). With the disturbance and replacement of original habitats in most of its surface, it is expected that plant and insect biodiversity and consequently their ecological interactions have been compromised (Rull et al., 2017).

Of the nine Azorean islands, Terceira is an ideal model system that enables to assess the impact of such perturbations on island ecosystems. Terceira has one of the largest areas of native forest (commonly known as laurel forest or "Laurissilva") in Azores and includes the largest and more pristine forest fragments. When it comes to terrestrial arthropods, Terceira is also the second most speciose island, with 1,235 species of which 132 are endemics, and with great representation of introduced species (Rego et al., 2015).

Mutualistic interactions are important for maintaining and generating biodiversity, as such this work aims to evaluate the effect of changes in native forest communities (presence of invasive species and human disturbance) on species richness of plants and pollinators and on their interactions. For this, we selected paired sampling sites (one well-preserved and one disturbed) in two study areas (Lomba and Pico Galhardo), in Terceira (Azores) and we

analysed plant-pollinator networks integrity. We also want to assemble scientific information to provide guidelines for future nature conservation interventions in this archipelago.

2. Material and Methods

2.1. Study area

The Azores are a volcanic archipelago situated in the north Atlantic Ocean (between 36°55′–39°43′ N and 25°00′–31°15′ W), being composed by nine principal islands and some islets. Originally, the Azorean landscapes were composed by different natural habitat types, including the emblematic native forest (usually named Laurissilva). Following human colonization, the natural habitats were strongly modified by human activities (Fernández-Palacios, 2010; Rego et al., 2015; Elias et al., 2016). The native forest that covered almost entirely the Azores islands upon their discovery in the XV century was largely destroyed and substituted by pastures and forest plantations (mainly *Cryptomeria japonica*). Nowadays, natural forest is reduced to very few and small patches (only 5% of the total area of the archipelago), usually located at higher altitude, where the orography and the harsh climate are unsuitable for human establishment (Fernández-Palacios, 2010; Triantis et al., 2010; Rego et al., 2015).

Fieldwork took place in Terceira, the third largest island (402 km²) and also one of the oldest of the archipelago (3.52 MY). Compared with the other Azorean islands, the native forest still covers a relatively large surface area (23 km²) in Terceira, including some of the largest and more pristine native forest fragments of the archipelago (Rego et al., 2015). These native forest fragments – Biscoito da Ferraria, Caldeira Guilherme Moniz, Pico Galhardo, Serra de Santa Bárbara, and Terra Brava – harbour a large number of Azorean endemic species, some of which are exclusive of the island. Due to their importance for the conservation of biodiversity, these native forest fragments were recently included in a network of protected areas – the Terceira Natural Park (Calado et al., 2009).

Fieldwork was carried out in two study areas (**Figure 1**), Lomba and Pico Galhardo, both located in the Terceira Natural Park. Lomba (L) is located in Serra de Santa Bárbara, an important area for biodiversity conservation in Azores (Dias et al., 2004; Borges et al., 2005), while Pico Galhardo (PG) is a small native forest fragment located in the center of the island.

In each of the two study areas, two sampling sites (one well-preserved and one disturbed) were set relatively close to each other (distanced by ~500m), to minimize geographic distance effects on community composition. So, there were four sampling sites, two well-preserved (L: UTM 26S 04752E 42874N and PG: UTM 26S 04803E 42871N) and two disturbed (Ld: UTM 26S 04752E 42866N and PGd: UTM 26S 04806E 42867N). The two sampling sites of each pair differed considerably in plant species composition and structure, since one presented almost exclusively native species (the well-preserved) while the other had several exotic species, which were often abundant (the disturbed).

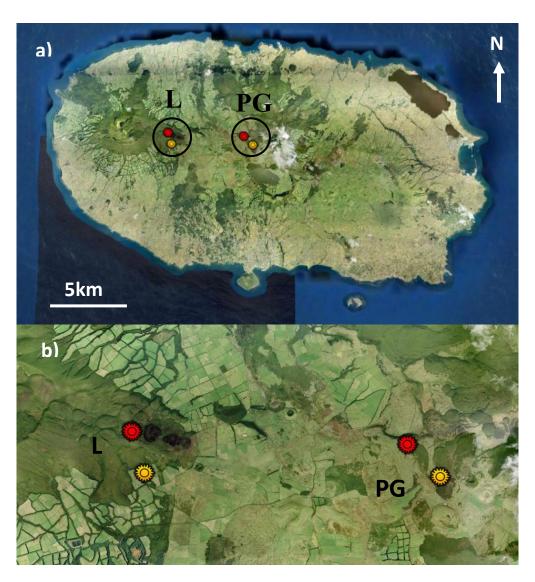


Figure 1 – a) Study areas in Terceira island: Lomba (L) and Pico Galhardo (PG) and b) the location of the sampling sites (red and yellow markings are conserved and disturbed areas, respectively).

2.2. Flowering plant composition and insect visitation networks

The flowering of most Azorean plant species occurs between June and August (Schäfer, 2005; Flora-on Açores, 2014), a period when most insect visitors are also active (Picanço et al., 2017b). Field data collection was carried out in 2016, from 4 to 27 July, aiming to encompass the flowering peak in the selected sampling sites. Nevertheless, the extended rainy season during this year was responsible for a delay in the flowering phenology, so our findings correspond to the early flowering period.

The sampling methodology followed the protocol proposed by Carvalheiro et al. (2008). Sampling points, each one consisting on 1 m radius semi-circle, were randomly chosen in each study site, to assess flowering species composition, flower abundance and insect visitation. A total of 100 samplings points were surveyed, 50 in each study area (25 per site). Insect surveys took place from 10h00 to 18h00 to cover the main period of insect activity.

In each sampling point, flowers were observed for 15 minutes and all the insects contacting with the plant reproductive structures were recorded. In this study, following Carvalheiro et al. (2008), we considered an inflorescence to be a flower unit from a flying insect perspective rather than by flower anatomy. During the 15-minute period all flowervisitor interactions were recorded and most of the insects were collected to identify or confirm species identification in the laboratory. Plant species richness and abundance, and insect visitors richness in each sampling point were assessed through the number of flower-visitor interactions, i.e. visits records were assumed to reflect numbers of species and individuals. The insect specimens were collected with sweeping nets and stored in vials with ethanol (70%). Samples were taken to the laboratory and all specimens were identified to family level. For some insect visitor groups that are usually considered important pollinators, like bees, bumblebees and wasps (Hymenoptera: Apidae, Vespidae), butterflies and moths (Lepidoptera), beetles (Coleoptera), and large-size flies (Diptera: Calliphoridae, Muscidae, Scathophagidae, Syrphidae), identification was performed to species level. Plant identification took place during fieldwork using specific literature (Schäfer, 2005), but for some specimens the identification was later confirmed in the laboratory. All plant and insect visitor species were classified according to three distributional categories (endemic, native non-endemic or introduced) following Borges et al. (2010).

2.3. Data Analysis

The differences in plant species richness, flower availability and insect visitor richness between study areas and sites were assessed by hierarchical ANOVA, with site as a nested factor in study area. Before performing the analyses the data were tested for normality and homocedasticity, and some variables (flower availability and insect visitor richness) needed to be transformed (using the logarithmic transformation), in order to meet the assumptions of ANOVA. The significant differences found with ANOVA were subsequently assessed by multiple comparisons of means using the Tukey HSD post hoc test with a 95% confidence level. The information on the absolute frequency of plant species richness and flower availability for the four study sites is presented graphically highlighting the contribution of endemic, non-endemic native and introduced plant species. Furthermore, the main groups of insect visitors in each study site are also presented.

The temporal variation in the average insect visitation to flowers during the day-period (from 10h00 to 18h00) was examined by plotting the average number of visits in each study area during four different time periods (10h-12h, 12h-14h, 14h-16h, 16h-18h). Data from the two sites in each study area were pooled and the observations made in different days were also included in each time-period category. Further, we tested the association between flower availability and insect visitor richness with the number of visits in each study site by Spearman rank correlation analysis. Then, data from the study populations were pooled for a general analysis. To further explore the relation between insect visitor richness and the number of visits in each site, we performed regression analyses aiming to identify differences between well-preserved and disturbed sites. The statistical analyses were performed using the SPSS software version 24.0 (IBM Corp. 2016).

Finally, the distribution of the insect visits to flowers of different plant species in the four study sites was analysed as separated bipartite networks, using the *bipartite* package for R (Dormann et al., 2008). The bipartite networks display the interactions between insect visitor species and plant species using the information on the observed number of visits (data for each site was pooled). We carried out preliminary bipartite analyses for all the insect visitors to flowers and then, considering that some visitors are not important as pollinators being frequently excluded from pollination network analyses (King et al., 2013), we performed the analyses on a subset just including the potential pollinator species.

Several network metrics (e.g. connectance, nestedness, specialization, interaction strength asymmetry) were computed for each study site using the *bipartite* package aiming to identify differences that may relate with disturbance level. These analyses were complemented with a comparative analysis of the most important insect visitors and on the association of their distributional status with study sites. The ecological information provided by five metrics of ecological networks used in this work is summarized below.

Both links per species and connectance assess the number of links of an ecological network. Links per species is given by the average number of links of interactions per plant and pollinator species (Dormann et al., 2017), while connectance is the number of interaction combinations that is actually observed in a community (Stouffer et al., 2014). More recently, some authors advocated the use of weighted connectance since it is a metric that also includes information on the non-interacting species (Dormann et al., 2017).

Insect flower interaction networks have structural properties, such as asymmetry and nestedness, which are known to provide information on network stability and resilience in the face of species extinctions and also able to assess the potential impact of invasive species on native insect-flower networks (Stout and Casey, 2014). Species assemblages are considered nested when the biotas of sites with lower numbers of species tend to be subsets of the biotas at richer sites (Wright and Reeves, 1992). Asymmetry can be obtained by interaction strength asymmetry (ISA) or dependence asymmetry, and is a measure of specialization across trophic levels that assesses the balance of a species affecting or being affected by other species. Positive values of ISA indicate higher species dependence in the higher-trophic level and negative values indicate more species dependence in the lower-trophic level (Dormann et al., 2017). Finally, we used a network level measure of specialization (H2) that ranges between 0 (no specialization) and 1 (complete specialization) and has the advantage of not being affected by network size or sampling intensity (Bluthgen et al., 2006).

3. Results

3.1. Plant species, flower availability and flower-visiting insects

During this study, we sampled a total of 22 flowering plant species in the four study sites (see **Annex 1**). We found small differences in average species richness of flowering plants between study areas (F=5.44, P=0.02), but not between sites in the same area (F=0.11, P>0.05). Introduced flowering plant species occurred in higher number and were more frequent in disturbed sites – D Sites, than in well-preserved sites – P Sites (**Figure 2**). In well-preserved sites the most frequent flowering plants were native species, like the endemics *Hypericum foliosum*, *Lysimachia azorica* and *Tolpis azorica*.

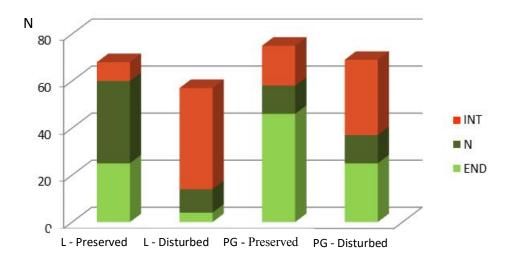


Figure 2 – Absolute frequency of flowering plant species in the study sites considering their distribution status (Lomba – L, Pico Galhardo – PG; introduced – INT, native non-endemic – N, endemic – END).

In regard to the average number of flowers, considerable differences were found between areas (F=22.54, P<0.001) with Pico Galhardo showing higher numbers than Lomba (**Figure 3**). Furthermore, in both study areas disturbed sites presented less number of flowers than the neighbouring well-preserved sites (F=19.72, P<0.001). A clear trend was observed for the origin of flower availability in our study sites since in well-preserved sites over 90% of the flowers belonged to native species (mostly Azorean endemics) while in disturbed sites the majority of flowers were from introduced species (**Figure 3**).

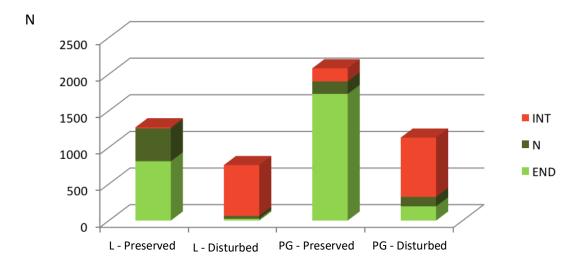


Figure 3 – Overall flower abundance in the study sites considering the distribution status of plant species (Lomba – L, Pico Galhardo – PG; introduced – INT, native non-endemic – N, endemic – END).

Lomba had higher diversity of flower-visiting insect species/groups (S=30 for the well-preserved site and S=37 for the disturbed) than Pico Galhardo (S=25 and 22 for the well-preserved and disturbed sites, respectively), but many visitors were common to all study sites (**Figure 4**). For example, flower visitors like the beetle *Anaspis proteus*, the bumblebee *Bombus terrestris*, and the hoverflies *Episyrphus balteatus*, *Eristalis tenax*, *Sphaerophoria nigra* and *Xanthandrus azorensis* were found in all study sites (see **Annex 1**). Also, we found that the average diversity of flower visitors per sampling period was much higher at Lomba than in Pico Galhardo (F=96.49, P<0.001), but within each study area no significant differences were found between well-preserved and disturbed sites (F=1.98, P>0.05, **Figure 4**).

The main group of flower visitors in all study sites was Diptera, particularly syrphids, sepsids and calliphorids, accounting over 65% of the visits to flowers (**Figure 5**). Beetles (Coleoptera) and bees (Hymenoptera) were locally important since the former were mostly associated to well-preserved areas while the latter were responsible for a considerable number of visits in the two disturbed sites (**Figure 5**). It is important to highlight the finding of two new species to Terceira island: *Sepsis fulgens* Meigen, 1826, which is reported to the Azores archipelago for the first time and *Sepsis biflexuosa* Strobl, 1893, previously known from Faial and São Miguel (Borges et al., 2010), but not recorded in Terceira.

The information on the overall and average flowering plant and visitor species/group richness is summarized in **Table 1** to ease the interpretation of the differences between study areas and sites.

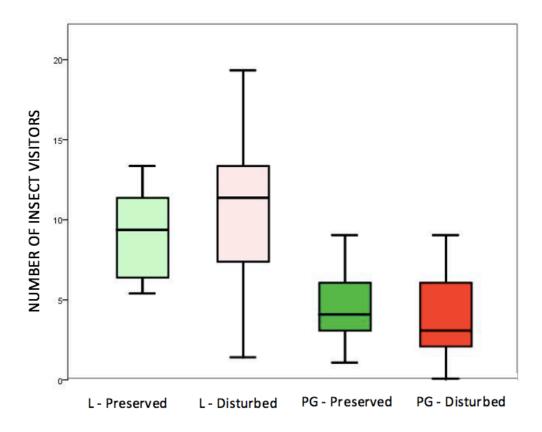


Figure 4 – Box plot indicating the median, maximum, minimum, and upper and lower quartiles of insect visitors to flowers in the four study sites (L-Lomba, PG-Pico Galhardo).

Table 1 – General information on the overall and average flowering plant species and visitor insect species/group richness in the study areas. The average flower availability in each site is also presented.

	LOMBA		GALHARDO	
	Preserved	Disturbed	Preserved	Disturbed
Flowering plant species richness	7	12	9	14
Average flowering plant species richness	2.8	2.8	3.5	3.4
Average flower availability	51.2	33.8	90.8	56.8
Flower-visiting insects	30	37	25	22
Average flower-visiting insects	9.9	11.0	4.4	3.5

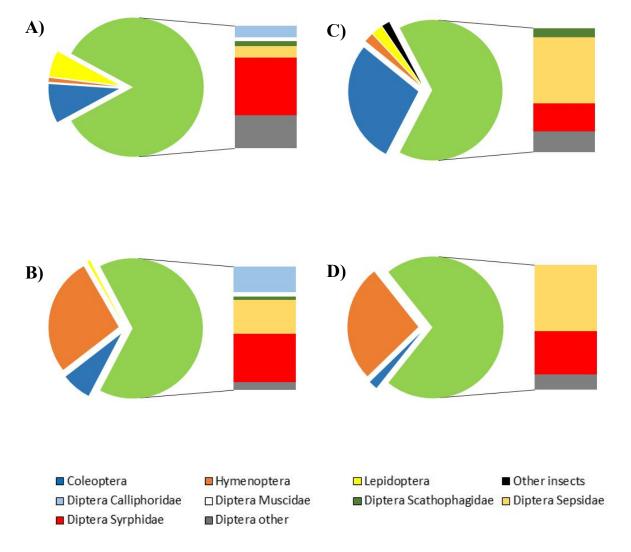


Figure 5 – The main groups of flower visitors in the four study sites: A) Lomba well-preserved, B) Lomba disturbed, C) Pico Galhardo well-preserved; D) Pico Galhardo disturbed.

3.2. Visitation rates between areas and throughout the day

During the sampling period (10h00-18h00), visits to flowers were regular throughout the day in both study areas (**Figure 6**). However, major differences were found in the average number of visits throughout the daily sampling period between Lomba and Pico Galhardo (**Figure 6**).

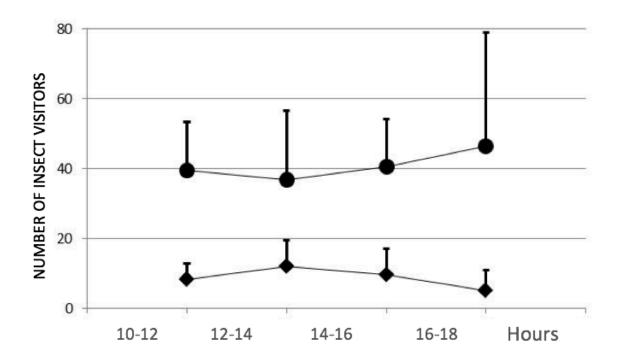


Figure 6 – Average visitation rates in Lomba (circles) and Pico Galhardo (diamonds) in different periods of the day (10h-12h, 12h-14h, 14h-16h, 16h-18h), with vertical bars representing standard deviation.

In this study, we found a high positive correlation between the diversity of flower-visiting insects and the number of visits (r=0.83, p<0.01; see also **Annex 2** for correlations at site level), but no consistent association was found between flower availability and the number of flower visits (**Annex 2**). Furthermore, we found that monotonic increases in insect visitor richness lead to exponential increases in the number of visits to flowers in all study sites (**Figure 7**).

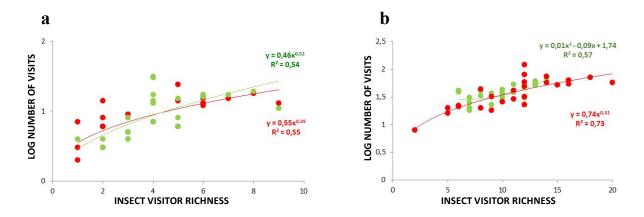


Figure 7 – Influence of insect visitor richness on the (log-transformed) number of visits in Lomba (a) and Pico Galhardo (b). Data from well-preserved (in green) and disturbed areas (in red) is shown jointly with the information (equation and R²) of the best regression model.

3.3. Insect visitation networks and plant-insect associations

The visitation networks from well-preserved and disturbed sites at Lomba and Pico Galhardo are shown in **Figures 8** and **9**, respectively. These networks include only the interactions between insect visitors that are usually considered effective pollinators (like bees, bumblebees, beetles, butterflies, moths and large size flies). Considerable differences were found in some network metrics between study areas and between sites in each study area (**Table 2**).

Both disturbed sites showed higher plant and insect visitor species richness than the neighbouring well-preserved sites in each study area. Consequently, the average number of links in the well-preserved sites was lower than in the disturbed ones. Furthermore, for other network metrics (nestedness, interaction strength asymmetry and H2) considerable differences were also found between well-preserved and disturbed sites in both study areas.

Lomba had higher links per species than Pico Galhardo. Although, links between study sites were similar (L and P; Ld and PGd), within same area, disturbed sites had higher values.

Regarding connectance, all areas and study sites appear having even values, with only well-preserved Lomba slightly standing out. Also in the case of H2, areas and respectively study sites are similar to each other, well-preserved Pico Galhardo showed higher values for this metrics.

Also, concerning nestedness and interaction strength asymmetry (ISA), in general Pico Galhardo showed higher values than Lomba, but more accentuated differences were found between sites within each area. Overall, well-preserved Pico Galhardo has the maximum values for both nestedness and ISA (see **Table 2**).

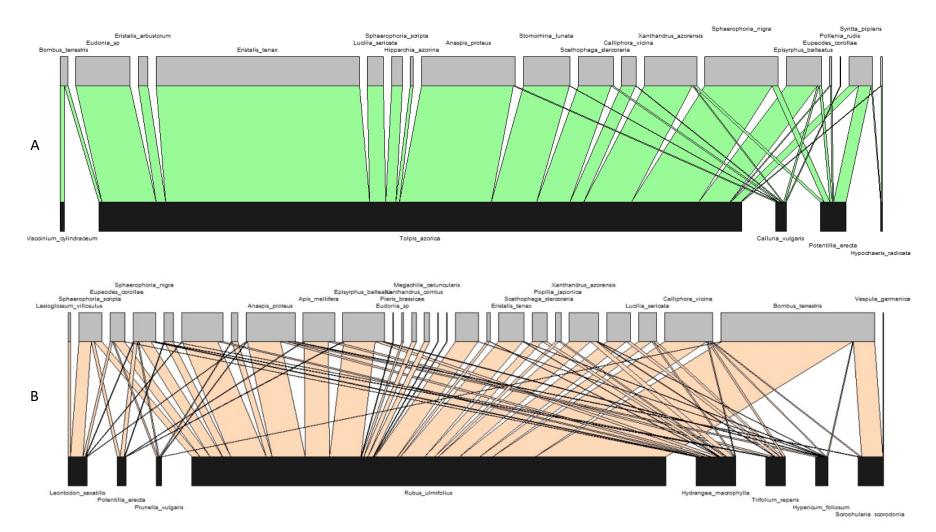


Figure 8 – Visitation networks at Lomba. The network from the well-preserved site is shown on top (A - with interactions in green) while the network from the disturbed site is presented at bottom (B - with interactions in rose). Insect visitor names are presented on the top of each graphic and plant names at the bottom. Insect visitation to each plant species is proportional to the area of the interaction that connects them.

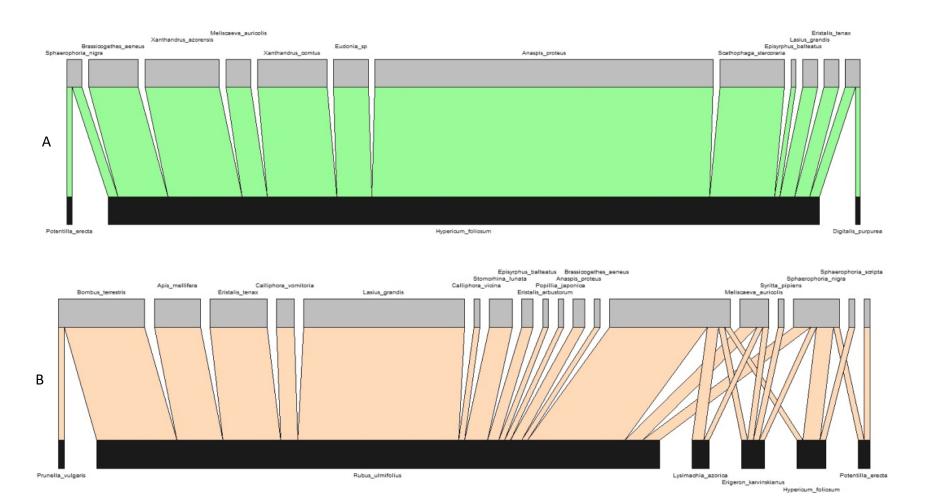


Figure 9 – Visitation networks at Pico Galhardo. The network from the well-preserved site is shown on top (A - with interactions in green) while the network from the disturbed site is presented at bottom (B - with interactions in rose). Insect visitor names are presented on the top of each graphic and plant names at the bottom. Insect visitation to each plant species is proportional to the area of the interaction that connects them.

Table 2 – Results of selected visitation network metrics for the four study sites in the two study areas.

	LOMBA		PICO GALHARDO	
	Preserved	Disturbed	Preserved	Disturbed
NERWORK METRICS				
Links per species	1.514	2.128	0.968	1.333
Connectance	0.353	0.270	0.200	0.227
Nestedness	18.130	13.584	22.098	14.312
Interaction strength asymmetry	0.404	0.229	0.720	0.207
H2	0.389	0.320	0.507	0.315

In each study site, most of the visits were directed to a plant species. In the well-preserved sites, the most visited plant species were the endemics *Tolpis azorica* (at Lomba) and *Hypericum foliosum* (at Pico Galhardo), while in both disturbed sites the introduced *Rubus ulmifolius* was the plant species that received the higher number of visits (**Figure 10**). The other plants, native and introduced, received much less visits. Interestingly, most of the visits were carried out by native insect species, particularly in the well-preserved sites. Only the disturbed site at Lomba showed a similar number of visits of introduced and native insect species.

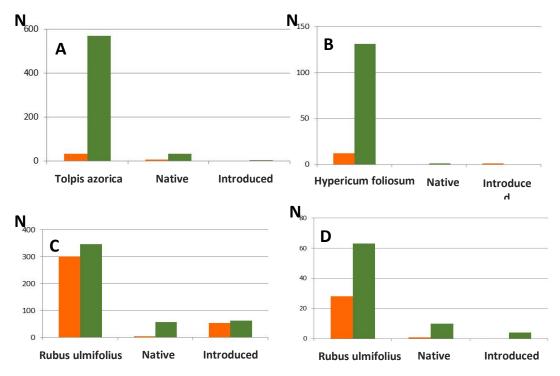
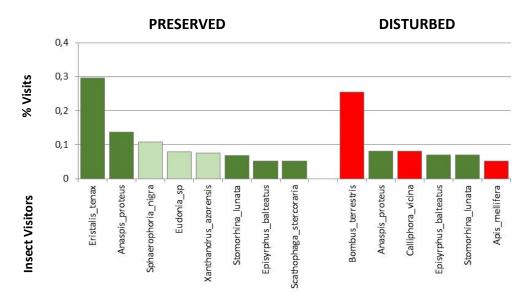


Figure 10 – Number of visits (N) by native (in green) and introduced (in orange) insect species to native (A and B) and introduced plants (C and D) in each study site. The most visited plant species in each study site is highlighted showing the differences in visitation by the two insect groups. A) Well-preserved site at Lomba; B) Well-preserved site at Pico Galhardo; C) Disturbed site at Lomba; D) Disturbed site at Pico Galhardo.

In the well-preserved sites, the large majority of visits were carried out by native insects, both endemic and non-endemic species (**Figure 11**), particularly the beetle *Anaspis proteus* and several hoverflies like *Eristalis tenax*, *Sphaerophoria nigra* and *Xanthandrus azorensis*. These species were also important visitors in disturbed sites, but introduced species like the bumblebee *Bombus terrestris*, the honeybee *Apis mellifera* and the blowfly *Calliphora vicina* also played an important role as flower visitors (**Figure 11**).

A) Lomba



B) Pico Galhardo

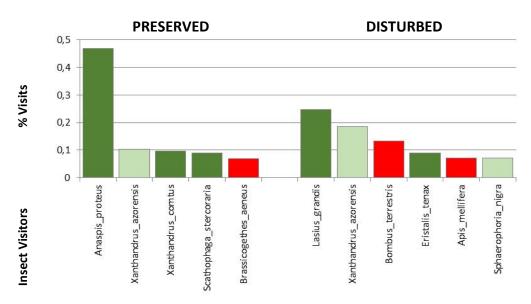


Figure 11 – Percentage of visits by the most important insect visitors (with >5% of visits) in the study sites at Lomba (A) and Pico Galhardo (B). The distribution status of each insect species (endemic in light green, native in dark green, introduced in red) is shown jointly with insect visitor species names.

4. Discussion

4.1. Plant species, flower availability and flower-visiting insects

Although Terceira island has some of the largest remnants of native forest, the present landscape is transformed or degraded by human presence, which is reflected in its biodiversity (Dias et al., 2005; Elias et al., 2016). Both endemic and non-endemic native species have colonized the Azores by natural long-distance dispersal mechanisms, but endemic species are exclusive to the archipelago and native non-endemic can also be found elsewhere. Introduced species colonized the Azores with the aid of man, some introductions were deliberated (e.g. some beneficial insects to agriculture), while others were accidental (e.g. urban, forest and agriculture pests) (Borges et al., 2010). Our records show, in total of sampled areas, a large number of introduced species, with more than half the plant species being introduced, and less than half the insect species being introduced (see **Annex 1**).

Alien plants and animals bring implications to pollination and reproductive success of native plants. Multiple factors and mechanisms are involved in the interactions through which native plants and pollinators can find ways to adapt and survive alongside the invaders. Depending on features of both native and alien species, plants tend to have characteristics to attract important pollinators, among which are changes in flower morphology and attractiveness, changes in blooming time and location (population size and density) and shifts to reproductive modes that are independent of animal visitation (Bjerknes et al., 2007; Pisanty and Mandelik, 2011).

Small differences were found in the average species richness of flowering plants between study areas (Lomba and Pico Galhardo), but no significant differences were found between sites in the same area. Even though, the species composition varied considerably between sites within the same study area. Larger differences in species composition between study sites (well-preserved and disturbed sites) were not surprising since, despite they were relatively close to each other (distanced by ~500 m), the disturbed sites were considerably changed by human activities that led to the introduction of exotics and loss of native species. So, due to anthropogenic changes, only some species are shared between the two sites in each study area: L and Ld only had 3 common species in a total of 16, and P and PGd only had 8 in 14.

Native plant species have different kinds of responses to disturbances, but in oceanic islands many were found to decline or even became locally extinct following habitat fragmentation and the introduction of invasive species (Sodhi and Ehrlich, 2010; Valiente-Banuet et al., 2015). In our study, the high number of alien plants in disturbed areas was due to the combined effects of habitat destruction and the establishment of introduced species that prevented recolonization by the native species.

Regarding the average number of flowers, Pico Galhardo showed higher values than Lomba, and disturbed sites presented fewer flowers than the adjacent well-preserved sites. This fact is probably related to the kind of species that are present in each area and site and their morphology. Lomba had more plant species (16) than Pico Galhardo (14) and disturbed sites (L: 12; PG: 13) had more species than well-preserved ones (L: 7; PG: 9). These results show that the occurrence of more species does not necessary mean higher abundance of flowers. It is the set of flower species in each area or site that translates in the number of existing flowers. Once there are species that produce more flowers than others, less species can form a bigger set of flowers. For example, plant species with inflorescences (flowers borne together in clusters) usually have more flowers per individual than species that produce solitary flowers (borne singly and separate from one another).

The trend observed for the origin of flower availability comes from the fact that P Sites were mostly represented by endemics like *Tolpis azorica*, *Vaccinium cylindraceum*, *Hypericum foliosum* and *Lysimachia azorica*; while D Sites were formed primarily by introduced species like *Rubus ulmifolius* and *Persicaria capitata*. These latter species have been associated to landscape disturbance in a recent study (Picanço et al., 2017a).

Our results about flower-visitor insect species/groups show higher diversity and number of visitors per sampling period in Lomba than in Pico Galhardo, but there were no significant differences in flower-visiting insect species between sites. Pico Galhardo has a higher level of disturbance than Lomba, because it is a small native forest fragment, isolated in the center of the island, surrounded by heavily changed habitats, due to human activities (mostly pastures and planted forests) (Dias et al., 2005; Sodhi and Ehrlich, 2010). Consequently, Pico Galhardo has more introduced plant species and less endemic or native non-endemic than Lomba. Despite Lomba also having representatives of alien species, endemics and native non-endemics seem to balance things, making Lomba a less affected area. Lomba is located in Serra de Santa Bárbara, which according to Dias et al. (2005) is one

of the most pristine native forest areas in Azores, i.e. with natural vegetation still in its original state. Serra de Santa Bárbara is extremely rich in rare habitats and species, representing one of the most important and well-preserved biological hotspots of Azores. This region gathers conditions like continuum of vegetation formations that vary in structure and in floristic composition, reflecting a great variety of forest microhabitats, each one showing different combinations of environmental factors (Dias et al., 2005). In turn, Pico Galhardo has higher habitat loss, subdivision of the remaining patches of vegetation and habitat modification, which might result from its location and forms of land-use (Dias et al., 2005; Sodhi and Ehrlich, 2010). Eventually, differences in land use may result in increasingly smaller fragments, leading to a disproportional amount of edge effects, with small or entirely absent unaffected core areas, hosting different levels of biodiversity (Kaiser-Bunbury et al., 2009). Consequently, by increasing the susceptibility to invasion by alien species, the composition and functioning of invaded ecosystems is affected and the ecological interactions altered (Traveset and Richardson, 2006; Whittaker and Fernández-Palacios, 2007). And so, a currently well-preserved site may soon be severely affected by these human-driven disturbances.

Most insect visitors that appeared in the study sites were native non-endemic and endemic species, except for the introduced *Bombus terrestris*. The main group of flower visitors in all study sites was Diptera (flies like syrphids, sepsids and calliphorids). Other two groups, beetles and bees, were locally more important: bees were responsible for a considerable number of visits in the two disturbed sites and beetles were mostly associated to well-preserved areas. Bee species were mainly native non-endemic and endemic, while a single beetle species was found, the Macaronesian endemic *Anaspis proteus*.

4.2. Visitation rates between areas and throughout the day

Plant populations are composed of individuals showing different flowering schedules, which vary in flowering intensity, timing, and duration. Pollinators also exhibit specific phenological patterns, and insect visits also depend on abiotic parameters, such as temperature, wind velocity and solar radiation (Herrera, 1990; Valverde et al., 2015).

These schedule differences in pollinator and flower availability cause a range of possible links in the set of pollinator species that interact with individual plants (Valverde et al., 2015). Terceira island climate is temperate oceanic, characterized by both high levels of relative atmospheric humidity and low temperature fluctuations throughout the year, further it benefits of a mild and agreeable climate, with small daily fluctuations in temperature and air humidity (Borges et al., 2010; Picanço et al., 2017a). Continents lack homogeneity in those factors during the day.

Our results show that both areas (L and PG) have even average visitation rates during the day, probably resulting from the homogeneity of climate factors of the island. The activity of most flower visitors is strictly associated with variations in climatic conditions (particularly temperature) and, contrary to the findings in mainland, the small fluctuations in temperature during the day allow insects to be active for a longer uninterrupted period.

Nonetheless, Lomba and Pico Galhardo follow different visitation patterns: Lomba had more insect visits in the hour intervals that correspond to solar peak and sunset (10-12 and 16-18 h), while in Pico Galhardo was completely the opposite (approximately between 12-16h).

The most important environmental factors in Lomba, are the wind exposure, high precipitation, humidity and continuous ground wetness, the geology and geomorphology are also important factors, creating high number of microhabitats (Dias et al., 2005). It is also important to mention the fact that Lomba has higher diversity of flower-visiting insects, and that is reflected in higher number of visits in this area than in Pico Galhardo, with lower diversity of visiting insects.

So, insect species diversity and weather conditions, influenced in some way the number of visits to flowers, either in areas or sites. But still, we can't directly associate increasing number of visits with higher flower availability.

4.3. Insect visitation networks and plant-insect associations

The introduction of alien organisms is commonly expected to have significant negative consequences for native plant species. However, depending on the level of invasion

and degree of degradation, the presence of alien species in insular habitats may have negative, neutral or positive consequences on island species and interactions (Whittaker and Fernández-Palacios, 2007; Kaiser-Bunbury et al., 2009).

Island pollination systems (IPS) have distinctive ecological characteristics compared to mainland systems because of the differential pool of species inhabiting it. Also, insular plant populations are usually visited by fewer pollinator species than their mainland populations (Olesen et al., 2002; Kaiser-Bunbury et al., 2009).

IPS are characterized by the prevalence of generalist species which are plant or animal species that interact with many other plant or animal species. Nevertheless, there are also some species-specific pollination relationships in insular systems, but are much rare and more vulnerable to extinction (Olesen et al., 2002; Kaiser-Bunbury et al., 2009; Valido and Olesen, 2010; Stouffer et al., 2014). Probably, most of the specialists have already disappeared, before ever being discovered. In fact, the ones found nowadays are those that survived the human colonization and the severe natural habitat destruction of Azores archipelago since the XV century (Rego et al., 2015; Elias et al., 2016).

About generalists, some authors stated that in oceanic islands there is a group coined as super-generalist species that establish links with a very high number of species when compared to the average in the community. Due to their role in ecological interactions they may also act as keystone species (Olesen et al., 2002; Kaiser-Bunbury et al., 2009). The most studied alien pollinators are the honey bee (*A. mellifera*) and the bumblebee (*B. terrestris*), both are frequently considered super-generalist species and are usually well integrated into the pollinator networks of many invaded island communities. Among the known islands supergeneralists there are also some beetle and fly species that have a cosmopolitan distribution (Kaiser-Bunbury et al., 2009). However, in a recent publication, Weissmann et al. (2017) mentioned that bees (Hymenoptera, Apidae) are represented with a low number of lineages in the Azores and suggested that the endemic flora of Azores might have evolved mainly without the presence of bees as pollinators and adapted to other pollinator groups. Our findings confirm that the aforementioned groups, alongside with butterflies, moths (Lepidoptera) and large size flies (Diptera: Calliphoridae, Muscidae, Scathophagidae and Syrphidae) are the main pollinators in Terceira island, Azores.

The finding that disturbed sites had higher flowering plant species richness than neighbouring well-preserved sites was not surprising, and matched the increasing biotic homogenization that is being witnessed in many oceanic islands where a set of introduced species have established and may co-occur with native species. Larger plant assemblages may attract more and different pollinators and potentially create more links between species. In disturbed sites, many plant species were introduced, and can compete for mutualistic services with native plants. Furthermore, their integration into resident communities can be facilitated by generalist pollinators. Some alien plants offer more floral resources (pollen and nectar) than native plant species, attracting greater numbers of pollinators (Traveset and Richardson, 2006; Kaiser-Bunbury et al., 2009).

Consequently, there are positive effects for both interactors, invasive plants and native pollinators, but the native plants may face negative consequences if they have to compete for pollination services with the invasive ones. For example, if an exotic pollinator is more or less effective than a native pollinator in pollinating a particular plant, then native (but also exotic) plants may either increase or reduce seed set and thus, hamper or facilitate native plants pollination (Whittaker and Fernández-Palacios, 2007; Pisanty and Mandelik, 2011). Whittaker and Fernández-Palacios (2007) gave the example of a Canarian endemic plant, *Echium wildpretii*, affected by the competition between the endemic *Bombus canariensis* (Canarian bumblebee) and the introduced *Apis mellifera*.

According to Olesen et al. (2002), interactions have many types of categories, endemic interactions (i.e. between endemic plants and endemic pollinators), non-endemic native interactions (i.e. between native plants and native pollinators), introduced interactions (i.e. between introduced plants and introduced pollinators) and mixed interactions (i.e. between introduced plants and endemic pollinators, etc.). Our study areas mainly showed mixed interactions.

Our interaction networks analysis showed some differences on the plants that were the target of most insect visits: the endemics *Tolpis azorica* and *Hypericum foliosum* in well-preserved sites (L and PG, respectively) and the introduced *Rubus ulmifolius* in both disturbed sites (Ld and PGd). Respectively to each site, those were the plant species that concentrated more insect visits.

However, some plants, either native and introduced, received much less or no visits. Some studies suggest possible plant species adaptations that might explain the incidence of insect visitors.

Dias et al. (2005) stated that some flowers of endemic plants suffer peculiar adaptations to insular land. For example, loss of strong colours, size decrease or the attraction of more generalist insects. Whereas Bjerknes et al. (2007) say that alien plants with a generalized pollination strategy (plants attracting many visitor species) invade existing plant–pollinator networks more easily than pollination specialists (plants attracting one or a few visitor species). In this study that is the case of *Rubus ulmifolius*.

In what concerns to visits, our results show a pattern: most of networks visits were carried out by native insect species, particularly in the well-preserved sites. Only the disturbed site at Lomba showed a similar number of visits by introduced and native insect species.

These patterns possibly mean that, on one hand, the present pool of insect species was not significantly affected by changes in plant biodiversity (biotic resistance) due to their generalist behaviour. Thus, native insect species may help alien plants to establish, by building new interactions with the new community members (biotic facilitation). On the other hand, we found that introduced pollinator species do not seem more efficient in visiting plants than the native pollinators, but further studies are needed to assess their impact in Azorean natural communities. (Olesen et al., 2002; Whittaker and Fernández-Palacios, 2007).

Generalist species (species with large diet breadth) are more likely to adapt to new environments, thereby reducing the impact of native species loss and increasing the stability of food webs. Alien species are frequently linked to many native species through shared pollinators or plants. Therefore, and as reported in the work of Carvalheiro et al. (2008), their removal must be carefully planned since may have unexpected and significant negative effects on native species.

Lomba was the area that had the largest number of links between plant and pollinator species, and both disturbed sites had higher linkage levels than the neighbouring well-preserved ones. Flower abundance and species richness of the interactor groups can account for much of the variation in linkage levels in pollination networks (Hagen et al., 2012) and, as found in our study, higher average of links per species were found in disturbed sites networks, where plant and insect visitor species richness were higher.

However, connectance values showed few oscillations between areas and sites, meaning that despite the differences in linkage level, in general, the communities of interactors assembled similarly. According to Hagen et al. (2012), often, a few common species engage in many interactions, and most rare species engage in few interactions but generalists and common species may be "forced" over evolutionary time towards being more specialized and rare.

Network structure is normally constrained by many factors as phenological uncoupling, abundance, body size and population structure. Some potential links between species are never observed, and their absence can be just as ecologically informative as their presence. Reasons why null observations might commonly happen are "temporal mismatching", weak coincidence or weak coupling and many times a consequence of sampling effect (Olesen et al., 2012).

Nestedness along with interaction strength asymmetry (ISA) provide information on network stability and resilience to impact of invasive species on native insect-plant networks and possible species extinctions (Padrón et al., 2009).

A network is nested when the species links are a subset of links of more connected species. So, an increase in nestedness of mutualistic networks may increase their robustness. Nonetheless, that doesn't prevent alien species from having a negative effect on individual native species. For instance, by decreasing the number of links and/or the number of visits from pollinators, their presence eventually translates into a lower reproductive plant success, which will also compromise stability and robustness of the entire network (Padrón et al., 2009).

Usually, large networks are more likely to be nested than small ones. However, by looking at well defined subsets of generalist species existent in our networks, and at the "Nestedness" metric values, we can say that our networks are relatively nested, with well-preserved sites standing out when compared to disturbed ones. Hence, due to the high degree of nestedness and redundancy in interactions, the overall structure of plant-pollinator networks may be robust and resilient to perturbations (Burkle et al., 2013). Even so, it is important to refer that when interactions are redundant, at first their loss will not be reflected in the ecological function of the network, for as much as other interactions play that role. But if more interactions are lost, the ecological function will eventually collapse. This lag between

environmental change and functional decay of interactions represents an extinction debt for ecological functions (Valiente-Banuet et al., 2015).

Asymmetric interactions describe links between specialist and generalist species. In nature, mutualistic relationships tend to be asymmetric, i.e. there is an imbalance, existing more species-specific and less generalist species or vice versa (Kaiser-Bunbury et al., 2009; Stouffer et al., 2014).

Data relating to network level measure of specialization (H2) and ISA, both showed low specialization in the studied networks, confirming the prevalence of species with a generalist behavior in the pollination networks from disturbed and well-preserved sites of Terceira native forest. This suggests that, although individual alien invasive species may have a negative impact on one or several native mutualistic interactions, in this case, their effect on the overall community may be neutral or even positive with regards to the outcome of mutualistic interactions (Kaiser-Bunbury et al., 2009).

Therefore, with the information available up until now, we have no way to assess if the numbers of links and visits have changed over time. So, with only the information provided by this work, we can not conclude if nestedness of networks were or not affected. Except from the study of Olesen et al. (2002), little research has been done about this topic in Azores, and even less in Terceira island (Picanço et al., 2017b). Thus, by providing baseline data on flowering plants, insect visitors and their interactions in both well-preserved and disturbed sites from an oceanic island, the present work is an important contribution to future studies on plant-pollinator interactions in Azores and provides information to guide conservation actions in this archipelago.

5. Conclusions

We conclude that even though the disturbed sites have introduced species, they still hold a considerable number of native flowering plants and insect visitors. We found that a single plant species received most of the insect visits in each area (well-preserved and disturbed) and regarding insect visitors we conclude that flies (families Calliphoridae, Muscidae, Scathophagidae and Syrphidae) are the main pollinators in Terceira island.

The ecological network analysis suggests that the extant plant-pollinator interactions seem to be stable and dominated by generalists.

6. Final Considerations

Pollination is an important ecosystem function and plays a key role in world sustainability (Fontaine et al., 2006; Carvalheiro et al., 2008; Kaiser-Bunbury et al., 2017). Plant-pollinator interactions are underlying global crop production, the maintenance of biodiversity, the conservation of rare plant and pollinator species, contribute to ecosystem resistance and resilience, and lowers the vulnerability of co-extinctions (Kremen et al., 2007). Pollination is seen at the level of an ecological community as a web or network of mutual beneficial interactions between species of two trophic levels, and thus impacts on certain species may propagate through the network, affecting directly or indirectly other species and even an entire ecosystem (Carvalheiro et al., 2008). The effects of interaction loss on ecological functions will depend on the proportional contribution and role of each interaction type to the global function provided by the whole interaction assemblage (Valiente-Banuet et al., 2015).

Therefore, it is important to highlight and protect these mutualistic interactions and preserve pollination services to ecosystems, currently threatened by human activities and invasive species.

A network approach in conservation research and management practices is a very useful tool. It shows how species are linked between them and could answer the question about if and how invasive species affect native species.

By studying pollinator and plant diversity and their links, our work identified some consequences of human disturbance on natural communities stressing the need for monitoring studies and if needed the establishment of conservation management plans.

However, there are still many questions to be answered regarding this topic, and generalizations to other island systems cannot be made without caution, since each one has its differences and peculiarities.

To further understand these issues and come to potential solutions, more studies and conservation efforts should be done. We emphasize the need to keep doing regular biodiversity surveys and monitor these interactions and to see if their relationships and role in the web change through time. It is also important, for conservation of island biodiversity, to prevent further species introductions and try to monitor and control the effects of those

already introduced. Eventually, taking action in case of sharp increase of direct threat from invasive to native species might be needed.

Some studies show that it would be impracticable to completely restore the ecosystem to its original state. However, it is important to conserve biodiversity and the integrity of interactions between native species since they play a pivotal role on islands.

The conservation of the Azorean natural heritage must involve the establishment of a large-scale strategy to manage invasive, native and endemic species, while protecting the remnants of native habitats (Azorean forest) and, ideally, increasing their extent.

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ANNEX 1: List of flowering plant species (A) and insect visitors (B) recorded in each study site (L: Lomba preserved; Ld: Lomba disturbed; PG: Pico Galhardo preserved; PGd: Pico Galhardo disturbed).

A - Flowering plant species (End: endemic; Native-non-end: Native non-endemic; Introd-Natur: Introduced Naturalized).

Diama Consultan	Distribution	Lomba		Pico Galhardo		
Plant Species		L	Ld	PG	PGd	
Calluna vulgaris	Native-non-end	Х				
Crepis capillaris	Introd-Natur			Х	Х	
Digitalis purpurea	Introd-Natur			Х	Х	
Erigeron karvinskianus	Introd-Natur				Х	
Hydrangea macrophylla	Introd-Natur		Х			
Hypericum foliosum	End		Х	Х	Х	
Hypericum humifusum	Native-non-end				Х	
Hypochaeris radicata	Introd-Natur	Х	Х			
Leontodon saxatilis	Doubtful		Х	Х		
Lobelia urens	Introd-Natur			Х	Х	
Lotus pedunculatus	Introd-Natur	Х		Х	Х	
Lysimachia azorica	End		Х	Х	Х	
Lythrum junceum	Introd-Natur				Х	
Persicaria capitata	Introd-Natur		Х		Х	
Potentilla erecta	Native-non-end	Х	Х	Х	Х	
Prunella vulgaris	Doubtful	Х	Х	Х	Х	
Ranunculus sp.	-		Х			
Rubus ulmifolius	Introd-Natur		Х		Х	
Scrophularia scorodonia	Introd-Natur		Х			
Tolpis azorica	End	Х				
Trifolium repens	Introd-Natur		Х			
Vaccinium cylindraceum	End	Х				

B - Insect visitors (End: endemic; Mac-end: Macaronesian endemic; Native-non-end: Native non-endemic; Introd: Introduced).

Order	Family	· .	D: ('1 - ('	Lomba		Pico Galhardo	
		Species	Distribution	L	Ld	PG	PGd
Coleoptera	Carabidae	Gen sp1	-	X			
		Gen sp2	-	X			
	Nitidulidae	Brassicogethes aeneus	Introd			X	X
	Scarabaeidae	Popilia japonica	Introd		X		X
	Scraptiidae	Anaspis proteus	Mac-end	X	X	Х	X
	Agromyzidae	Unidentified	-			X	
	Anthomyiidae	Unidentified	-	X	X	X	
	Chloropidae	Unidentified	-			Х	
		Calliphora vicina	Introd	X	X		X
		Calliphora vomitoria	Introd		х		X
	Calliphoridae	Lucilia sericata	Introd	X	х		
		Pollenia rudis	Introd	X	х		
		Stomorhina lunata	Native-non-end	X	х		Х
	Dolichopodidae	Unidentified	-	X	Х	Х	Х
	Empididae	Unidentified	-	X			
	Ephydridae	Unidentified	-			Х	
	1 7	Eudasyphora cyanella	Introd	X	х	Х	
		Hydrotaea dentipes	Introd		х	х	
	Muscidae	Musca osiris	Introd	X	х		
	Wasciaac	Stomoxys calcitrans	Introd	X			
		Gen sp1	-	X	х	Х	х
era	Phoridae	Unidentified	_			X	X
Diptera	Sepsidae	Sepsis biflexuosa	Native-non-end	X	х	X	
Q		Sepsis fulgens	Native?	X	x	X	х
		Sepsis thoracica	Native-non-end	X	x	X	X
	Scathophagidae	Scathophaga stercoraria	Native-non-end	X	x	X	A
	Sciaridae	Unidentified	-	A	X	A	
	Syrphidae	Episyrphus balteatus	Native-non-end	X	x	Х	х
		Eristalis arbustorum	Native-non-end	X	X	A	X
		Eristalis tenax	Native-non-end	X	x	х	X
		Eupeodes corollae	Native-non-end	X	x	A	A
		Meliscaeva auricolis	Native-non-end	А	x	X	х
		Myathropa florea	Native-non-end		x	А	A .
		Sphaerophoria nigra	End	X	X	х	x
		Sphaerophoria scripta	Native-non-end	X	X	Λ	X
		Syritta pipiens	Native-non-end	X	x		X
		Xanthandrus azorensis	End	X	X	х	X
		Xanthandrus comtus	Native-non-end	A	X	X	A
	Tephritidae	Unidentified	- Tative-non-end	X	X	X	
	Теритичае	Apis melifera	Introd	Λ	X	Λ	v
Hymenoptera	Apidae	Bombus terrestris	Introd	X	X	X	X X
		Megachile cetuncularis	Native-non-end	Λ	X	^	^
		Lasioglossum villosulum	Native-non-end	X	X		X
	Formicidae	Lasius grandis	Native-non-end	Λ	X	X	X
	Vespidae	Vespula germanica	Native-non-end		X	^	^
ra	_						
	Crambidae	Eudonia sp.	End	X	Х	X	
dopte	Noctuidae	Unidentified	-		Х		
Lepidoptera	Nymphalidae	Hipparchia azorina	End	X			
	Pieridae	Pieris brassicae azorensis	End		Х		

ANNEX 2: Correlations between the richness of flower-visiting insects and flower availability with the number of visits to flowers in each study area – the r-values are shown jointly with their significance (p-values).

	Flower-visiting insects and Number of visits to flowers	Flower-availability and Number of visits to flowers
LOMBA Preserved	0.75**	-0.29 n.s.
LOMBA Disturbed	0.64**	0.34 n.s.
PICO GALHARDO Preserved	0.47*	0.40*
PICO GALHARDO Disturbed	0.77**	0.43*

P-values: n.s. not significant; * p<0.05; ** p<0.01