



The impact of habitat loss on pollination services for a threatened dune endemic plant

Sara Beatriz Mendes¹ · Sérgio Timóteo¹ · João Loureiro¹ · Sílvia Castro¹

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Abstract

Habitat loss is currently a major threat to biodiversity, affecting species interactions, such as plant–pollinator interactions. This is particularly important in self-incompatible plants relying on pollinators to reproduce and sustain their populations. Here, we evaluated how habitat loss affects the pollination system, plant individual–pollinator species interaction network, and plant reproductive fitness of the self-incompatible *Jasione maritima* var. *sabularia*, a threatened taxon from dune systems. This plant is a pollinator generalist, visited by 108 species from distinct taxonomic groups. Results suggest that increasing habitat loss led to a significant decline in pollinator richness, increased pollen limitation, and a decrease in reproductive fitness of *J. maritima* var. *sabularia*. Visitation rate per individual did not significantly change with available area, indicating that the quality of pollen differed across populations. The topology of the network between *J. maritima* var. *sabularia* individuals and its pollinator species did not change, which may be attributed to the stability in the core of pollinator species. This suggests that the lower fitness of plants with increasing habitat degradation may be explained not only by the lower richness of peripheral pollinators but also by the genetic structure of the plant populations, as there is a possible higher transference of less quality pollen by pollinators, ultimately compromising the persistence of plant populations. Our study highlights the need of future studies to integrate the fine details provided by individual-level networks, which will increase our understanding of the pattern of species interactions and its consequences for the fitness of threatened plant populations.

Keywords Threatened species · Individual-based networks · Pollen limitation · Reproductive fitness · Self-incompatible plant

Introduction

Habitat loss and fragmentation of natural habitats are a major threat to biodiversity worldwide, currently being the primary cause of species extinction (Pimm and Raven 2000; IPBES 2018). It leads to changes in land cover composition and configuration, and to a gradual degradation of habitat quality (Fischer and Lindenmayer 2007; Hanski 2011; Hadley and Betts 2012). Landscape changes affect plant and animal populations alike, reducing their sizes and/or increasing their isolation and, consequently, increasing inbreeding depression and the risk of extinction (Kearns et al. 1998;

Vanbergen 2014). Habitat transformation jeopardizes not only biodiversity itself but also disrupts interactions between species on which ecosystem functioning depends (Haddad et al. 2015). Among these interactions are mutualisms between flowering plants and their pollinators, which play a critical role in shaping much of Earth's biodiversity and complexity (Bascompte 2009). Pollination is crucial for the sexual reproduction of plants (more than 87.5% of flowering plants require, to some degree, animals for pollination; Ollerton et al. 2011), as well as for the maintenance of insect populations relying on wild plants nectar and/or pollen as food resources (e.g., Carvell et al. 2006; Müller et al. 2006; Baude et al. 2016). Habitat degradation may disrupt pollination functioning through the reduction of plant and pollinator diversity in a given area (positive species-area relationship theory; MacArthur and Wilson 1967) and by changing pollinator's foraging behavior (Hadley and Betts 2012; Blaauw and Isaacs 2014; IPBES 2016).

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✉ Sérgio Timóteo
stimoteo@gmail.com

¹ Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Pollinator decline at local, regional and global scales is currently threatening the persistence of wild plant populations that rely on animal vectors for pollination (Kearns et al. 1998; Ollerton 2017), and among its causes is anthropogenic-driven habitat disturbance (Kearns et al. 1998; Potts et al. 2010; IPBES 2016). The combination of multiple stressors increases the overall pressure on living organisms and triggers complex negative feedback loops between animals and the plants they pollinate (Hadley and Betts 2012). Habitat loss and fragmentation may influence pollination directly through changes in plant and pollinator densities, and indirectly through changes in pollinator foraging behavior. First, the reduction of available habitat, and subsequent loss of nesting, oviposition and foraging sites, directly results in a decline of diversity and abundance of pollinators at local scales (Potts et al. 2010; Winfree et al. 2011). In addition, with the decline in plant density and diversity, food resources for pollinators become scarcer and scattered. These changes in food resources alter pollinator's movements and foraging strategies, resulting in changes in visitation patterns as well as flight distances. Consequently, this also changes the costs to meet pollinator's energetic needs (Wilcock and Neiland 2002; Xiao et al. 2016), ultimately impacting pollinator communities in the landscape (Tewksbury et al. 2002; Kremen et al. 2007).

Declines in pollinators will inevitably decrease the pollination service to wild plant populations and increase pollen limitation (Burd 1994; Potts et al. 2010). In addition, habitat degradation also results in impoverished plant populations and lower genetic diversity of conspecific plants, impacting the availability and quality of mating partners (Hadley and Betts 2012; Xiao et al. 2016). Consequently, there is a decrease in seed quantity and quality, plant reproductive output, and ultimately leading to the demographic collapse of plant populations (Aizen et al. 2002; Wilcock and Neiland 2002). Nevertheless, flowering plants present a wide array of reproductive strategies and the dependence on the pollination mutualism is conditioned by the plant's breeding system (Bond 1994; Richards 1997). Self-incompatible plants are highly dependent on the availability of pollen vectors and mating partners since they can only use outcross pollen to produce seeds (Aizen et al. 2002; Hiscock and McInnis 2003). Therefore, self-incompatible plants pollinated by biotic vectors are expected to be highly vulnerable to habitat degradation, with extra concerns in the case of endemic, threatened and/or rare plant species (Bond 1994; Aguilar et al. 2006).

Over the last decade, there has been an increase in the number of studies that analyzed biological interactions using a network approach (Ings et al. 2009; Heleno et al. 2014). Ecological networks present a framework to simultaneously explore the role of species and their interactions, while simultaneously exploring emergent community properties

(Bascompte and Jordano 2007; Heleno et al. 2014). Moreover, a network approach has proved to be a powerful tool to foresee the indirect effects of biodiversity loss (Tylianakis and Morris 2017), which may trigger extinction cascades and threaten the persistence of natural ecosystems (Kaiser-Bunbury et al. 2010; Rumeu et al. 2017). To date, however, most ecological network studies focused on interactions between species (Heleno et al. 2014; Timóteo et al. 2018). Although the potential of habitat loss to disrupt ecological interactions and to alter network structure at the community level is well documented (e.g., Grass et al. 2018; Heleno et al. 2020; Udy et al. 2020), to date its effect at the individual level, and the consequences for the functioning and stability of populations is still largely overlooked (but see Dáttilo et al. 2015). Recent studies have highlighted the importance of understanding interaction patterns at different levels of organization because network patterns may be scale-dependent (reviewed in Guimarães 2020). This includes downscaling ecological networks to the individual level (Dupont et al. 2011; Gómez et al. 2011; Gómez and Perfectti 2012; Tur et al. 2014; Valverde et al. 2016), because in the end it is the pattern of interactions between individuals that will dictate the dynamics and persistence of each population (Dupont et al. 2014; Tur et al. 2014; Arroyo-Correa et al. 2021). In the case of pollination networks specifically, downscaling networks to the individual plant level may be viewed as a map of pollen flow (Fortuna et al. 2008), revealing the dynamics of a population and how interaction patterns will impact plant fitness.

Here, we investigated the vulnerability to habitat loss of an insect-pollinated plant species endemic to dune habitat. Coastal sand dunes are characterized by an environmental gradient that determines a characteristic coast-to-inland plant community zonation (Acosta et al. 2007) and harbor a structurally and floristically distinct community of native plants. The environmental isolation of the dune system has been associated with speciation processes, resulting in a high proportion of endemic plant species (Neto et al. 2007). Coastal sand dunes are considered one of the most vulnerable and disturbed landscapes in Europe, and the Portuguese coastal dunes are no exception, being strongly subjected to natural and anthropogenic pressures (Marchante 2007; Martínez et al. 2008; Fantinato 2019). In Portugal, coastal zones are the most densely populated areas of the country, with more than 75% of the population living on the coast (DGA 2000; Calvão et al. 2013). Moreover, the increasing touristic activity and associated pressures (e.g., infrastructures, dune trampling, removal and substitution of native by alien species, and agricultural tillage) (Marchante 2007; Calvão et al. 2013) have contributed to decrease the heterogeneity of this landscape. These activities lead to a significant reduction in dune area, and consequently, of the availability of suitable habitat for native dune vegetation (Curr et al. 2000;

Calvão et al. 2013), which may jeopardize the persistence of insect-dependent plants and its pollinators (e.g., Traveset et al. 2018).

In this study, we used the threatened *Jasione maritima* var. *sabularia* (Cout.) Sales & Hedge (Campanulaceae) as a study system to understand how habitat loss may affect the topology of the network between individuals of *J. maritima* var. *sabularia* and its pollinator species, as well as the plant fitness. *Jasione maritima* is a self-incompatible plant (Siopa et al. 2020; Castro et al. unpublished data) from the west coast of the Iberian Peninsula dune systems (Sales and Hedge 2001a; ICNF 2002), relying on pollinators for successful reproduction. The dependence on flower visitors suggests that this plant could be particularly vulnerable to the loss of dune habitats, not only through the obvious lack of habitat but also by the reduction of flower visitors and, consequently, of its reproductive fitness. Moreover, downscaling networks from species to individuals may allow new insights into how habitat loss affects the pattern of interactions at the individual plant level and how this translates into changes in the fitness of populations (Gómez and Perfectti 2012).

In this context, we studied the pollination ecology of the threatened *J. maritima* var. *sabularia* at 15 localities along the Portuguese coast, that differ in the availability of suitable area for *J. maritima* var. *sabularia*. First, we evaluated how habitat loss (represented as the percentage of available area for the focal plant species) affects the populations of *J. maritima* var. *sabularia* and its pollinators. We hypothesize that *J. maritima* var. *sabularia* density and the richness of its pollinator community will increase with increasing available area and, consequently, the number of visits per plant will also increase. Second, we assessed whether habitat loss reduces the fitness of *J. maritima* var. *sabularia* due to limited pollination services. For this, we quantified plant reproductive fitness and pollen limitation through a hand-pollination experiment at each population. We expect that pollen limitation will decrease, and *J. maritima* var. *sabularia* fitness will increase with increasing available area. For each locality, we built plant individual–pollinator species networks to assess whether individual-level network structure changes with the available area. We expect that networks will become more generalized as the available area increases, with individual plants receiving visits from more partners. Since *Jasione maritima* var. *sabularia* was the only resource flowering by the end of spring, these networks represent a proxy for pollinator foraging patterns in each locality. This allowed us to explore whether and how the pattern of interactions at the plant individual level differs with available area, and how it will affect the fitness of its individuals and ultimately of its populations. Finally, we investigated the network structural role of pollinator species, based on pollinators connectivity, along the gradient of available area. We expect that the pollinator community will be composed

by a stable core of reliable pollinators along the gradient of available area (Dáttilo et al. 2013).

The results provide significant insights to our understanding of the dynamics of the interaction between insect-pollinated plants and the community of its pollinators across environments with different degradation levels.

Materials and methods

Study plant

Jasione maritima var. *sabularia* (Cout.) Sales & Hedge (Campanulaceae) is an endemic taxon from the west coast of the Iberian Peninsula (Sales and Hedge 2001a, b), with a distribution confined to the coastal sand dunes, mainly to the interdunal space and grey dunes among open vegetation (ICNF 2002). This taxon has received several taxonomic treatments; however, its recognition as a distinct taxon is supported by morphological traits (indumentum and leaf shape; Sales and Hedge 2001a, b) and by molecular analyses (Pérez-España et al. 2005). It is classified by the IUCN as Threatened due to the current destruction of the dune systems of the Iberian Peninsula (Bilz 2011). In Portugal, it occurs between Caminha and Aveiro, with an estimated area of occupancy of 1300 km² (Commission of the European Communities 2009).

Jasione maritima is a perennial plant flowering from June to July. Controlled self-pollinations reveal that this species is self-incompatible (seed set, mean \pm SE ($n = 8$): self-pollinations 8.8 ± 5.9 ; outcross pollinations 241.6 ± 68.7) and failed to produce seed under pollinator exclusion (Castro et al. unpublished data), similar to its sister species (*J. montana*, Parnell 1982, 1987; *J. maritima* subsp. *maritima*, Siopa et al. 2020). Consequently, this plant relies on pollinators for successful reproduction. Individual flowers are protandrous and open acropetally, with inflorescences lasting several days depending on their size, and have 33 ± 2 ovules (mean \pm SE, $n = 10$; Castro et al. unpublished data). Nectar is secreted from the top of the ovary in small quantities, accessible to a large variety of insects, and pollen is actively collected as a reward by several bee species (author's field observations). In addition, it exhibits secondary pollen presentation on a distal cylindrical stylar brush of small hairs (Faegri and Van Der Pijl 1979; Yeo 1993), and pollen removal by flower visitors triggers the female phase.

Study site and experimental design

The present study was carried out in the northern Portuguese coastal sand dunes, between Silvalde (40°59'9.93"N, 8°38'43.99"W) and Torreira Sul (40°44'27.55"N,

8°43'6.02"W), stretching 29 km along the north-west coast of Portugal. The study area is characterized by a low sandy shore and is considered one of the most vulnerable habitats along the Portuguese coastline (Martins et al. 2012). This dune system has been greatly impacted by human activity, from infrastructures construction directly on the dunes, to the removal of natural vegetation, usually starting in the grey dune and continuing further inland, with conversion of land to agriculture and other uses (Martins et al. 2013).

The study area is located in the transition between the Eurosiberian and the Mediterranean biogeographic regions (Costa et al. 1998), which influences the type of vegetation present in the dune system (Martins et al. 2013). The native vegetation of the interdunal space and the grey dunes, the main habitat of *J. maritima* var. *sabularia*, is characterized by low plant cover with herbaceous species [e.g., *Malcomia littorea* (L.) R.Br., *Helichrysum italicum* subsp. *picardi* (Boiss. & Reut.) Franco, *Silene* L. species, *Linaria* Mill. species, *Anagallis monelli* L.] and a few shrubs [e.g., *Corema album* (L.) D. Don and *Artemisia campestris* subsp. *maritima* Arcang.]. Moreover, the dune system is affected by an extensive proliferation of invasive species, mainly *Acacia longifolia* (Andrews) Willd. and *Carpobrotus edulis* (L.) N.E. Br., considered the most impactful threats to the Portuguese native flora (Marchante 2007). The native vegetation flowers earlier in the spring, whereas invasive species flower in winter and early spring, and thus flowering periods did not overlap with *J. maritima* var. *sabularia* blooming.

Characterization of habitat quality and *J. maritima* var. *sabularia* density

Along the study area, 29 1-ha quadrangular plots parallel to the sea, and 1 km apart, were established to prospect for *J. maritima* var. *sabularia*. Each plot was characterized according to: (1) geographical coordinates; (2) presence/absence of *Jasione maritima*, and (3) type of habitat anthropogenic-driven disturbance (urbanization and altered dune vegetation, such as presence of invasive species or grasses) (Supplementary material; Table S1). *Jasione maritima* var. *sabularia* was only present in 15 of the 29 sites, further selected for this study.

Habitat loss was characterized using the percentage of available habitat for the focal plant, being proportionally inverse to available area. In each 1-ha plot, the percentage of available area for *J. maritima* var. *sabularia* growth was quantified with the aid of satellite images from Google Earth v7.3.2, and later validated in the field (Supplementary material; Fig. S1). The available area for *J. maritima* var. *sabularia* was defined as the dune area with native dune vegetation (Table S1). Thus, the area occupied by urbanization and altered vegetation due to invasive plant expansion or other vegetation (e.g., grasslands) changes due to

anthropogenic activities (both forming dense stands) was calculated using the Earth Point tool for Google Earth (<http://www.earthpoint.us/>) and subtracted from the total plot area. Both the changes in vegetation considered here and the urbanization impede completely the growth of *J. maritima* var. *sabularia* and other native dune vegetation. Bare sand paths present along each plot were not considered unavailable area because we frequently observe this taxon thriving in these paths.

The density of *J. maritima* var. *sabularia* in each 1-ha plot was estimated in April of 2018, along four transects perpendicular to the sea and 25 m apart. Along each transect, a 1 m² quadrat was placed at every 15 m and the number of reproductive individuals of *J. maritima* var. *sabularia* was counted (Table S1). A total of 28 quadrats per plot were analyzed (seven quadrats per transect). Thus, density of *J. maritima* var. *sabularia* is defined as the number of individuals of *J. maritima* per square meter in each 1-ha plot.

Flower visitor assemblage

The assemblage of floral visitors of *J. maritima* var. *sabularia* was characterized by direct observation during the flowering peak of 2017 (from June to July), on sunny and low to moderate windy days. In each of the 15 sites, observations were made in 10 patches of 2.25 m² (1.5 m × 1.5 m), randomly distributed in areas where the studied plant grows. The observer was positioned at approximately 1 m from the patch, with small range binoculars, being able to monitor all floral visitors without interfering with the foraging activity of visitors. Visits were recorded in 15 min census for one day (from 0900 to 1800 h, GMT), or in two consecutive days when the lack of sunny conditions due to fog limited the number of censuses in the first day, totalling 384 censuses and 96 h of observations. In each patch, the following parameters were registered: (1) number of *J. maritima* var. *sabularia* individuals; (2) number of open inflorescences per individual plant; (3) identity and number of each flower visitor that interacted with the reproductive organs of *J. maritima* var. *sabularia*; (4) number and sequence of inflorescences visited by each flower visitor. One specimen of each insect type was collected for further identification to the lowest taxonomic level possible (hereafter called species for simplicity). Visitation rate per plant was calculated as the number of inflorescences of each plant individual visited per minute (Castro-Urgal et al. 2012).

Reproductive fitness and pollen limitation

To determine the effect of habitat loss on the reproductive fitness of *J. maritima* var. *sabularia* and on pollen limitation, the following treatments were applied at each site, during the flowering period of 2017: (1) open pollination (control),

i.e., flowers without treatment, left open for natural levels of pollination; (2) supplementary pollination, i.e., flowers left open to natural levels of pollination and additionally supplemented with fresh pollen from five different genotypes of *J. maritima* var. *sabularia*. For this, at each site, 30 individuals were arbitrarily selected to receive both treatments, with one inflorescence marked to receive natural levels of pollination, while another received pollen supplementation. These plants were permanently labeled. Inflorescences with the exterior row of receptive flowers (visible by the lack of pollen in the stylar brush and by the bilobed stigmatic surface) were selected for pollen supplementation. Only this row was considered in the treatment due to time constraints to perform pollinations in all the study sites. Pollen supplementation was made by gently rubbing the selected inflorescence with inflorescences from five different genotypes. When mature, but prior to dehiscence, infructescences were collected to estimate the number of fruits and seeds. In the laboratory, fruit set (percentage of flowers that developed into fruits) and seed set (number of viable seeds per fruit) were quantified for each individual and treatment. To calculate the reproductive fitness of each individual of *J. maritima* var. *sabularia*, seed set was multiplied by fruit set. Only the exterior row of fruits of the infructescences from both the control and supplement treatments was used to estimate reproductive fitness to avoid differential resource allocation within the inflorescence. Although this fitness variable might not account for differences in internal rows, considering external rows in both treatments enabled us to obtain comparable results for the purpose of our objectives (i.e., calculate pollen limitation values). Pollen limitation (PL) was estimated for each individual based on the reproductive fitness according to Larson and Barrett (2000) as: $PL = 1 - C/S$, where C is the reproductive fitness of the control treatment and S is the reproductive fitness of the pollen supplementation treatment. Positive values resulting from higher reproductive fitness in pollen supplemented than in control treatment indicate the existence of pollen limitation in natural populations, while zero or negative values indicate no pollen limitation.

Network analysis

Quantitative plant individual-floral visitor species interaction matrices were built for each of the 15 sites, pooling the observations from the ten monitored plots. Each *Jasione maritima* individual and each floral visitor species observed in the day of pollinator monitoring were considered as a node in the visitation networks. These networks represent the potential mating events between individuals of *J. maritima* in a given population that may result from visiting insect species. Link weight was quantified as frequency of interaction between floral visitors and plants and calculated

as the number of insect visits per minute (Castro-Urgal et al. 2012; Traveset et al. 2018).

To compare the structure of the individual-based plant–pollinator networks along our gradient of increasing available area, the following network-level descriptors were calculated: (1) weighted connectance, the linkage density divided by the number of species in the network, and reflecting the fraction of realized interactions (Tylianakis et al. 2007); (2) interaction evenness, based on Shannon diversity, reflects the uniformity of the interactions between species at network level (Bersier et al. 2002) often negatively associated with habitat disturbance (Tylianakis et al. 2007); (3) network specialization (H_2'), a measure of the selectivity of interaction partners across the network, derived from Shannon entropy, and is a robust metric not affected by network size and sampling effect (Blüthgen et al. 2006); (4) generality, the mean number of preys per predator; in the present work interpreted as the mean number of plant individuals per pollinator (Bersier et al. 2002; Tylianakis et al. 2007); (5) vulnerability, the number of predators per prey, in the present work interpreted as the mean number of pollinators per plant individual (Bersier et al. 2002; Tylianakis et al. 2007); (6) pollinator robustness, a metric quantifying how much the pollinator community can withstand the random loss of plant individuals; and (7) plant robustness, quantifying how much the plant population can withstand the random loss of floral visitor species (Memmott et al. 2004). Moreover, two node-level descriptors for plants and for pollinators were calculated: (1) normalized degree for plants and for pollinators, number of pollinator species visiting each plant individual and the number of plant individuals visited by each pollinator species, respectively, divided by the number of possible interaction partners (Martín González et al. 2010); (2) species strength for plants and for pollinators is the sum of dependencies, i.e., is a measure of plant individual's importance for the pollinator community and a measure of pollinator species' importance for plant population, respectively (Bascompte et al. 2006). Since in our networks plant nodes are individuals rather than species, we will refer to individual plant strength from now on.

Many network descriptors can be affected by differences in sampling effort and network size (Fründ et al. 2016). To overcome this potential issue, network-level descriptors were corrected with a null model, which allows reliable comparisons between the networks (Vázquez and Aizen 2003; Costa et al. 2016). Due to the presence of decimal values in the matrix and to allow the use of a quantitative null model, networks were standardized by dividing its link weight by the lowest non-zero link weight in the matrix and rounded to the nearest integer (Timóteo et al. 2018). Afterwards, we generated 1000 randomizations of the original networks and, for each network descriptor, we calculated its z-score ($z = [\text{observed} - \text{null mean}] / \text{null standard deviation}$)

(Sebastián-González et al. 2015; Dalsgaard et al. 2017), which were used in subsequent analysis. The randomized networks were obtained using the Patefield's null model (Patefield 1981), which fixes network size (species richness) and marginal sums (total interaction frequency of each species). Network and species level descriptors were calculated using the *bipartite* R package (Dormann et al. 2008, 2009).

Finally, pollinators were classified for their role in each network as core or peripherals, according to the number of links of each species, i.e., number of individuals of *J. maritima* they visited (Dáttilo et al. 2013, 2015). Following Dáttilo et al. 2013, this classification is based on standardizing the number of links of each pollinator species through: $G_c = (k_i - k_{\text{mean}}) / \sigma_k$, where k_i is the number of links of the species in a network, k_{mean} is mean number of links of all pollinator species in a network, and σ_k is the standard deviation of the number of links of a species in a network. Pollinator species with $G_c > 1$ have higher number of links than other pollinator species, thus structural core species of the pollination network, whereas those with $G_c < 1$ have lower number of links than the other species, being structural peripheral species in.

Statistical analysis

All statistical analyses were performed in R version 3.5.0 (R Core Team 2018). To evaluate the level of sampling completeness of pollinator species, we calculated species accumulation curves for each study site [*vegan* package (Oksanen et al. 2017)]. We estimated the minimum asymptotic richness of pollinators using the non-parametric estimator Chao 2 (Chao 1987), which is known to be robust for reduced sample sizes, and more reliable than other estimators (Walther and Moore 2005). The percentage of sampling completeness was calculated as the observed number of species divided by the estimated number of species (Costa et al. 2016).

To assess the effect of the available area on the number of open inflorescences per patch of pollination observation, number of flowers per inflorescence, number of inflorescences per plant individual, reproductive fitness, pollinator normalized degree and pollinator species strength we resorted to Generalized Linear Mixed Models [GLMM, *lme4* (Bates et al. 2014) and *lmerTest* (Kuznetsova et al. 2017) packages]. To explore the effect of available area on *J. maritima* var. *sabularia* density, visitation rate per plant, pollen limitation, plant normalized degree and plant strength, we used Robust Linear Mixed Models [RLMM, *robustlmm* package (Koller 2016)]. Latitude was included as a random factor to control for the potential variability associated with the geographic location of the sampling locations. In the analyses regarding pollinator node-level metrics, we also included pollinator species as a random factor to account for differences in species composition between locations and to

avoid pseudo-replication. Moreover, for response variables based on pollination observations, we included observation patch as a nested factor within each latitude, i.e., within location, to account for the nested structure of the experimental design (patches of pollination observation within location).

For each network-level metric [weighted connectance, network specialization (H2'), vulnerability and generality, interaction evenness, pollinator and plant robustness], we have only obtained one value per location, and latitude could not be entered as a random factor. Thus, we used Generalized Linear Models (GLM) and latitude was included as a covariate instead.

The models for the number of open inflorescences, number of flowers per inflorescence, number of inflorescences per plant individual, and pollinator species richness were fitted with Poisson errors distribution, with a square-root link function for the first model, an identity link function for the next two models, and a log link function for the last model. The model for reproductive fitness was fitted with Gaussian errors distribution and a square-root link function. The models for network specialization H2' and pollinator normalized degree and species strength were fitted with Gamma errors distribution, with an inverse link function for the first model, and a log link function for the latter two models. The models for the remaining variables were fitted with Gaussian errors distribution and an identity link function.

For all GLMMs, RLMMs and GLMs mentioned before, *J. maritima* var. *sabularia* density (number of individuals of *J. maritima* per square meter) was also included as a covariate to account the effect of *J. maritima* density (the density of *J. maritima* in 1-ha plot), to test if the results are independent of population density.

The effect of pollen supplementation on reproductive fitness for all populations combined was analyzed using a GLMM, including pollination treatment as fixed factor, latitude and individual as random factors, fitted with Gaussian errors distribution and an identity link function. To assess the effect of pollen supplementation for each locality, differences were also investigated for each population using linear models (LMs).

To test if the community of pollinators changed across the gradient of available area, we used a Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson 2001), based on a Bray–Curtis' distances with 9999 permutations, with function *adonis* () from the R package *vegan* (Oksanen et al. 2017). Besides available area, G_c classification was included to test for differences between the composition of the core and peripheral communities. Finally, we tested whether each of those subcommunities changed across the gradient of available area, and whether the number of core pollinator and peripheral species was affected by the available area, using a GLM fitted with Poisson errors distribution with a log link, including density of *J. maritima*

to control. Core species are those that contribute most to pollination with visits to several *J. maritima* individuals, and likely the most effective pollinators.

For all the LM, GLM and GLMM analyses, residuals were plotted and analyzed for departures from normality and homoscedasticity, and response variables were transformed to ensure the best fit to the assumptions of standard regression. When the assumptions for linear models were not met, Robust Linear Mixed Models were used as they down-weight influential points on the general trend of the data and provide better estimates of the regression parameters and their standard errors (Koller 2016). Given that likelihood ratio tests are not available for robust models, significance of the explanatory variables was calculated using Satterthwaite approximations of degrees of freedom (Luke 2017; Geniole et al. 2019). The details and significance of all models are presented in Tables S2 to S9.

Regarding visitation per plant and network analyses, two extreme values related to the frequency of interaction of ant species during two observation periods were tested as outliers (once they corresponded to observations performed in monitoring patches located above ant colonies) and removed to meet the assumptions of normality and homoscedasticity.

Pearson correlation analysis was performed to evaluate the correlation between the following variables: *J. maritima* var. *sabularia* density, pollinator richness, reproductive fitness and pollen limitation.

Results

Effect of available area on *Jasione maritima* populations

The density of *J. maritima* var. *sabularia* varied between 0.04 and 8.61 plants per square meter (Table S1), but we did not find a significant effect of available area on *J. maritima* var. *sabularia* density ($t = 1.862$, 13 df, $P = 0.085$; Fig. 1a and Table S2). Nevertheless, data suggest a slight trend for higher *J. maritima* var. *sabularia* density with increasing area.

The number of open inflorescences of *J. maritima* var. *sabularia* per patch significantly increased with available area ($\chi^2 = 6.548$, 1 df, $P = 0.011$), ranging between 13.8 and 59.9 inflorescences per patch (Fig. 1b; Table S2). Available area did not significantly affect the number of flowers per inflorescence ($\chi^2 = 0.015$, 1 df, $P = 0.902$; Table S2 and Table S3), or the number of inflorescences per individual plant ($\chi^2 = 0.315$, 1 df, $P = 0.574$; Table S2 and Table S3).

In addition, *J. maritima* var. *sabularia* density had no effect on any response variable ($P > 0.101$ for all response variables; Table S2, Table S4–Table S8) analyzed in the

following sections of the results, suggesting that the results are independent of population density.

Effect of available area on pollinator assemblage and visitation rate per plant

A total of 1258 insects, belonging to 108 morphospecies, were observed visiting the flowers of *J. maritima* var. *sabularia*. Floral visitors included ants, wasps and bees [Hymenoptera (38.9%), 42 morphospecies]; flies [Diptera (39.8%), 43 morphospecies]; beetles [Coleoptera (4.6%), 5 morphospecies]; and butterflies [Lepidoptera (16.7%), 18 morphospecies] (Fig. 2; Table S9 for a detailed list of species). Overall, 3044 interactions between *J. maritima* var. *sabularia* and its visitors were recorded across the 15 sites. Most of the interactions were with Hymenoptera (1687 interactions—55.4%), followed by Diptera (918 interactions—30.2%), Coleoptera (353 interactions—11.6%) and Lepidoptera (86 interactions—2.8%). Sampling completeness ranged between 32.6% and 89.6% (Table S1). Nevertheless, in most sites (11 out of 15 studied), we achieved a moderate to high level (64.0% or above) of sampling completeness.

Pollinator species richness ranged from 10 to 25 species, whereas the abundance of pollinators ranged between 33 and 176 individuals (Table S1). Available area for *J. maritima* var. *sabularia* had a significant positive effect on pollinator richness ($\chi^2 = 7.677$, 1 df, $P = 0.006$; Fig. 1c and Table S4), but not on the visitation rate per plant ($t = -0.142$, 12 df, $P = 0.889$; Fig. 1d and Table S5).

Effect of available area on reproductive fitness

Regarding reproductive fitness, available area showed a significant effect on the number of seeds *J. maritima* var. *sabularia* produced ($\chi^2 = 6.579$, 1 df, $P = 0.010$; Fig. 1e and Table S5 and Table S11).

At the regional level, strong significant differences were detected between open pollinated and pollen supplemented flowers ($\chi^2 = 208.65$, 1 df, $P < 0.001$; Table S8), with supplemented flowers reaching a higher reproductive fitness, i.e., producing more seeds per flower than open pollinated flowers (mean \pm SE; 18.95 ± 0.42 vs 12.21 ± 0.44 , respectively, Table S11). At the population level, in 13 of the 15 sites studied, supplemented flowers had a significantly higher reproductive fitness than open pollinated flowers (Fig. S2, Table S9 and Table S11). In the remaining two populations, supplemented flowers had a greater reproductive fitness than open pollinated ones, but no significant differences were observed between the two pollination treatments (Fig. S2 and Table S10).

Pollen limitation index increased as available area for *J. maritima* decreased ($t = -2.197$, df = 10.66, $P = 0.051$; Table S5; Fig. 1f).

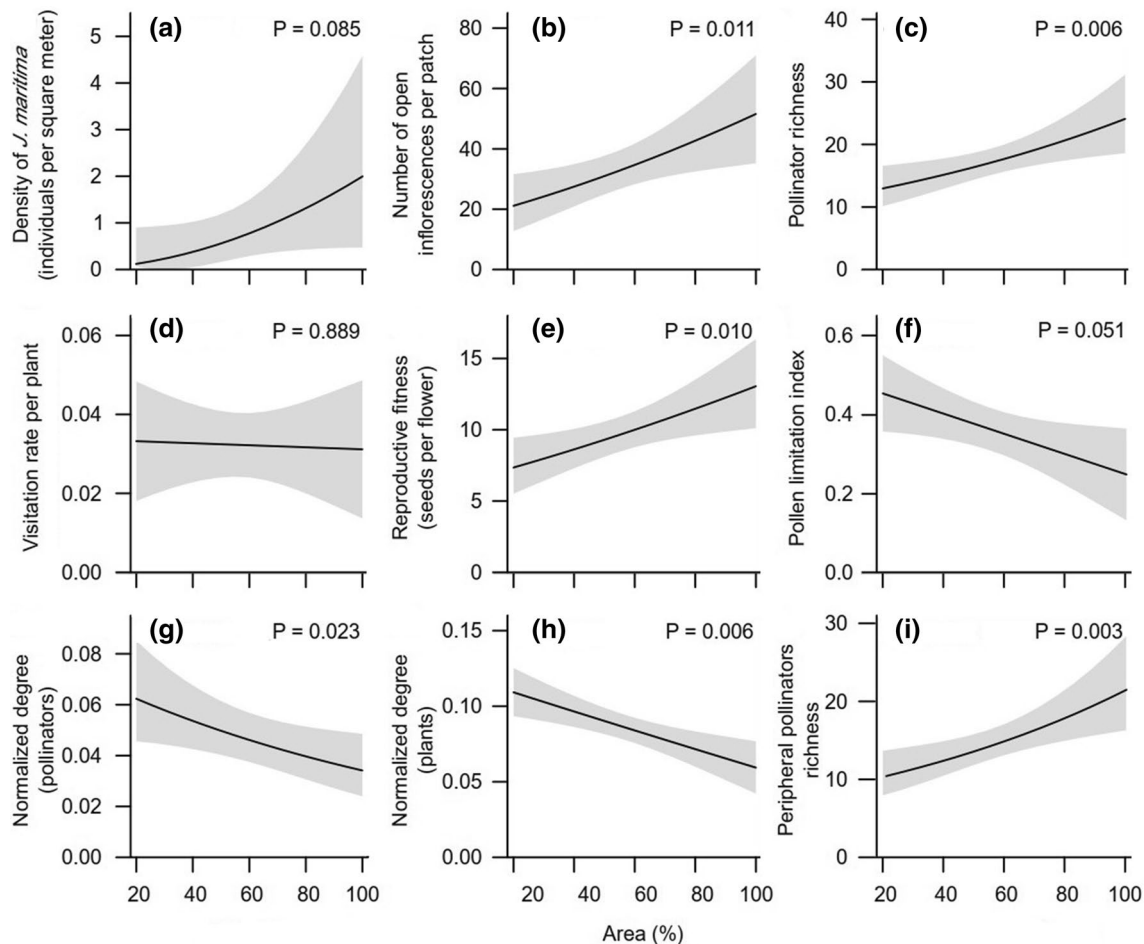


Fig. 1 Effect of the available area on (a) *Jasione maritima* var. *sabularia* density (number of individuals per square meter), (b) number of open inflorescences of *J. maritima* var. *sabularia* per monitored patch, (c) pollinator species richness (number of floral visitor's taxa), (d) visitation rate per plant (number of inflorescences of each plant individual visited per minute), (e) reproductive fitness (number of

seeds per flower), and (f) pollen limitation (PL) across the increasing gradient of available area. Values were back-transformed. Solid lines represent the predicted relationships from the respective model and shaded areas correspond to 95% confidence interval. Significance values for the effect of area on each variable are given at the upper left corner

Correlation between plant and pollinator variables

Pollinator richness was significantly correlated with reproductive fitness of *J. maritima* var. *sabularia* ($r=0.5204$, $P=0.047$). None of the other correlations between the studied variables was significant (Table 1).

Effect of available area on network structure and node-level descriptors

Contrary to our expectations, available area did not have a significant effect on any network-level descriptor ($P > 0.260$ for all network-level metrics; Table S6; Table S12 and Table S13). Regarding node-level descriptors, available area had a significant negative effect on both pollinator normalized degree ($\chi^2=5.187$, 1 df, $P=0.023$) and plant normalized degree ($t=-3.382$, 10.60 df,

$P=0.006$), a marginally significant positive effect on pollinator species strength ($\chi^2=3.454$, 1 df, $P=0.063$), and no significant effect on individual plant strength ($t=-1.562$, 12.60 df, $P=0.143$; Table S7, Table S14 and Table S15).

Globally, pollinator community composition did not change across the gradient (PERMANOVA: Pseudo-F=1.070, $R^2=0.006$, $P=0.298$), and no differences were detected between the composition of the community of core and peripheral pollinators (PERMANOVA: Pseudo-F=1.067, $R^2=0.007$, $P=0.300$). Moreover, when looking into each of these communities of pollinators, neither the core nor the peripheral community changed with available area (PERMANOVA: core—Pseudo-F=0.933, $R^2=0.025$, $P=0.646$; peripheral—Pseudo-F=1.096, $R^2=0.008$, $P=0.244$). However, while the number of core species did not change across the gradient ($\chi^2=0.011$, 1 df, $P=0.916$; Table S4), the number of peripheral species

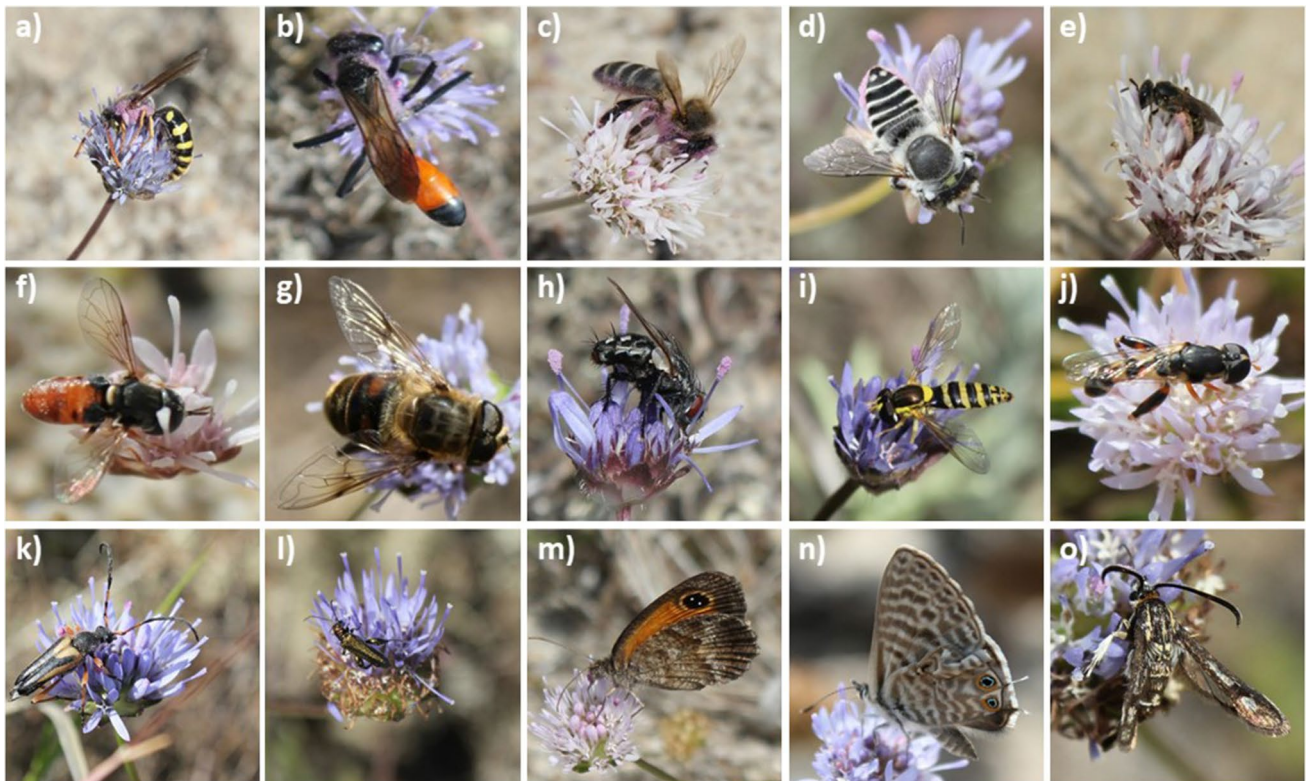


Fig. 2 Examples of pollinators of *Jasione maritima* var. *sabularia*: (a) *Stizus ruficornis*, (b) *Podalonia hirsute*, (c) *Apis mellifera*, (d) *Megachile* morphospecies 1, (e) *Lasioglossum* morphospecies 1, (f) *Paragus* morphospecies 1, (g) *Erystalis tenax*, (h) *Sarcophaga* mor-

phospecies, (i) *Sphaerophoria* sp., (j) *Syritta pipiens*, (k) *Paracorymbia stragulata*, (l) *Oedemera flavipes*, (m) *Pyronia cecilia*, (n) *Lepototes piriethous*, and (o) *Pyropteron hispanica*

Table 1 Pearson correlation coefficients for comparisons between *Jasione maritima* density, pollinator richness, reproductive fitness and pollen limitation

Variables	<i>Jasione maritima</i> density	Pollinator richness
Pollinator richness	−0.187 ^{n.s}	–
Reproductive fitness	−0.214 ^{n.s}	0.520*
Pollen limitation	−0.377 ^{n.s}	−0.411 ^{n.s}

Asterisks indicate significant correlations between the variables: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. denote non-significant correlations

increase with available area ($\chi^2 = 8.842$, 1 df, $P = 0.003$; Fig. 1i, Table S4).

Discussion

Jasione maritima var. *sabularia* is a pollinator generalist, with its flowers being visited by 108 species, mostly Hymenoptera and Diptera. This threatened endemic taxon is self-incompatible and, thus, it highly relies on pollinators

for its successful reproduction. Here, we observed a decline in pollinator richness and pollination service (indicated by increased pollen limitation levels), resulting in a reduction in reproductive fitness as available habitat for *J. maritima* decreases (i.e., with increasing habitat loss). Consequently, the ongoing expansion of urbanization and of invasive plant species in Portuguese dune systems can lead to the decline of the pollinators of *J. maritima* var. *sabularia* and compromise the persistence of its populations. In addition, the vulnerability to the loss of pollinators due to habitat loss observed here could be extended to other insect-pollinated plants growing in disturbed habitats.

Species sensitivity to habitat degradation depends on the combination of species traits, such as, for example, in plants, the competitive and dispersal abilities, reproductive potential across generations, iteroparity or clonal reproduction (Henle et al. 2004). However, habitat area is fundamental to determine the persistence of plant and pollinator populations, since smaller areas will commonly contain fewer species and individuals (MacArthur and Wilson 1967; Bender et al. 1998; Hagen et al. 2012). Indeed, several authors (e.g., Aizen and Feinsinger 1994; Blaauw and Isaacs 2014; Jauker et al. 2019) have demonstrated decreases in pollinator species

richness due to smaller areas generated by habitat loss and fragmentation. Larger habitat areas are structurally more heterogeneous and usually support larger communities of pollinators by presenting a higher availability and quality of nesting opportunities (Potts et al. 2005; Hopfenmüller et al. 2014), as well as higher diversity and abundance of food resources (Blaauw and Isaacs 2014; Hopfenmüller et al. 2014). Although the increase in the density of *J. maritima* var. *sabularia* with available area was not statistically significant, larger areas presented significantly higher number of open inflorescences, which may affect attractiveness to pollinators. This result supports the consensus that smaller populations are less attractive or less detectable, which is a fundamental factor in the diversity of pollinators and also the patterns of pollen flow (Sih and Baltus 1987). Importantly, in the populations studied in this work, at the time of its peak flowering (late spring to early of summer), *J. maritima* var. *sabularia* was nearly the only food resource for the pollinators. Indeed, and except for a very few late flowering individuals of other native plants in a few localities, there were no other co-flowering plants. This lack of alternative food resources highlights the critical importance of *J. maritima* var. *sabularia* as a key resource for the maintenance of the pollinator's community in these dune systems.

The present study shows that habitat loss influences the reproductive fitness of *J. maritima* var. *sabularia*, as the decrease in available area was associated to lower number of seeds per flower. The reduction in the reproductive fitness of *J. maritima* var. *sabularia* with habitat loss is consistent with other studies that document decreases in reproductive fitness of self-incompatible plants due to habitat degradation (Steffan-Dewenter and Tschardt 1999; Moody-weis and Heywood 2001; Aguilar et al. 2006). A review by Ghazoul (2005) showed that, in 12 out of 16 studies, small populations of self-incompatible plants had lower fitness than large ones. Although seed and fruit set can be influenced by other ecological and genetic factors (Caruso et al. 2005; Campbell and Husband 2007), pollen limitation is a key factor for reproductive fitness (Ashman et al. 2004) and has been pointed out as one of the main causes for the reduction of plant fitness in fragmented habitats (Aguilar et al. 2006). Our pollen supplementation experiment showed that *J. maritima* var. *sabularia* plants were strongly pollen limited at the regional level, with plants supplemented with outcross pollen presenting higher fitness when compared to control plants. However, pollen limitation can vary temporally and spatially within the same species (e.g., Knight 2003; Castro et al. 2008) and two of the populations from habitats with larger available area were not pollen limited. Moreover, habitats with larger available area presented lower pollen limitation when compared to habitats with smaller areas. Pollen limitation is a consequence of the reduction, in quantity and quality, of the pollen deposited on stigmas (Wilcock and

Neiland 2002). Successful pollination depends on factors related with pollinator traits, such as pollinator diversity, abundance and efficiency and pollinator foraging strategies, as well as plant traits, such as breeding system and population genetic composition (Ghazoul 2005; Castro et al. 2009; Hadley and Betts 2012). Thus, one may hypothesize that increased levels of pollen limitation with increased habitat loss may result from (1) changes in pollinator communities and plant–pollinator interaction networks, and/or (2) genetic dilapidation in this self-incompatible plant populations.

Changes in pollinator communities and plant–pollinator networks may affect pollen transfer between plant individuals and, consequently, impact its reproductive fitness. However, contrary to our expectations, the overall topology of the individual-based plant–pollinator networks did not change along the gradient of available area, with no changes detected for any network-level descriptor. Available area did not significantly affect the diversity or distribution of interactions across networks, as no trend was found for weighted connectance, interaction evenness, network specialization, vulnerability and generality. Furthermore, available area did not affect pollinator and plant robustness, whereby the structure of the individual networks was robust to loss of available area. Nonetheless, we found an effect of the gradient of available area on the number of interaction partners (normalized degree) for both pollinators and plants. In smaller areas, pollinators tended to visit more plant individuals, whereas each plant individual was also visited by a greater number of pollinator species. Moreover, the core pollinators did not change across the entire gradient of available area, being reliable deliverers of the pollination service. Core pollinators contribute with most interactions to networks, visiting more individuals of *J. maritima* var. *sabularia* and being likely the most effective pollinators and the reason why we did not detect changes in network topology (Dáttilo et al. 2015). To date, very little information exists about the effect of habitat degradation on individual-based networks, but the pattern we found here is consistent with a previous study from tropical rainforests showing that reliable core pollinators helped to stabilize network topology (Dáttilo et al. 2015). However, we also observed that along the gradient of available area the number of peripheral pollinator species increased, leading to a higher richness of pollen vectors. For plants depending on pollinators for seed production, visits from a more diverse pollinator community, with distinct foraging patterns, has been associated with better reproductive success and fitness (Gómez and Perfectti 2012; Tur et al. 2013). A diverse pollinator community introduces functional redundancy and weakens mutual dependencies, strengthening community resilience and robustness to local species loss (Tylianakis et al. 2007; Kaiser-bunbury et al. 2017). In this study system, the higher reproductive fitness along the gradient of available area may be partially explained by the cumulative

positive effect of peripheral pollinator species, whose richness increases along the gradient. Successful pollination in a population is determined not only by individual pollinator abundance or pollinator richness, but also by pollinator efficiencies, and because pollinator efficiencies are variable, the relation between those factors and reproductive success might not be straightforward.

The fact that visitation rate per individual plant did not change across the gradient of available area also suggests that the quality of pollen may differ across populations, raising the hypothesis of genetic dilapidation of *J. maritima* var. *sabularia* populations with habitat loss. Smaller areas tend to hold smaller populations of *J. maritima* var. *sabularia* and plant individuals are probably genetically more related, which may lead to increase inbreeding levels and reproductive failures, thus facing higher fitness costs (Fischer and Lindenmayer 2007; Sletvold et al. 2012). This is likely associated with transference of lower quality pollen (incompatible or genetically related pollen in self-incompatible species) by pollinators that can result in stigmatic clogging and a consequent decrease in reproductive success (Ehlers 1999; Waites and Ågren 2004). Larger areas are likely to have higher genetic diversity with most of the pollen received by individual plants being compatible, thus increasing the reproductive fitness in such populations. Future studies accounting for the genetic structure and inbreeding levels may provide further insights into the processes involved in the dynamics of plant populations under disturbance. Given that the pollinator community is more homogeneous in smaller areas, *J. maritima* var. *sabularia* could still be at risk of a higher reproductive failure if it relies heavily on a guild of pollinators that responds similarly to a future anthropogenic effect (Kremen et al. 2007). Anthropogenic actions and ecological processes acting together can result in a selective decline of important species of the system, leading to detrimental effects on the plant population, and ultimately to the irreversible collapse of the plant–pollinator network (Kaiser-Bunbury et al. 2010).

Conclusions

This is one of the first studies investigating the effect of habitat loss on the reproductive fitness of the threatened *J. maritima* var. *sabularia*, and how the pattern of interactions of its individuals with the pollinator community is affected. Here, we show that the loss of suitable habitat within the dune system is a main factor leading to a decrease in pollination services, and consequently in the reproductive fitness of *J. maritima* var. *sabularia*. This self-incompatible species highly relies on the pollinator community for sexual reproduction, and although the

structure of its network of interaction did not change, future disturbances impacting its core pollinators may further compromise the persistence of viable populations. The sensitivity of plant–pollination interactions across disturbance gradients may constitute a major threat to animal-pollinated plant populations growing in habitats highly susceptible to disturbance. This study provides information on the pollination ecology of this endemic plant, which can be a first step to devise a strategy for the implementation of conservation measures.

Our results provide a novel insight to the study of individual-based networks in degraded environments, highlighting the need to develop more comprehensive experimental designs that include for example the evaluation of the genetic structure of the populations, to better understand how environmental gradients may affect the structure of mutualistic networks and how it may translate into the fitness of its individual constituents and of the overall population.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00442-021-05070-y>.

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Author contribution statement SC, ST and JL conceived and designed the experiment. SM conducted field and laboratory work with the assistance of the remaining authors. SM, ST and SC analyzed the data and SM and ST developed the plant–pollinator networks. SM wrote the manuscript with the contributions of all the other authors.

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Data availability Data supporting the results will be made publicly available upon acceptance.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Permits to collect samples and undertake scientific research were obtained from ICNF—Instituto da Conservação da Natureza e das Florestas, Reference 866/2018.

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