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DNA metabarcoding unveils the effects of habitat fragmentation on pollinator diversity, plant-pollinator interactions, and pollination efficiency in Maldive islands

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Abstract

Habitat fragmentation affects biodiversity, but with unclear effects on pollinators and their interactions with plants in anthropized landscapes. Islands could serve as open air laboratories, suitable to disentangle how land-use alteration impacts pollination ecology. In Maldive islands we investigated how pollinator richness, plant-pollinator interactions and pollination efficiency are influenced by the green area fragmentation (i.e., gardens and semi-natural patches). Moreover, we considered the mediating role of pollinator body size and the plant trait of being invasive in shaping interactions. To do this, we surveyed pollinator insects from 11 islands representing a gradient of green area fragmentation. A DNA metabarcoding approach was adopted to identify the pollen transported by pollinators and characterize the plant-pollinator interactions. We found that intermediate levels of green area fragmentation characterized pollinator communities and increased their species richness, while decreasing interaction network complexity. Invasive plants were more frequently found on pollinator bodies than native or exotic noninvasive ones, indicating a concerningly higher potential for pollen dispersal and reproduction of the former ones. Intriguingly, pollinator body size mediated the effect of landscape alteration on interactions, as only the largest bees expanded the foraging diet in terms of plant richness in the transported pollen at increasing fragmentation. In parallel, the pollination efficiency increased with pollinator species richness in two sentinel plants. This study shows that moderate landscape fragmentation of green areas shapes many aspects of the pollination ecosystem service, where despite interactions being less complex and mediated by pollinator body size, pollinator insect biodiversity and potential plant reproduction are supported.

KEYWORDS
DNA barcoding, ecosystem services, habitat degradation, pollen DNA metabarcoding, pollinator foraging, species interactions

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1 | INTRODUCTION

Land-use intensification, mainly induced by the expansion of urbanization and agricultural activities, is often considered a major threat to biodiversity and specifically to pollinator species conservation (Biella, Tommasi, et al., 2022; Dicks et al., 2020; Potts et al., 2010; Tommasi, Biella, et al., 2021). This is because landscape intensification leads to habitat loss and green areas fragmentation, especially in urban environments (Kovács-Hostyánszki et al., 2017; Rathcke & Jules, 1993; Senapathi et al., 2017). As a result, pollinator community composition is impoverished by species loss in fragmented landscapes, mainly due to the disappearance of specialist pollinator taxa (Xiao et al., 2016). In turn, plant-pollinator interactions are expected to become more generalized, possibly due to changes in floral composition and distribution (Andrieu et al., 2009; Fortuna & Bascompte, 2006; Matthews et al., 2014). Local conditions such as flower diversity and abundance are important drivers of pollinator community features since they could even mitigate the negative impacts posed by land-use intensification both on pollinator community composition and its interactions with plants (Tommasi, Biella, et al., 2021).

In landscapes altered by human practices, green areas become of high importance for biodiversity and the effects of this fragmentation on pollinators could vary at different geographical and taxonomic scales. This translates into changes in pollination efficiency that have already been documented, albeit with idiosyncratic responses depending on the investigated species (Xiao et al., 2016). At a small scale (i.e., 20 m radius), the diversity of bees appears negatively associated with the fragmentation of green areas (Hennig & Ghazoul, 2012). Conversely, at wider scales (i.e., 200 or 1000 m radius), the fragmentation of green patches corresponds to increased pollinator species richness, flower visitation rates and pollination efficiency (Hennig & Ghazoul, 2012; Theodorou et al., 2020). This variability in responses to green area fragmentation highlights difficulties at predicting how land-use intensification affects pollinator communities and the quality of the ecosystem service they provide. Furthermore, different species could greatly diverge in their foraging strategies and contribute differently to pollination. Thus, the analysis of interspecific and intraspecific variation in plant-pollinator interactions in fragmented habitats is necessary to comprehend the role of target species, and their changes in response to anthropic disturbance (Biella, Akter, et al., 2019; Biella, Tommasi, et al., 2022; Fuster & Traveset, 2020). Therefore, by studying the effects of green area fragmentation on pollinators it is possible to suggest reliable strategies for mitigating the impact on green ecosystems.

In this framework, islands offer unique opportunities to investigate the effects of pressures on biodiversity related to land-use (Biella, Ssymank, et al., 2022; Castro-Urgal & Traveset, 2014; Kaiser-Bunbury & Blüthgen, 2015; Picanço et al., 2017; Steibl et al., 2021). Islands can be considered open air laboratories for ecological studies for several reasons. First, they host simplified and isolated biotic communities, which ease the evaluation of species roles in ecosystem functioning (Kaiser-Bunbury et al., 2010; Warren et al., 2015). Secondly, environmental changes spread more rapidly on islands than in the mainland context, favoured by small population sizes (Castro-Urgal & Traveset, 2014). These aspects also apply to pollinator and plant assemblages, which are usually simplified in insular ecosystems (Kaiser-Bunbury et al., 2010; Traveset et al., 2016). An additional, yet relevant aspect is that dispersal events among islands are occasional or rare, and this is a favourable property when studying the effects that land-use changes have on plant-pollinator interactions (Kaiser-Bunbury & Blüthgen, 2015). Given these assumptions, islands are suitable scenarios to solve ecological questions related to the effects of land-use intensification on pollinators foraging.

To date, several studies on pollination in insular contexts have been focused on the effects of exotic and invasive species (e.g., Bartomeus et al., 2008; Padrón et al., 2009). This is because non-native species often represent a large component of insular communities and they are considered among the major threats to conservation issues (Kaiser-Bunbury et al., 2011). However, a limitation in many insular systems is that they are largely neglected, especially in light of ecological research on terrestrial biodiversity and interactions with plants. This is the case of Maldives, in the Indian Ocean, where studies on terrestrial biodiversity are extremely rare (Steibl et al., 2021). In addition, studies in insular systems could be biased by limited taxonomic and species distribution knowledge. These aspects are met even for pollinators in Maldives (but see Kevan, 1993). There, islands are homogeneous in terms of climatic conditions and island physical features, while varying in the degree of human exploitation and impact (Fallati et al., 2017). This context results in a gradient of green area fragmentation and provides a suitable model to better understand and interpret the impact of this fragmented landscape on pollinators, allowing knowledge transfer to other geographical contexts of landscape alteration.

In this framework, modern molecular approaches can efficiently support investigation on species biodiversity and biological interactions. In recent years, molecular tools such as DNA metabarcoding have been increasingly applied to pollination ecology research for describing plant-pollinator interactions (Bell et al., 2017; Pornon et al., 2016; Tommasi, Biella, et al., 2021). By foraging on flowers, pollinators carry pollen grains that keep trace of their foraging activity (Bosch et al., 2009). Standard DNA barcode loci, associated with the use of high throughput sequencing technologies (HTS), can be used to characterize such pollen and understand which plants were visited (Tommasi, Ferrari, et al., 2021), to reconstruct the plant-pollinator networks and assess the resource preferences of flower visitors (Biella, Tommasi, et al., 2019). This approach ensures significant advantages, allowing to reduce the time spent for field direct observation of interactions or to reduce the time spent for pollen characterization in laboratories, while improving the number of observed interactions (Bell et al., 2017). However, the potential of DNA metabarcoding for identifying pollen can be amplified when it is applied to contrasting scenarios in order to further illuminate the effects of human disturbance (Soares et al., 2017). Moreover, since flower visitation does not necessarily lead to conspecific pollen deposition (Ashman et al., 2020), the combination of DNA metabarcoding-based network analysis with measurements of pollination efficiency (e.g., pollen deposition, pollen tube growth, fruit, or seed set) (Stavert et al., 2020) could provide a comprehensive overview of the effects of human disturbance on such ecosystem interactions.

In this study, we combined the experimental advantage of an island model with the application of DNA metabarcoding to increase our understanding on how the fragmentation of green areas affects (i) the pollinators species richness and their community composition, (ii) the plant-pollinator interactions at the community and species level, considering the role of non-native plants, and (iii) the efficiency of the pollination service.

As the green areas fragmentation may shape the distribution and availability of flower resources (including those provided by invasive plants) (Hansen et al., 2018) to pollinators, we hypothesise that changes in pollinator community composition and decreases in pollinators species richness will occur in the insular context. Moreover, as small fragmented patches may host depauperated plant-pollinator networks with fewer links (Sabatino et al., 2010), we expect to observe a shift towards less complex plant-pollinator interactions with the increase of green habitat fragmentation, with possible consequences for the success of pollinator foraging and plant pollination.

2 | MATERIALS AND METHODS

2.1 | Study area and landscape characterization

The study was conducted on 11 islands of the Maldivian archipelago, located in two adjacent atolls, namely the southern part of Faafu and the northern part of Dhaalu atolls, about 150 km from the capital Malé (Figure 1). The temperature in these islands is stable throughout the year, with almost no seasonal variation and a mean of 28°C (Bailey et al., 2015). The vegetation is characterized by dense, unfragmented coastal forests, where association of *Scaevola taccada*, *Pemphis acidula*, and *Pandanus* spp. are typically found together with coconut palm coastal plantations. Differently, the inner land of inhabited islands is characterized by scattered coconut palms along with large trees such as *Ficus benghalensis*, *Artocarpus altilis*, and several cultivated fruit or ornamental species. Exotic plant species compose almost 60% of the vegetation of the islands (Sujanapal & Sankaran, 2016).

The islands investigated in this study were selected in order to cover a gradient of land-use intensification related to anthropic activities, intended as the proportion of buildings, such as housing or infrastructures devoted to human activities. The distance between islands range between 1110 m (between islands 8 and 9, Figure 1) and 12,000 m (between islands 6 and 10, Figure 1). They also differ in terms of inhabitants (0–1600 people ca, National Bureau of Statistics, 2014) that leads to a gradient of land-use and habitat fragmentation across islands (Figure S1). The fragmentation was estimated through the edge density index (i.e., green patches edge length divided by total area), specifically calculated for the inland green patches (i.e., gardens, parks and semi-natural green covered patches excluding the usually continuous coastal forest). To estimate edge density, index polygons enclosing the inland green patches have been manually drawn for each island using QGIS 3.16 and Bing Aerial base map updated to 2019. The edge density of inland green patches was calculated through the LecoS QGIS plugin (Jung, 2013). In the five largest islands (i.e., those with a major axis greater than 500 m) two sampling sites (three in one case) were selected. Overall, 17 sampling sites were included in the survey (Table S1).

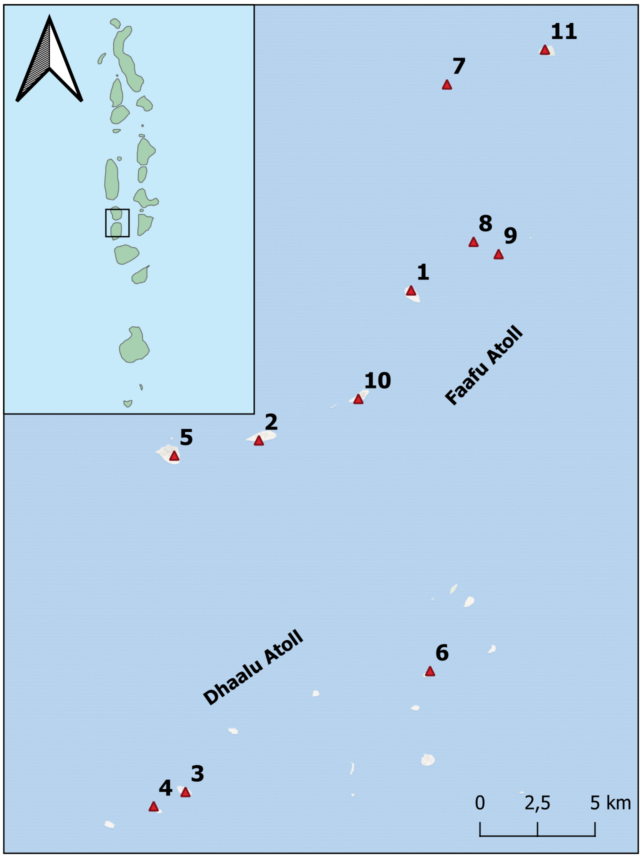

FIGURE 1 The studied 11 islands of the Faafu (north) and Dhaalu (south) atolls. Island full names and coordinates are provided in Table S1. The gradient of green area fragmentation is reported in Figure S1

2.2 | Pollinator insects and plant characterization

Sampling activities were performed in October 2019, from 9:00 AM to 16:00 PM only with good climate conditions. Pollinator insects were sampled by hand-collecting flower visitors through entomological nets along free transects in areas of continuous vegetation about 50 x 50 m at each sampling location. Pollinator insects were collected after being observed foraging on flowers for a few seconds and surveys were performed during a fixed time of 3 h to allow unbiased comparison between sampling sites (similar to Tommasi, Biella, et al., 2021). Specifically, Hymenoptera and Diptera (mainly Syrphidae) were targeted during sampling because of their well-known importance as pollinators and because these are major target in studies on pollinator monitoring (Biella, Tommasi, et al., 2022; Hennig & Ghazoul, 2012; Ssymank et al., 2008; Tommasi, Biella, et al., 2021). After collection, each insect was individually stored in a clean tube and filled with 70% ethanol. A clean entomological net was used in each sampling site to prevent cross contamination between samples from different sites.

Insect identification was performed through morphological inspection and via standard DNA barcoding (Table S2). Specifically, insects were first sorted at the lowest possible taxonomic level (family or genus) following morphological criteria and identification keys (e.g., Batra, 1977; Gupta, 2003; Thompson, 1981). Morphospecies grouping was subsequently confirmed by analysing 1–11 individuals representative for sex and atoll of provenance for each morphospecies through a standard DNA barcoding approach. The primer pair LCO1490-HCO2198 (Folmer et al., 1994) and the laboratory protocols described in Galimberti et al. (2021) were adopted. For doubtful identifications, where two or more candidate DNA barcode identifications were equally likely, a detailed morphological scrutiny was additionally performed by an expert (author PB). Flower species richness was also estimated by identifying all the flowering species observed during insect sampling following Sujanapal and Sankaran (2016). The flowering plant species were also categorized as native, exotic noninvasive and invasive following Sujanapal and Sankaran (2016) and Thomas (2011). Only those plants identified at the species level were assigned to these categories, otherwise they were excluded from subsequent analyses. Sampling localities and details are available in BOLD under the project code ZPLML (https://www.boldsystems.org/index.php/MAS_Management_DataConsole?codes=ZPLML) and are also reported in Table S1.

2.3 | Pollen DNA metabarcoding and plant-pollinator networks

The taxonomic composition of the pollen carried on insects' bodies was used to retrieve information about their interactions with plants, following a procedure similar to Tommasi, Biella, et al. (2021). Briefly, pollen was recovered from insects by vortexing the tubes containing insects and ethanol for at least 10 s. Insects were removed from the ethanol and tubes were centrifuged at 20,000 *g* for 10 min. Afterwards, the ethanol was removed through evaporation in a chemical hood. Pollen was grinded through a Tissue Lyser (Qiagen) after being frozen in liquid nitrogen, then DNA was extracted following DNeasy Plant mini kit (Qiagen) according to the manufacturer's protocol. Negative controls were also produced for each day of laboratory activity during the DNA isolation phase. Primers S2F and S3R (Chen et al., 2010), tailed with Illumina overhang sequence adapters were used to amplify the internal transcribed spacer 2 (ITS2) region. Illumina standard protocol was used for Library preparation and sequencing was performed using an Illumina Miseq 600 V3 (2 ×300-bp paired-end sequencing) technology. Raw sequencing reads were paired using QIIME2 (version 2019.4; https://qiime2.org/) (Bolyen et al., 2019). After primer trimming, removal of chimera and low-quality reads, a 0.97 clustering was performed, keeping only features between 200 and 500 bp (Torbjørn et al., 2016).

DNA reference sequences of the plant species identified at the study sites but not available in NCBI GenBank, were obtained by collecting leaf samples and sequencing the ITS2 region as described in Tommasi, Biella, et al. (2021) (details on the produced DNA sequences are available in Table S3). Reads taxonomic assignments were carried out using the BLAST algorithm (Camacho et al., 2009) on the NCBI nucleotide database and on the local database including the reference sequences we produced. Only assignments with a maximum identity and a query coverage ≥98% were accepted. Species occurrences were filtered by removing the implausible matches found both in negative controls and pollen samples (i.e., plants found not to be present in the study area), and by removing those represented by a reads count lower than the maximum number of reads matching plant species (i.e., 24 reads) produced by negative controls (Tommasi, Ferrari, et al., 2021).

The taxonomic identification of pollen samples was used to retrieve interactions between plants and pollinators. First, the interaction matrix between pollinator insects and plants obtained from DNA metabarcoding was used to calculate the network index of Connectance, through the R package Bipartite (Dormann et al., 2008). This, calculated as the number of actually observed interactions divided by the number of the possible interactions (Biella et al., 2017), provides an overall estimation of network specialization and complexity. Furthermore, to evaluate intraspecific changes in the foraging strategies in response to habitat fragmentation, we selected three pollinator species belonging to three different body size classes (estimated by measuring the intertegular [IT] distance of 30 individuals per species) and that were uniformly distributed across islands, namely *Braunsapis picitarsis* (small, mean IT 1.3 mm), *Lasioglossum albescens* (intermediate, mean IT 1.6 mm), and *Xylocopa fenestrata* (large, mean IT 7.1 mm). Specifically, the number of plant taxa found in pollen samples retrieved from each individual pollinator (individual "degree") was calculated.

To evaluate the role of native, invasive and exotic noninvasive plant species in plant-pollinator interactions at each site, all plants identified on pollinator bodies were categorized as explained above, then the number of links of each plant species was calculated as the number of insect samples where the plant pollen was detected (plant "degree"). The proportional degree for each plant species was obtained by dividing the number of realized links by the number of possible links, multiplied by a hundred.

2.4 | Pollination efficiency

Pollination efficiency was estimated by counting pollen tubes in pistils. This approach is commonly employed for this purpose since it provides a good proxy of conspecific pollen deposition (Akter et al., 2020; Biella, Akter, et al., 2019) and seed production (Alonso et al., 2012). Twenty pistils of the more abundant flowering species were collected at each sampling site, stored in a solution of EtOH and CH_2O (66.5/33.5/2 EtOH/H_2O/CH_2O v/v/v) and preserved at room temperature until further analysis. Once in laboratory, pistils were softened in 4 M NaOH and stained with 0.1% aniline blue in 0.1 M K2HPO4 for 12 h. After being washed with distilled water, pistils were mounted on slides with glycerine and observed with a fluorescence microscope. When pollen tubes were not visible, we counted the number of pollen grains on stigmas considering that only pollen with tubes still attached to the stigma should remain after sample preparation (Biella, Akter, et al., 2019) and that this number is expected to be correlated to the amount of germinated tubes (Stavert et al., 2020). Pollen tube counts were performed for those species uniformly distributed among sampling sites, specifically *Tridax procumbens* and *Wollastonia biflora*, both reported as exotic invasive species in the country, and the native *Scaevola taccada*.

2.5 | Statistical analysis

Changes in pollinator species richness were evaluated in response to green area fragmentation (i.e., the variable edge density) and flower richness (i.e., the number of flowering species per site). To do this, a generalized linear mixed model (GLMM) regression with Poisson distribution (that accounts for the assumptions of count data) was used, with island identity as a random effect. The flower richness was included as a predictor along with the edge density, since it could represent an important local driver of pollinator richness (Blüthgen & Klein, 2011). Variation in pollinator community composition in response to green area fragmentation and to the proportions of invasive and of exotic noninvasive plant species present in the field was evaluated with a redundancy analysis (RDA). Specifically, a binary matrix reporting the presence or absence of pollinator species at the sampling sites was used. The effects of the edge density of green areas, the proportion of invasive and of exotic noninvasive plant species in shaping pollinator community composition was evaluated using the Monte Carlo permutation test (999 permutations).

The edge density of green areas and flower richness were used along with the network size as predictors of change of the Connectance network index. Network size, calculated as the product between the number of insects and plants included in the networks for each site, was included to account for its effect on Connectance variation (as in Biella et al., 2020). In this case, a GLMM with beta distribution (commonly used for indices varying between 0 and 1) and island included as a random effect was used.

Changes in individual pollinator degree were evaluated in response to green area fragmentation and flower richness. The effects of these covariates were evaluated in interaction with the pollinator species identity to highlight differences among the considered pollinator species. A GLMM with Poisson distribution (that accounts for count data) was used, with sites nested in the island as a random effect.

To investigate the presence of non-native plants in the field and in the pollen in response to the green area fragmentation, the proportions of invasive and of exotic noninvasive plants present in the field at the sampling sites were investigated separately in response to the edge density. For the pollen data, proportions were calculated for each sample. GLMMs with binomial distribution (commonly used for proportional data) were used for testing the edge density of green areas. In the models with the field data, the island was included as a random effect, while the sites nested in the island were used as a random effect in the model with the pollen data. Moreover, to investigate if native, exotic noninvasive, and invasive species were equally visited by pollinators, a GLMM approach with gamma distribution (accounting for non-normal distribution with positive values) was used including plant proportional degree as response variable, the plant categories as predictors and the site nested within island as a random effect. A comparison among the proportional degree of the plant categories was performed through a post hoc test (Tukey's HSD test) with the multcomp package (Hothorn et al., 2008) for R (version 3.6.1; R Core Team, 2019).

Variation in the pollination efficiency for three selected plants was evaluated in response to the pollinator richness, and the Connectance. Moreover, the plant degree (number of pollinator species interacting with the selected plant species) was calculated from DNA metabarcoding data to estimate the plant generalism and included as model covariate. The role of these covariates was evaluated in interaction with the plant species identity, to highlight differences among the investigated plants. A GLMM with negative binomial distribution was used to account for overdispersion in count data. The site nested in the island was included as a random effect.

All the analyses were performed with R. Regressions were performed with glmmTMB R package (Magnusson et al., 2017). Predictor significance was evaluated through a log likelihood ratio test ($p < .05$). To exclude collinearity among variables, the variance inflation factor was calculated with the vif function of the car package (Fox & Weisberg, 2019), choosing an exclusion threshold of 3 (Zuur et al., 2007). In all cases, the final models were obtained by removing the variables that did not improve the model fit through backward stepwise regression based on second-order Akaike information criterion (AIC) (Zuur et al., 2009) calculated with the package MuMIn (Barton, 2009).

3 | RESULTS

3.1 | Pollinator and plant communities

The field survey yielded 333 pollinator insects belonging to 25 morphospecies. Specifically, 72.4% of the individuals were Anthophila bees, 12% wasps, 10.2% Syrphidae and 5.4% were Bombyliidae flies. These were identified with DNA barcoding (Table S2). The most represented and widely distributed taxa were bees, namely *Lasioglossum albescens* with 79 individuals, followed by *Braunsapis picitarsis* with 53 individuals and *Xylocopa fenestrata* with 42 individuals. Considering plants, 48 flowering species were observed in the sampling sites during the survey of flower species richness, with a minimum of three and maximum of 14 flowering species present at each site. Among these, 20.8% were categorized as exotic noninvasive and 31.3% as invasive. The most widespread species among sampling sites were *Tridax procumbens*, *Cyanthillium* sp., *Scaevola taccada*, and *Wollastonia biflora*. Details on the flowering plants observed in sampling sites are reported in Table S3. Green area fragmentation was not associated with an increase in the proportion of exotic noninvasive ($\chi_1^2 = 0.16$; $p = .6$) and invasive plant species ($\chi_1^2 = 1.08$; $p = .29$) present in the field at the sampling sites. Conversely, concerning the pollinator communities (Table 1), the edge density had a significant, positive effect in increasing pollinator species richness (Figure 2a), while no significant effects were found in response to flower species richness. Moreover, pollinator community composition varied in response to green area fragmentation (RDA, F = 2.3, $p = .008$), while the proportions of exotic noninvasive and invasive plant species present in the field were not determinant in shaping this feature (Figure 2b).

3.2 | Pollen DNA metabarcoding and plant-pollinator interactions

Pollen DNA metabarcoding yielded 10,357,652 reads (average 30,285.53 reads per sample) grouped in 3147 molecular features. After their taxonomic assignment and the exclusion of 23 plant taxa known to be absent in the study area, interactions between the collected insects and 112 plant taxa were found. Among these, 81 (72.3%) were assigned at species level, 28 (25%) at genus level, and three (2.7%) at family level. Plant taxa found in the overall pollen transported by insects ranged between nine and 58 across sites (mean 26.4 ±16.7), while the mean number of pollen plant taxa transported by each sampled insect was 3.5 ± 2.1. Among the plant taxa found in pollen, 16.9% belong to exotic noninvasive species, and 18.8% to invasive ones. The green area fragmentation was not significant in increasing for the amount of pollen from exotic noninvasive ($\chi^{1}_{2} = 0.79$; $p = .37$) or invasive species ($\chi^{1}_{2} = 0.03$; $p = .58$) carried by pollinator individuals.

In plant-pollinator networks constructed from the DNA metabarcoding data, the Connectance index resulted negatively associated with the edge density ($\chi_1^2 = 4.03$; $p = .04$) (Figure 3a) and network size ($\chi_1^2 = 7.95$; $p = .01$). However, the proportional degree of native, exotic noninvasive, and invasive plants differed ($\chi_1^2 = 45.2$; $p < .001$). Specifically, the proportional degree was higher for the invasive species when compared both with the exotics noninvasive (estimated difference = 0.91; $p < .001$) and the natives (estimated difference = −0.76; $p < .001$), with no significant difference when comparing the proportional degree of native and exotic noninvasive species (estimated difference = 0.14; $p = .62$) (Figure 3b). The individual pollinator degree resulted positively related to the edge density ($\chi_3^2 = 27.63$; $p < .001$) (Table 1). However, the responses of different widespread and most abundant insect species were not homogeneous in this case, as the values of this index significantly increased in the biggest bee *Xylocopa fenestrata* ($\beta_i = 14.9$; $p = .001$), while it was not significant in the case of the two smaller bees *Lasioglossum albescens* ($\beta_i = 2.94$; $p = .52$) and *Braunsapis picitarsis* ($\beta_i = -6.02$; $p = .24$) (Figure 3c).

3.3 | Pollination efficiency

Overall, 242 pistils were analysed, 96 for *Wollastonia biflora*, 77 for *Tridax procumbens*, and 69 for *Scaevola taccada*. Only the interaction between local pollinator richness and plant species identity resulted positively associated with the number of pollen tubes counted on stigmas ($\chi_3^2 = 40.37$; $p < .001$), with different trends among the investigated species as highlighted in Figure 4. Specifically, pollination efficiency increased in *Wollastonia biflora* ($\beta_i = 0.19$; $p = .001$), and *Scaevola taccada* ($\beta_i = 0.19$; $p = .002$), while it was constant in *Tridax procumbens* ($\beta_i = -0.02$; $p = .7$).

TABLE 1 Outputs of several generalized linear mixed models involving pollinator species richness, Connectance and individual pollinator degree

Response variable	Initial model covariates	Final model covariates	ΔAIC	B_i	χ^2; *df*	*p*-value
Pollinator richness	Edge density Flower richness	Edge density	2.49	16.5	11.01; 1	**.02**
Connectance	Edge density	Edge density	0	−15.5	4.03; 1	**.04**
	Flower richness	Flower richness		0.05	3.57; 1	.06
	Network size	Network size		−0.3	7.95; 1	**<.001**
Individual pollinator degree	Edge density × (pollinator species) Flower richness x (pollinator species)	Edge density × pollinator species	4.28	a = 14.9 b = 2.94 c = −6.02	27.63; 3	**<.001**

Note: Final models were selected through backward stepwise selection using AIC criterion. ΔAIC reports the difference in AIC values between full and final models. *p*-value stands for statistical significance after log likelihood ratio test, and is in bold if below .05. In the model of the individual pollinator degree, the βi for each of the three pollinator species considered in interaction with the predictors correspond to “a”: *Xylocopa fenestrata*, “b”: *Lasioglossum albescens*, and “c”: *Braunsapis picitarsis*.

Abbreviations: *df*, degrees of freedom; βi, regression coefficient of a given variable; χ^2, chi square values.

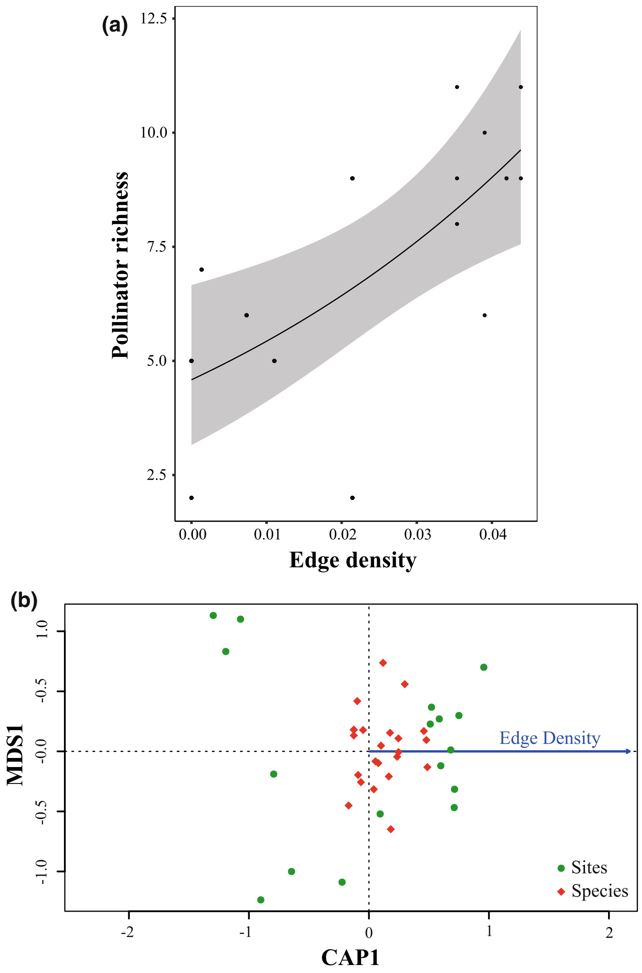

FIGURE 2 (a) Green area fragmentation (edge density) and pollinator species richness, the black line and grey areas indicate the estimated relationship and its confidence intervals from generalized linear mixed models. (b) RDA ordination analysis of pollinator communities in relation to the edge density of green areas, green dots represent sites and red squares the species; MDS1 indicates the unconstrained axis, CAP1 the constrained one

4 | DISCUSSION

In this study, a DNA metabarcoding approach to describe plant-pollinator interactions has been coupled with pollinator traits and deposited pollen data to disentangle the effects of green habitat fragmentation in an oceanic insular system. In this way, we described the direct effects of green areas fragmentation and non-native plants on several aspects, ranging from pollinator species richness and community composition, to their interactions with plants, and their pollination efficiency. Alongside, we provided a characterization of the flower-visiting insects (i.e., Hymenoptera and Diptera) of Maldives islands and the associated flora that to the best of our knowledge were largely unexplored in this biodiversity hotspot. Green areas fragmentation increased pollinator species richness and shaped community composition in the surveyed oceanic islands. In insular contexts, the effects of green areas fragmentation was little studied in the past. The increase in species richness did not agree with our initial hypotheses but it is consistent with the observations from other studies from the mainland at comparable spatial scales. For instance, Theodorou et al. (2020), found that bee richness increased with local edge density of green spaces both in urban and rural landscapes. A similar positive trend in species richness was observed elsewhere at levels of disturbance comparable to those observed in our study system (Kremen, 2005; Rader et al., 2014). However, it seems that other guilds, such as soil macroinvertebrates, have been found to decrease with fragmentation in oceanic islands (Steibl et al., 2021). Nevertheless, differences between pollinator and macroinvertebrate responses could be explained by the different natural histories of these groups. In accordance with our hypotheses, green area fragmentation also led to a significant variation in the pollinator community composition. This could be related to the previously mentioned increase of pollinator species richness and to the direct effect of habitat disturbance that promotes some pollinator insect functional traits while disadvantaging those species less tolerant to disturbance and landscape anthropic changes (Ayers & Rehan, 2021). Given the intermediate levels of edge density in the Maldives (range 0.00–0.04), compared to the mainland ones, our results could be explained by the intermediate disturbance hypothesis, according to which species diversity is expected to increase when the disturbance is of moderate entity (Huston, 2014; Rutgers-Kelly, 2005). This may occur as a trade-off between the competitive exclusion that characterizes the absence of disturbance and the abiotic limitation found in highly disturbed conditions (Lazarina et al., 2019; Lazaro et al., 2016). Moreover, an intermediate disturbance could potentially promote foraging and nesting heterogeneity of pollinator insects, thus permitting to host more species and guarantee their survival and reproduction (Wenzel et al., 2020; Winfree et al., 2009). Our interpretation is further supported because the highest conditions of fragmentation in our study system corresponded to a proportion of surfaces occupied by infrastructures of about 30% (see Figure S2), that still represents an intermediate degree of disturbance compared to more urbanized areas (Wenzel et al., 2020). At higher disturbances, the pollinator richness is supposed to decrease, as observed by Rader et al. (2014), and the community composition to change even more (Xiao et al., 2016).

Our study found that green area fragmentation clearly shapes plant-pollinator interactions at both the community and individual levels. We expected to find less complex plant-pollinator interactions with the increase of green areas fragmentation and we observed a decrease in Connectance, indicating a lowered proportion of realized interactions that highlights a simplification of plant-pollinator networks. As Connectance is often related to network complexity and stability (May, 1972), we can expect that an increasing fragmentation will lead to impoverished, more simplified networks. Simpler networks could have a low functional redundancy, an unfavourable property of ecosystems (Kaiser-Bunbury et al., 2017). Although in other insular contexts the invasive plants played a key role in shaping interactions (Kaiser-Bunbury et al., 2011; Kaiser-Bunbury et al., 2017; Padrón et al., 2009), we found that green area fragmentation did not

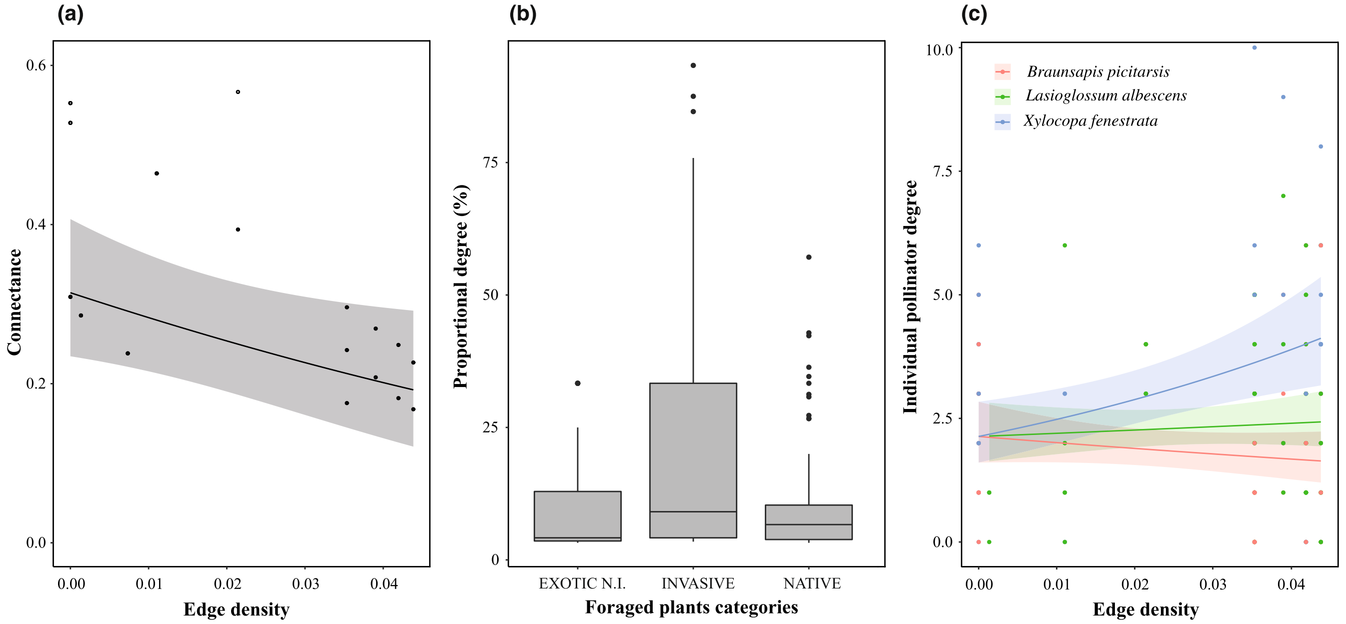

FIGURE 3 (a) Relationship between green area fragmentation (edge density) and the plant-pollinator network Connectance. The black line and grey areas indicate the estimated relationship and its confidence intervals from generalized linear mixed models. (b) Comparison of foraging preference of pollinator insects among exotic, exotic noninvasive ("exotic N.I.") and native plant species, estimated as the proportional degree calculated from pollen DNA metabarcoding data. (c) Individual pollinator degree, derived from the number of plant species carried on the pollinator (identified with pollen DNA metabarcoding) as a function of edge density for three pollinator species of different body size; The coloured lines and areas indicate the estimated relationship and the confidence intervals from generalized linear mixed models

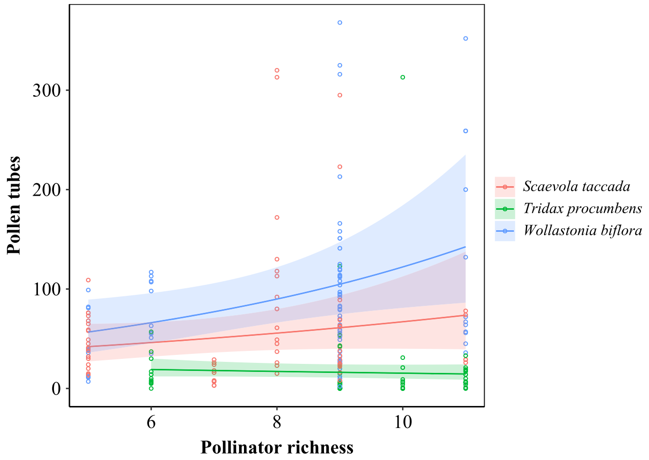

FIGURE 4 Number of pollen tubes as a function of environmental pollinator species richness. The coloured lines and areas indicate the estimated relationship and its confidence intervals with generalized linear mixed models, see Methods for further details

influence the richness of non-native plant species at the investigated sites. However, when comparing invasive and exotic non-invasive or native plants, the pollen of the former ones were more frequently found on pollinator bodies, thus indicating a higher potential for pollen dispersal than native plants. This finding is supported by other studies indicating invasive plants as a problem for island pollination systems (Kaiser-Bunbury et al., 2011). Interestingly, exotic noninvasive plants were as common in the pollen samples as the native ones, and they are probably not a threat for natural biodiversity conservation. In spite of the effects of fragmentation at the community level, idiosyncrasies emerged when considering the interactions of pollinators of three different body sizes. The largest one among these bees, *Xylocopa fenestrata*, was the only one that modified, and specifically increased, the number of foraged plant species in response to increased fragmentation, as indicated by DNA metabarcoding of pollen. Conversely, smaller species did not show diet expansions or contractions as number of foraged plant species. While a single large and two small species were investigated, the observed pattern seems to reflect a differential response depending on body size, since size is a functional trait related to flight performance (Greenleaf et al., 2007). In fact, small pollinators usually forage in smaller patches and potentially benefit from inhabiting small habitats (Tscharntke et al., 2002) and thus could not suffer from such fragmentation levels in oceanic islands, as opposed to larger species moving across fragments acquiring more flower resources as observed in *X. fenestrata*. Species responding in different ways to land-use change were already observed, according to their functional traits (Wenzel et al., 2020). Therefore, it is a priority to couple community trends and the responses of single species, as they are highly important to preserve community structure and functionality, for instance by establishing new interactions after a disturbance event (e.g., Biella et al., 2020).

The effects on the ecosystem service of pollination by green area fragmentation were evaluated by quantifying pollination efficiency in a panel of widely distributed plant species used here as sentinel cases. Specifically, the pollination efficiency was associated with pollinator species richness at the sites. This agrees with an increasing amount of evidence supporting a positive relationship between pollinator richness and plant reproduction (Fontaine et al., 2006; Garibaldi et al., 2013; Mallinger & Gratton, 2015; but see Biella, Akter, et al., 2021). Furthermore, pollinator species richness in an area may increase pollination efficiency through other mechanisms such as facilitation (i.e., the interactions between different species affect their foraging behaviours, enhancing the deposition of pollen), and temporal and/or spatial complementarity (Mallinger & Gratton, 2015). However, in our data set, the positive trend of pollination efficiency with environmental pollinator richness was independent from the plant degree (the amount of pollinators visiting a given plant), a measure of plant interaction generalism. This suggests that not all the plants respond in a similar way to pollinator availability (see Biella, Akter, et al., 2019). Overall, these results highlight the importance of safeguarding pollinator biodiversity for maintaining the equilibrium of pollination service.

5 | CONCLUSIONS

Integrating field observations, laboratory approaches and molecular tools for identifying species and interactions, this study provides interesting insights about the effects of green area fragmentation on pollinator insects and pollination service. The results obtained here are important for Maldives islands, and they could be easily transferred to the mainland contexts threatened by human activities to address proper mitigation solutions or predict the effects of land-use alterations.

In the Maldives, the rapid development of tourism and human settlement expansion represent the main drivers of change in the landscape composition and configuration (Fallati et al., 2017). Therefore, promoting suitable solutions to support biodiversity and ecological functioning is a priority. Indeed, we proved that a moderate green area fragmentation could even promote the biodiversity of pollinators, suggesting the need to assess tolerable disturbance thresholds in specific environmental contexts to develop local land-use planning aimed at promoting pollinator biodiversity. An additional element of concern is represented by invasive plants that seemed to be favoured by pollinators in terms of collected pollen. Therefore, a careful evaluation of how managing them could be useful for preventing further impact. Moreover, we claim for a higher mitigation of harmful land-uses and favouring pollinator-friendly interventions (e.g., promoting floral resources and availability of heterogeneous nesting sites). This would probably increase pollination success and thus improve the efficiency of the pollination service.

AUTHOR CONTRIBUTIONS

Conceptualization, Nicola Tommasi, Paolo Biella and Andrea Galimberti; Investigation, Nicola Tommasi, Paolo Biella, Davide Maggioni and Luca Fallati; Formal analysis, Nicola Tommasi, Paolo Biella and Giulia Agostinetto; Writing—original draft, Nicola Tommasi; Writing, review and editing, Nicola Tommasi, Paolo Biella, Andrea Galimberti, Davide Maggioni, Luca Fallati and Giulia Agostinetto; Funding acquisition, Paolo Galli and Andrea Galimberti.

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DATA AVAILABILITY STATEMENT

The DNA metabarcoding data generated for this study have been made available on EBI metagenomics portal (https://www.ebi.ac.uk/metagenomics/) study ID: PRJEB49045. Plants DNA barcoding sequences produced are available in GenBank under accession numbers OL672899-OL672949. Pollinator DNA barcode sequences produced are available in BOLD system under the project name “ZPLML Pollinator insects of Maldives”. All other data used in this article are available in figshare doi: 10.6084/m9.figshare.19755256. Benefits from this research accrue from the sharing of our data and results on public databases as described above.

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://www.ebi.ac.uk/metagenomics/.

BENEFIT-SHARING STATEMENT

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

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