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

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Abstract

Habitat fragmentation is known to affect biodiversity, but the impact on pollinators and their interactions with plants is still unclear in anthropized landscapes. Islands are open-air laboratories for ecological studies with simplified communities and interactions, suitable to disentangle how land-use alteration impacts pollination ecology and its ecosystem service. Here, we used Maldives islands as model systems to investigate how pollinator richness, their mutualistic interactions with plants, and pollination efficiency are shaped by the degree of green area fragmentation (i.e., gardens, parks and semi-natural green covered patches), by considering both community- and species-level responses. To do this, we surveyed pollinators from 11 islands showing a gradient of green area fragmentation. In order to characterize the interactions between plants and pollinators and obtain a novel and comprehensive view of the key ecological dynamics, a DNA metabarcoding approach was adopted to identify the pollen carried by pollinators. We found that green area fragmentation at intermediate levels played positive effects on pollinator richness. However, fragmentation decreased interaction network complexity. Intriguingly, body size mediated the effect of landscape alteration on plant-pollinator interactions, as only the largest bee species expanded the foraging breath in terms of transported pollen richness 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 the ecosystem service of pollination, where in spite of interactions being less complex and mediated by pollinator body size, pollinator biodiversity and potential plant reproduction are supported.

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 (Dicks et al., 2020; Potts et al., 2010, Tommasi et al., 2021 a). This is because landscape intensification leads to habitat loss and green areas fragmentation, especially in urban environments (Kovács-Hostyanszki et al., 2017; Rathcke & Jules, 1993; Senapathi et al., 2017). As a result, pollinator community composition is impoverished by a decreased diversity of species in fragmented landscapes, as specialist pollinators easily disappear (Xiao et al., 2016). In turn, plant-pollinator interactions are expected to become more generalised, possibly due to changes in floral composition and distribution (Andrieu et al., 2009; Fortuna, and Bascompte 2006; Matthews,Cottee-Jones & Whittaker 2014). Local conditions related to floral resources (*e.g.* , flower diversity and abundance) are important drivers of pollinator community features, and have previously been found to mitigate the negative impacts posed by land-use intensification both on community composition and interactions (Tommasi et al., 2021 a).

In landscapes intensively altered by human practices, green areas became 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 higher scales (*i.e.,* 200 or 1000 m radius), the fragmentation of green patches corresponded to increased pollinator species richness, flower visitation rates and pollination (Hennig & Ghazoul, 2012; Theodorou et al., 2020). This variability in responses to green habitat fragmentation highlights difficulties at forecasting how land-use intensification affects pollinator communities and the ecosystem service they provide. Furthermore, species can greatly diverge in their foraging strategies and contribute differently to pollination. Thus, the analysis of intraspecific variation in plant-pollinator interaction in fragmented habitats is necessary to comprehend the role of target species, and their changes in response to anthropic disturbance (Biella et al., 2019 b; Fuster & Traveset, 2020). Therefore, it is urgent to improve our comprehension of the effects of green habitat fragmentation on pollinators to suggest ways 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 (Castro-Urgal & Traveset, 2014; Kaiser-Bunbury & Bluthgen, 2015; Picanco et al., 2017; Steibl, Franke & Laforsch, 2021). Islands can be considered open air laboratories for ecological studies for several reasons. First, islands host simplified and isolated biotic communities, which allow to easily evaluate species roles in ecosystem functioning (Kaiser-Bunbury, Traveset & Hansen, 2010; Warren at al., 2015). Second, environmental changes spread earlier and more rapidly on islands than in the continent, also favored by small population sizes (Castro-Urgal & Traveset, 2014). These aspects apply also to pollinator and plant assemblages, which are usually simplified in insular ecosystems (Kaiser-Bunbury, Traveset & Hansen 2010; Traveset at al., 2016). An additional, 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 as green areas fragmentation have on plant-pollinator interactions (Kaiser-Bunbury & Bluthgen, 2015). Therefore, islands are suitable scenarios for investigating the effects of land-use intensification on pollinator foraging and thus on their interactions with plants, which further supports the adoption of this model system to solve ecological questions.

Many insular systems are peculiar and yet largely neglected, especially in light of ecological research on terrestrial biodiversity and interactions between taxa. This is the case of Maldives, in the Indian Ocean, where studies on terrestrial biodiversity are extremely rare (Steibl, Franke, & Laforsch, 2021). In addition, studies in insular systems could be biased by poor taxonomy and species distribution knowledge. 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 in pollination ecology research to achieve the goal of describing plant-pollinator interactions (Bell et al., 2017; Pornon et al., 2016; Tommasi et al., 2021 a). By foraging on flowers pollinators carry pollen grains that keep trace of their foraging activity (Bosch, Martin Gonzalez, Rodrigo, & Navarro 2009). Standard DNA barcode loci can be used to characterize this pollen and understand which plants were visited (Tommasi et al., 2021 b). In this way, it is possible to reconstruct the interaction networks among plants and their pollinators, as well as to better assess the resource use preferences shown by flower visitors (Biella et al., 2019 a). 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, Ferreira, & Lopes, 2017). This molecular information can be easily translated into network indices permitting reliable comparisons. 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, and seed set) (Stavert, Bailey, Kirkland, & Rader, 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 habitats (*e.g.* green patches or parks in urbanized conditions) affects pollinator diversity, their mutualistic interaction with plants, and the resulting efficiency of the pollination

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service. To do so, we investigated pollinator communities in the Maldives islands, an insular context largely neglected under a pollination ecology perspective (but see Kevan, 1993). There, islands are homogeneous in terms of composition of biotic communities and geographical conditions, while varying in the degree of human exploitation and impact (Fallati, Savini, Sterlacchini, & Galli, 2017). This context results in a gradient of green area fragmentation and provides a model condition that ensures better understanding and interpretation of the impact of this fragmented landscape on pollinators, allowing knowledge transfer to other geographical contexts of landscape alteration.

Standing at the need to improve the comprehension of the effects of green habitat fragmentation on pollinator communities, here we aimed at evaluating how this phenomenon affect the ecosystem service of pollination in tropical islands by investigating several aspects: i) the pollinator species richness, ii) the plant-pollinator interactions, considering both community and intraspecific variations, and iii) the pollination efficiency.

2. MATERIALS AND METHODS

2.1 Study area and landscape characterization

The study was conducted on 11 islands of the Maldivian archipelago (a number of islands comparable to those investigated by Steibl, Franke & Laforsch, 2021), located in two adjacent atolls, namely the southern part of Faafu and the northern part of Dhaalu atolls, about 150 km from the capital Male (Fig. 1). The temperature in these islands is homogeneous throughout the year, with almost no seasonal variation and a mean of 28degC (Bailey, Khalil, & Chatikavanij, 2015). The natural 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 investigated islands were selected in order to cover a gradient of land-use intensification related to anthropic activities, intended as the proportion of buildings, such as houses or infrastructures devoted to human activities. The distances among adjacent islands range between 1110 m (between islands 8 and 9, Fig 1) and 12000 m (between islands 6 and 10, Fig 1). They also differ in terms of inhabitants (0-1600 people ca, Maldives population & housing census, 2014) that leads to a gradient of land-use and habitat fragmentation across islands (Supporting Information, Fig S1). 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 biggest islands (*i.e.* , those with a major axis greater than 500 m) two sampling sites were selected. Overall, 17 sampling sites were included in the survey (Supporting information -Table S1).

[Fig.1]

2.2 Pollinator insects and plant characterization

Sampling activities were performed in October 2019, from 9:00 to 16:00 only with good climate conditions. Pollinator insects were sampled by hand-collecting flower visitors through entomological nets along free transects around and crossing over an area of continuous vegetation about 50 m x 50 m at each sampling location. Pollinator insects were captured after being observed foraging on flowers for a few seconds and captures were performed during a fixed time of three hours to allow unbiased comparison between sampling sites (similarly to Tommasi et al., 2021 a). 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.

Insects identification was performed through morphological inspection and via standard DNA barcoding

(Supporting information - Table S2). Specifically, insects were first sorted at the lowest possible taxonomic level (family or genus) following morphological criteria and identification keys (*e.g.*, Bratra, 1977; Gupta, 2003; Thompson, 1981). Morpho-species grouping was subsequently confirmed by analysing representative individuals (1- 11 individuals representative for sex and atoll of provenance) for each morpho-species through a standard DNA barcoding approach using primers LCO1490 and HCO2198 (Folmer et al., 1994) and the laboratory protocols described in Galimberti et al., 2021. For doubtful identifications where two or more candidate DNA barcode identification were equally likely, a detailed morphological scrutiny was additionally performed by an expert (author PB). Flower species richness was also estimated identifying all the flowering species observed in the whole area involved in insect sampling and plants were identified in the field or in the lab following Sujanapal & Sankaran (2016). 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 Supporting information - 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 et al (2021 a). 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 14000 rpm for 10 minutes. Afterwards, the ethanol was removed through evaporation in a chemical hood. Pollen was grinded through a Tissue Lyser (r) (Qiagen) after being frozen in liquid nitrogen, then DNA was extracted following DNeasy Plant mini kit (r) (Qiagen) according to 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) were used with the addiction of the Illumina overhang sequence adapters to amplify the internal transcribed spacer 2 (ITS2) region. Illumina standard protocol was used for Library preparation and sequencing was performed through Illumina Miseq 600 V3 (2 x 300-bp paired-end sequencing). Raw sequencing reads were paired using QIIME2 (ver. 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 500bp (Torbjorn 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 et al. (2021 a) (details on the produced DNA sequences are available in Supporting information, 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 max identity and a query coverage [?] 98% were accepted. Species occurrences were filtered by removing implausible matches (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 (i.e 24 reads) produced by negative controls (Tommasi et al., 2021 b). 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, Gruber, & Frund, 2008). This, calculated as the number of actually observed interactions divided by the number of the possible interactions (Biella, Ollerton, Barcella & Assini, 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 measuring inter tegular (IT) distance of thirty individuals per species) and that were uniformly distributed across islands: *Braunsapis picitarsis* . (small, mean IT 1.3 mm), *Lasioglossum albescens* (intermediate, mean 1.6 mm), and *Xylocopa fenestrata* (large, mean 7.1 mm). Specifically, the number of plant taxa found in pollen samples retrieved from each individual pollinator (individual degree) was calculated.

2.4 Pollination efficiency

Pollination efficiency was estimated by counting pollen tubes on pistil. This approach is commonly employed

for this purpose since it provides a good proxy of conspecific pollen deposition (Biella et al., 2019 b, Akter, Biella, Batary, & Klečka, 2020) and seed production (Alonso, Herrera, & Ashman, 2012). Twenty pistils of the more abundant flowering species were collected at each sampling site, stored in a solution of EtOH and CH$_2$O (66.5/33.5/2 EtOH/H$_2$O/CH$_2$O 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 hours. 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 et al., 2019 b) and that this number is expected to be correlated to the amount of germinated tubes (Stavert, Bailey, Kirkland, & Rader 2020). Pollen tube counts were performed for those species uniformly distributed among sampling sites, specifically *Tridax procumbens, Wollastonia biflora* , and *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 Generalised linear mixed model (GLMM) regression (glmmTMB R package; Magnusson et al., 2017) with Poisson distribution was used, with island included 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). The same variables (edge density, 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 also included as a predictor in the model to account for its effect on Connectance variation (as in Biella et al., 2020). In this case, a GLMM with beta distribution 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 was used, with sites nested in the island as a random effect. Variation in the pollination efficiency was evaluated using the pollinator richness and the Connectance as covariates. Moreover, the plant degree (mean number of pollinator species interacting with each plant species considered in pollination efficiency analysis) was calculated from DNA metabarcoding data to estimate the mean 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. Also in this case, the site nested in island was included as a random effect.

All the analyses were performed with R (version 3.6.1; R CoreTeam 2019). Predictor significance was evaluated through a log likelihood ratio test (P < 0.05). The Vif function of the car package (*i.e.* , Variance Inflation Factor with an exclusion threshold of 3 (Zuur, Ieno, & Smith, 2007)) was used to exclude collinearity among variables. 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.

3. RESULTS

3.1 Pollinator and plant communities

The field survey yielded 333 pollinator insects belonging to 25 morpho-species. 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, in Supporting information, Table S2 - List of collected pollinator species and DNA barcode sequence. The most represented and widely distributed taxa were *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 3 and maximum of 14 flowering species present in each

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site. 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 Supporting information, Table S3. Concerning the effects of habitat fragmentation on pollinator communities (Table 1), the edge density had a significant, positive effect in increasing pollinator species richness (Fig 2 a), while no significant effects were found in response to flower species richness.

3.2 Pollen DNA metabarcoding and plant-pollinator interactions

Pollen DNA metabarcoding yielded interactions between the collected insects and 112 plant taxa. Among these, 81 (72.3%) were assigned at species level, 28 (25%) at genus level, and 3 (2.7%) at family level. Plant taxa found in the overall pollen transported by insects on each island ranged between 9 and 58 (mean 26.4 ± 16.7), while the mean number of pollen plant taxa transported by each sampled insect was 3.5 ± 2.1. In plant-pollinator networks constructed from the DNA metabarcoding data, the Connectance index resulted significantly and negatively associated with the Edge density (Fig 2 b) and network size (Table 1). Individual pollinator degree resulted significantly, positively related to the Edge density. However, the responses of different insect species among the widespread and most abundant ones were not homogeneous in this case, as the values of this index significantly increased in the biggest bee *Xylocopa fenestrata* (β_i = 14.9 ; p = 0.001), while it was not significant in the case of the two smaller bees: *Lasioglossum albescens* (β_i = 2.94 ; p = 0.52) and *Braunsapis picitarsis(* β_i = -6.02; p = 0.24) (Fig 3).

[Fig. 2]

[Fig. 3]

[Table 1]

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 significantly and positively associated with the number of pollen tubes counted on stigmas (χ_3^2 = 40.37; p < 0.001), with different trends among the investigated species as highlighted in Fig 4. Specifically, pollination efficiency increased in*Wollastonia biflora (* β_i= 0.19; p= 0.001)*,*and *Scaevola taccada (* β_i= 0.19 ; p= 0.002)*,* while it was constant in *Tridax procumbens (* β_i= -0.02; p= 0.7*)*.

[Fig. 4]

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 habitat fragmentation in an oceanic insular system. In this way, we described the direct effects of green areas fragmentation at several levels, ranging from pollinator species richness to their interactions with plants and the 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. This result is consistent with the observations from other studies at comparable spatial scales. For instance, Theodorou et al. (2020), found that bee richness increased with 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 in our study (Kremen, 2005; Rader, Bartomeus, Tylianakis & Laliberté, 2014). However, it seems that other guilds, such as soils macroinvertebrates, have been found to decrease with fragmentation in oceanic islands (Steibl, Franke & Laforsch, 2021). Nevertheless, differences between pollinator and macroinvertebrate responses could be searched in different natural histories of these groups. Given the intermediate levels of Edge density on Maldives Islands (range 0.00 - 0.04) compared to continental levels, it seems appropriate to explain our results to 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

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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, Grass, Belavadi, & Tscharntke, 2020; Winfree et al., 2009). Our interpretation is further supported because the maximum fragmentation in our study system corresponded to a proportion of surfaces occupied by infrastructures of about thirty percent (see Supporting Information, Fig S2), that still represents an intermediate degree of disturbance (Wenzel, Grass, Belavadi, & Tscharntke, 2020). At higher disturbances, the pollinator richness is supposed to decrease, as observed by Rader et al. (2014).

Our study found that green area fragmentation clearly shapes plant-pollinator interactions both at the community and individual levels. 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), it is likely to interpret that increasing fragmentation will lead to impoverished, more simplified networks. We understand this as an alarming aspect as a high complexity could mean high functional redundancy and it is a desirable property of functioning ecosystems (Kaiser-Bunbury et al., 2017). 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 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 expansions or contractions of the number of foraged plant species. Differential responses depending on body size offer the key to interpret the effects of fragmentation, since size is a functional trait related to flight range (Greenleaf, Williams, Winfree, & Kremen, 2007).In fact, small pollinators usually forage in smaller patches and might even benefit from having small habitat fragments (Tscharntke, Steffan-Dewenter, Kruess & Thies, 2002) and they likely did not suffer from the level of fragmentation in oceanic islands. Differently, a larger species flying across fragments could acquire more flower resources and this is what we observed in *X. fenestrata* . Species responding in different ways to land-use change were already observed, according to their functional traits (Wenzel, Grass, Belavadi, & Tscharntke, 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, pollination efficiency associated with pollinator species richness at the sites, also related to green area fragmentation. This agrees with an increasing amount of evidence supporting a positive relationship between pollinator richness and plant reproduction (Fontaine, Dajoz, Meriguet. & Loreau, 2006; Garibaldi, Steffan-Dewenter & Winfree, 2013; Mallinger & Gratton, 2015; but see Biella et al., 2021). Furthermore, pollinator species richness in an area may increase pollination efficiency through other mechanisms such as facilitation (the interactions between different species affect their foraging behaviours enhancing the pollen deposition), and temporal and/or spatial complementarity (Mallinger & Gratton, 2015). However, in our dataset, the positive trend of pollination efficiency with environmental pollinator richness was independent to the amount of pollinator species visiting a given plant, a measure of plant interaction generalism. This suggests that not all plants respond in a similar way to pollinator availability (see Biella et al., 2019 b). Overall, these results highlight the importance of safeguarding pollinator biodiversity for maintaining the equilibrium of pollination service.

CONCLUSIONS

This study represents a valuable case of integrating field observations, laboratory approaches and molecular tools for species and interaction identification. An additional relevance is given by the study system, which comprises small oceanic islands offering unique conditions to investigate direct ecological issues in isolated conditions with limited dispersal of individuals elsewhere. Moreover, the presence of usually simplified island communities makes it easier to quantify effects and causes of land-uses on indigenous biodiversity (Picanço et al., 2017; Jupiter, Mangubhai & Kingsford 2014). Overall, these features make small oceanic islands efficient

model systems to comprehend the multi-level impacts of green area fragmentation. These could offer the possibility to transfer the obtained results to mainland contexts threatened by human activities and then to address proper mitigation solutions or to 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, Savini, Sterlacchini, & Galli, 2017). These changes highlight the need to find and promote suitable solutions to support biodiversity and ecological functioning. 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. Moreover, we stress 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 increase pollination success and thus improve the efficiency of the pollination service. Furthermore, as species-specific responses often take place in spite of community-averaged trends, the heterogeneity of pollinator life histories should be considered when choosing how to mitigate the effect of landscape alteration. In this view, the implementation of modern molecular tools such as DNA metabarcoding in ecological studies is of growing interest in the study of mutualistic interactions. As demonstrated by this study, it provides suitable information that can be easily integrated with field data to improve the efficiency of monitoring programs even in those contexts largely neglected by ecological research.

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DATA ACCESSIBILITY

The DNA metabarcoding data generated for this study are 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 code ZPLML *[ACCESSION NUMBER OF INSECTS SEQUENCES WILL BE RELEASED UPON PAPER ACCEPTANCE]*.

BENEFIT-SHARING

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

Author Contributions

Conceptualization, NT, PB and AG ; Investigation,NT, PB, DM, LF; Formal Analysis, NT, PB,GA; Writing - Original Draft, NT; Writing - Review and Editing, NT, PB, AG,DM, LF, AG, GA ; Funding acquisition,PG, AG.

Tables and Figures

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Fig1: Localization of the 11 islands of the Faafu (North) and Dhaalu (South) atolls. Island full names and coordinates are provided in Supporting information, Table S1. The gradient of green habitat fragmentation is reported in Supporting Information, Fig S1. This map was created using QGIS version 3.16.10 (https://www.qgis.org/it/site/).
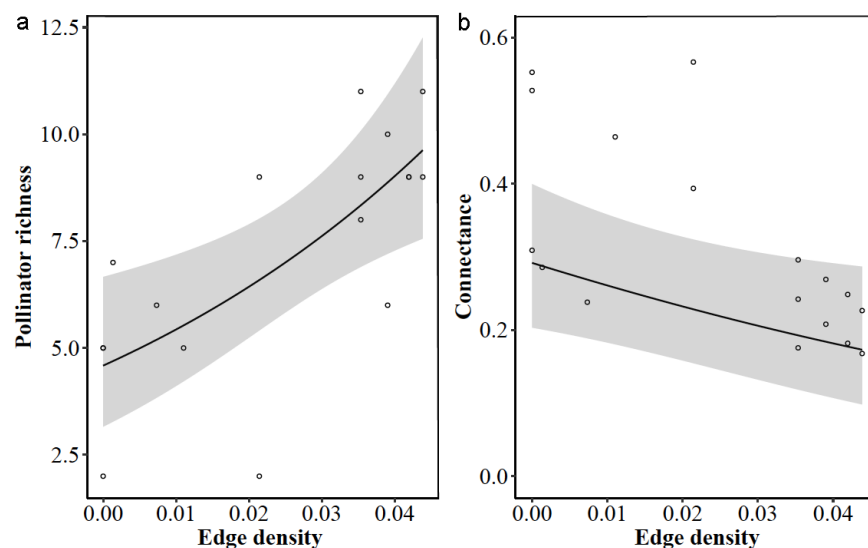
Fig 2: Relationship between habitat fragmentation represented by the Edge density of green areas and (a) pollinator species richness, and (b) network Connectance. The black line and grey areas indicate the estimated relationship and its confidence intervals from Generalised linear mixed models.
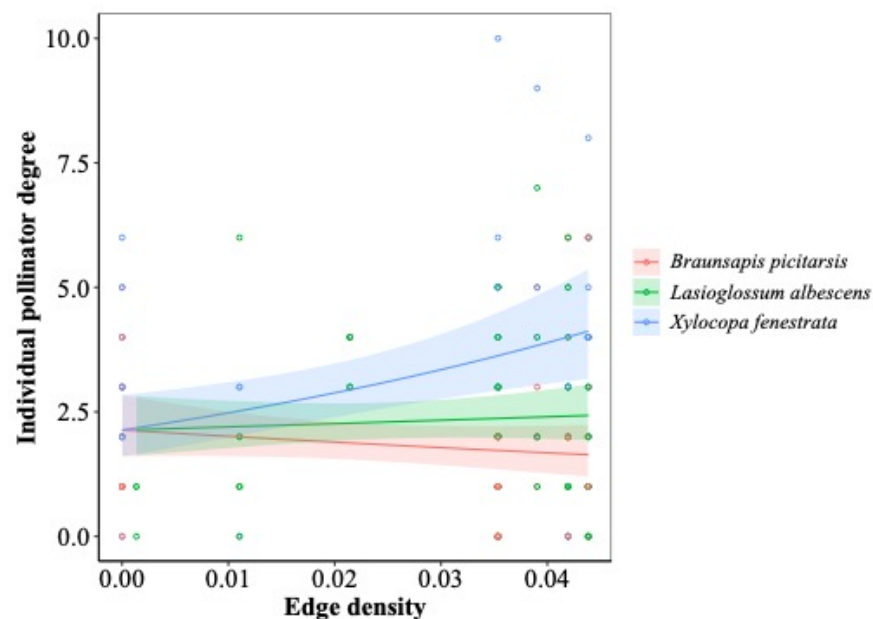

Fig 3: Individual pollinator degree, from the number of pollen species carried on the pollinator body, as a function of Edge density, a measure of habitat fragmentation, for three pollinator species of different body size. The coloured lines and areas respectively indicate the relationship and its confidence intervals as estimated with Generalised linear mixed models.
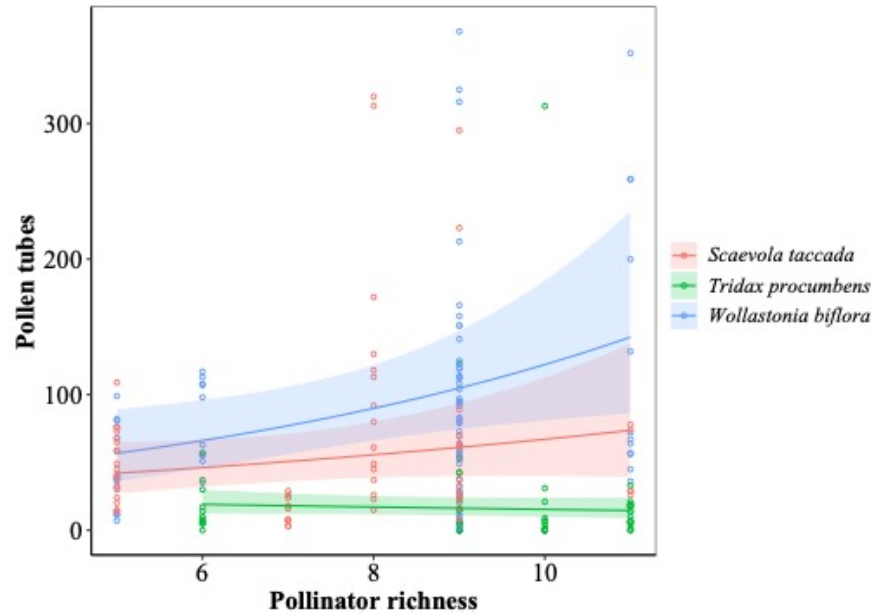
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Fig 4: Regression analysis of the number of pollen tubes on stigmas as a function of environmental pollinator species richness. The coloured lines and areas respectively indicate the estimated relationship and its confidence intervals with Generalised linear mixed models, see methods for further details.

Table 1: Output of different Generalised linear mixed models of pollinator species richness, Connectance, individual pollinator degree. Final models were selected through backward stepwise selection using AIC criterion. ΔAIC reports the difference in AIC values between full and final models. βi: regression coefficient of a given variable; χ2: chi square values; df: degrees of freedom. P value stands for statistical significance after log likelihood ratio test, and it is in bold if below 0.05. The regression coefficients for each of the three pollinator species considered in interaction with the predictors in the individual pollinator degree model correspond to a - *Xylocopa fenestrata*, b - *Lasioglossum albescens* , and c - *Braunsapis picitarsis*.

Response variable	Initial model covariates	Final model covariates	ΔAIC	B_i	X2; df	p value
Pollinator richness	Edge density	Edge density	2.49	16.5	11.01; 1	**0.02**
	Flower richness					
Connectance	Edge density	Edge density	0	-15.5	4.03; 1	**0.04**
	Flower richness	Flower richness		0.05	3.57; 1	0.06
	Network size	Network size		-0.3	7.95; 1	**<0.001**
Individual pollinator degree	Edge density x (pollinator species)	Edge density x pollinator species	4.28	a = 14.9 b = 2.94 c = -6.02	27.63; 3	**<0.001**
	Flower richness x (pollinator species)					

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