



ECOSYSTEMS

Tree cover and palm population structure determine patterns of palm-pollinator interaction networks in a grassland-forest ecotone

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Abstract: Pollen dispersal by insects determines the patterns of reproductive encounters between plants with flowers that have spatially or temporally segregated sexes. Pollinators exhibit varied responses to environmental gradients, such as those in grassland-forest ecotones. Individual-based interaction networks are useful yet underexplored tools to understand how interactions vary across these gradients. To test how a grassland-forest ecotone gradient affects these interactions, we studied pollination networks of *Butia odorata* individuals, a key palm tree species in a threatened South American grassland ecosystem. We assessed, through Structural Equation Models (SEMs), how network metrics (specialization and modularity) and pollinator richness and abundance (total, peripheral, core) respond to gradients of habitat and population structure in the grassland-forest ecotone. Networks with more spatially isolated palms showed greater specialization and modularity. Pollinator richness was dependent on the habitat context and pollinator role. The peripheral pollinators were negatively affected by palm density. The core pollinators were positively affected by tree cover, which, in turn, was positively associated with palm density and proximity to the forest. Our results suggest that palm population density can modulate the presence of peripheral pollinators, while increased tree cover in the grassland matrix can promote pollinator diversity by decreasing the dominance of core species.

Key words: *Butia odorata*, modularity, Pampa, plant-pollinator interaction, pollen dispersal, specialization.

INTRODUCTION

Insect pollination is essential to gene flow and genetic diversity structuring of plant populations (Butcher et al. 2020). Pollen dispersal depends on foraging patterns of pollinators and determines reproductive encounters between cross-pollinating plants (Mitchell et al. 2009). Environmental gradients affect the structure, dynamics, and functionality of ecological interaction networks by altering local species composition, their interaction frequencies/efficiency, or the spatial and temporal overlap

of partners (Tylianakis & Morris 2017). Pollinator diversity responses to environmental gradients have recognized effects on richness and abundance that vary among functional groups (e.g., dominant or rare species; Dáttilo et al. 2015, Arroyo-Correa et al. 2021). Understanding how environmental gradients can influence patterns of pollinators diversity and floral visitation is important to predict possible impacts on the genetic structure of plant populations (by gene flow) and plant-pollinator networks topology, that imply in ecological and evolutionary

consequences in response to selective pressures (e.g., climate change; Tylianakis & Morris 2017, Butcher et al. 2020).

The responses of animal-plant interactions to latitudinal and altitudinal environmental gradients (Adedaja et al. 2018, López-Segoviano et al. 2021, Wang et al. 2024), or habitat fragmentation (Dáttilo et al. 2015) are well established in the literature. However, the responses of these interactions to highly dynamic gradients, such as directional ecotones (Myster 2012), still require more comprehensive investigations. In this context, subtropical grassland-forest ecotones deserve attention as natural experiments, given the tendency of forest expansion over grasslands under present climatic conditions and land use (Chaneton et al. 2012, Anadón et al. 2014). Despite being a natural process, it may be accelerated by anthropogenic activities, threatening the unique grassland biodiversity (Calambás-Trochez et al. 2021), ecosystem functions, and traditional uses (Anadón et al. 2014). In the southern hemisphere, despite numerous studies dedicated to understanding the grassland-forest dynamics (mostly focused on plants; Chaneton et al. 2012, Müller et al. 2012), few have investigated the impact of these environmental changes on invertebrates, especially pollinators (but see Lara-Romero et al. 2016 for northern hemisphere data). Filling this knowledge gap is necessary to establish a solid theoretical baseline to guide conservation policies focused on grassland ecosystems (Potts et al. 2016, IPBES 2019).

Current approaches of ecological interaction networks focused on the community level have provided advances in the understanding of interactions in different environmental contexts and biologic systems (Bascompte & Jordano 2007, Tylianakis & Morris 2017, Guimarães 2020). However, “species-species” networks are generalizations of interactions observed at the

individual level (Dupont et al. 2014, Tur et al. 2014, Guimarães 2020), the basic units of interactions. In mutualism, individual interactions must translate into fitness, which is reflected in demographic and evolutionary patterns (Guimarães 2020) and may reveal emergent properties undetectable in species-level networks (Tonos et al. 2021), related to individual traits (Bolnick et al. 2003) and response to local environmental variations (Dupont et al. 2014). Individual-based network approaches have been originally developed and widely applied to systems of antagonistic interactions (Pires et al. 2011, Tur et al. 2014, Guimarães 2020). However, they remain little explored for mutualisms such as pollination, especially regarding plants with spatially or temporally segregated sexes (Mitchell et al. 2009, Arceo-Gómez et al. 2020, but see Arroyo-Correa et al. 2021).

In plants with reproductive segregation, therefore, with dependence on cross-pollination, certain network metrics directly describe the sharing of pollinators (upper trophic level) among plant individuals (lower trophic level), revealing patterns that determine fitness and the genetic structuring of these plants populations (Olesen et al. 2007, Dupont et al. 2014, Tur et al. 2014, Guimarães 2020). For example, specialization at the network level (H_2') describes ‘complementary specialization or selectiveness’ of nodes (i.e. representation of the biological unit in the network, linked to other nodes by “links” that represent their interactions) between trophic levels, so that the more selective the nodes, the higher the network specialization (Blüthgen et al. 2006). This indicates conditions that may be shaping different niche partitioning scenarios (Bolnick et al. 2003, Tur et al. 2014), which in pollination networks can indicate how plant individuals share the pool of available pollinators (Bolnick et al. 2003). Similarly, modularity describes

whether and to which extent interactions are organized into groups (modules) of partners that interact more frequently with each other than with other partners (Olesen et al. 2007). Different conditions (e.g., habitat heterogeneity and partner composition) among networks within the same system may lead to varying module configurations, indicating context-dependent preferred interactions between pollinators and plant individuals (Olesen et al. 2007, Fortuna et al. 2008, Dupont et al. 2014).

Butia is a genus of Neotropical palms (Arecaceae) with 24 species (Soares 2015, Rivas et al. 2023), many of which are extinction-threatened (Rio Grande do Sul 2014, Brasil 2022, IUCN 2023). *Butia* palms produce unisexual flowers, both sexes occurring in the same inflorescence with protandrous anthesis, thus depending on cross-pollination for reproduction (Fonseca 2014). In the Rio de la Plata grasslands (southern Brazil, Uruguay, and Argentina; Soriano et al. 1992), these palms shape ecosystems known as *Butia* palm groves (locally called “butiazais” or “palmares”), with an emergent stratum dominated by palms and an underlying grassland stratum (Sosinski et al. 2019, Rivas et al. 2023). *Butia* palm groves that occur in the Uruguay River basin and the coastal plains of Rio Grande do Sul, Brazil, are often found in ecotones with riparian forests that are expanding over grasslands due to current disturbance regimes alteration and climatic conditions (Chaneton et al. 2012, Sosinski et al. 2019, Salgado et al. 2021). Additionally, the population structure of these palm trees comprises different levels of density (individuals per hectare; Chaneton et al. 2012, Sosinski et al. 2015, Rivas et al. 2023) and isolation (distance between individuals), producing a gradient of woody species over a grassland matrix (Chaneton et al. 2012, Sosinski et al. 2015).

This gradient exposes pollinators to different contexts of habitat structure and complexity (Leibold & Miller 2004), which can represent environmental filters for their permanence (i.e., species-sorting) or limiting conditions for their dispersion (matrix permeability, i.e., patch-dynamics; Leibold & Miller 2004). Therefore, one should expect that these conditions or filters should determine which pollinators have access to flowering individuals along a gradient of populational density and individual isolation (Leibold & Miller 2004, Thompson & Gonzalez 2017), affecting the structure of the pollination networks of *Butia* palms (Fortuna et al. 2008, Dupont et al. 2014). On the other hand, the proximity/presence of forests or shrubs tends to favor pollinator-plant interactions in open ecosystems (Pinto et al. 2020, Arroyo-Correa et al. 2021), following the pattern expected by ecotone theory (Risser 1995).

In this work, we evaluate how interaction networks of palm tree individuals with male flowers and their pollinator species respond to a structural gradient of habitat (described by distance from the forest edge and tree cover) and population (density and isolation between conspecific palm trees) in a grassland-forest ecotone. Specifically, we evaluate how these gradients influence pollinator diversity, network specialization, and network modularity. We hypothesize that i) networks in transitional areas, i.e., closer to the forest and with increased tree cover, will present increased pollinator species diversity in comparison with networks with less trees and further away from the forest (Risser 1995, Pinto et al. 2020, Arroyo-Correa et al. 2021), ii) networks in areas with low palm population density and greater isolation between palms will present lower pollinator species diversity in comparison with networks in areas with denser population, due to the influence on pollinators displacement (Leibold & Miller 2004, Thompson

& Gonzalez 2017), and iii) networks in more complex habitat conditions, i.e., with more forest elements and denser palm population, will be less specialized and modular in comparison with networks in less complex habitats, with fewer palm trees (Bolnick et al. 2003, Fortuna et al. 2008).

MATERIALS AND METHODS

Study site

We conducted this work in a *Butia odorata* palm grove of ca. 170 ha, in the municipality of Tapes, Rio Grande do Sul, Brazil (30°31'47"S/ 51°21'50"W; Fig. 1a). The study region has a subtropical humid climate (Köppen's Cfa), with an average temperature in the hottest months reaching

26°C (Ramos et al. 2007). The study area is part of a private farm, where the native herbaceous stratum is used as a forage source for free-range livestock breeding that combines conservation of natural landscapes and income generation (Sosinski et al. 2015).

Butia odorata and the palm grove

Butia odorata (Arecaceae) occurs mainly from the coastal plains of Rio Grande do Sul, Brazil, to southeastern Uruguay (Soares 2015). This species makes up the largest remnants of *Butia* palm groves known in Brazil, with those from the study region among them (Fig. 1b). This palm tree is included in the regional redlist (Category "endangered" (EN), Criteria "A4cd"; IUCN 2012, Rio Grande do Sul 2014). Palm groves in the study

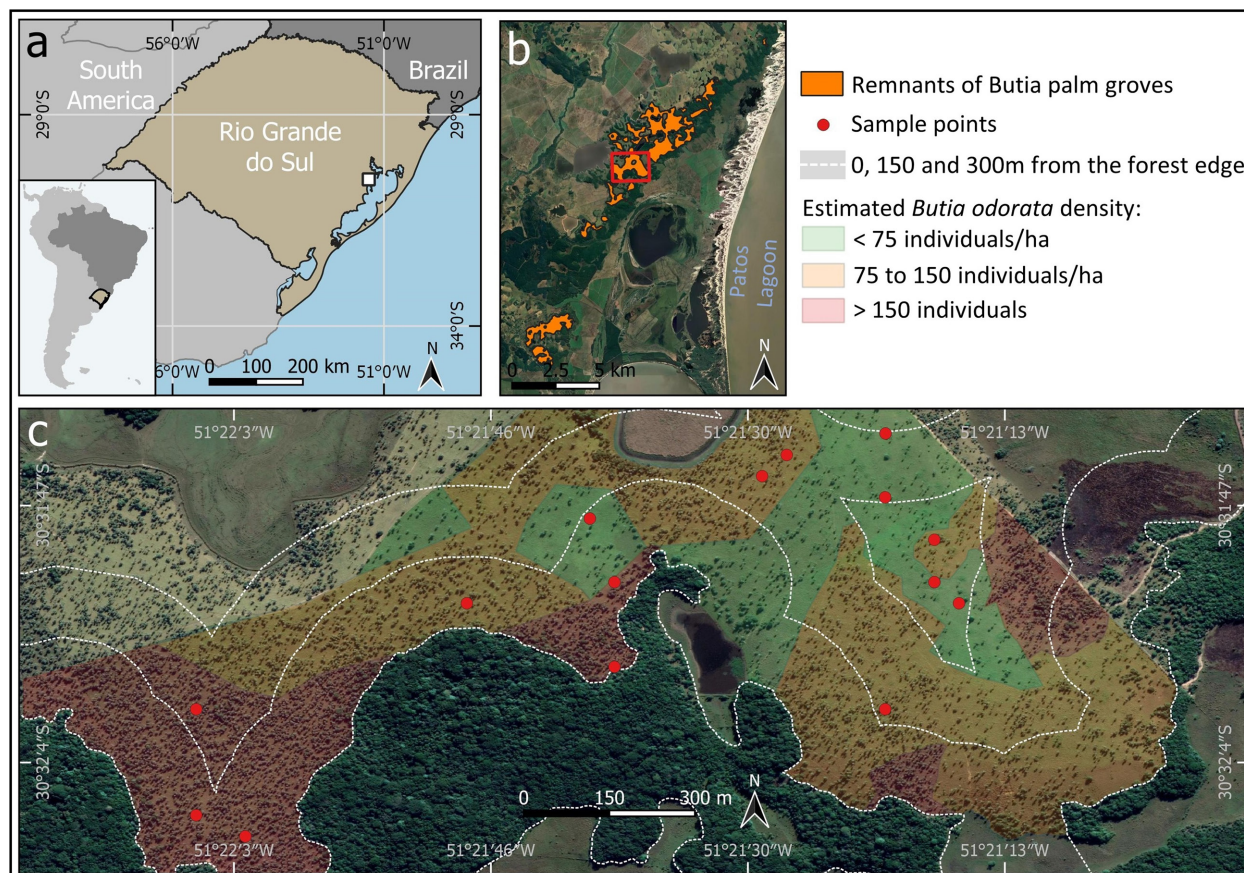


Figure 1. Location of the study area. (a) Regional context of the study area (white square); (b) Remnants of *Butia* palm groves (Ramos et al. 2007) and study area (red square); (c) Sampling points and criteria for sampling stratification. Geographical Coordinate System, Datum SIRGAS 2000.

region have been severely converted in the past (Sosinski et al. 2019, Calambás-Torchez et al. 2021), and currently are inserted in a mosaic of natural and converted areas, with the presence of forests, wetlands, silviculture, soybean, and rice plantations. At the study site, density of palm trees varies between 75 and 300 individuals per hectare (Sosinski et al. 2015), reach an average height of 8m, and the flowering season is from October to April, with flowering peak in December (field observations).

Individuals of *B. odorata* produce 2-7 inflorescences per flowering season, each one with ca. 20,000 flowers (Fonseca 2014). Flowers of both sexes produce nectar, plus pollen in the male ones (Fonseca 2014). The male anthesis lasts 6-11 days, whereas female anthesis lasts 4-6 days and the interval between both in the same inflorescence is ca. 5 days (Fonseca 2014). Male and female anthesis can coincide in different inflorescences of the same individual, albeit rarely (Fonseca 2014), but this synchronous flowering was not observed in this work. Additionally, we did not observe individuals with two inflorescences in anthesis simultaneously.

Sampling design and data collection

We collected data in 15 sample points defined from a stratified random design, from a 50m × 50m coordinate grid, considering three bands of distance from the forest edge (0-150, 150-300, and >300 m), and three classes of estimated density of *B. odorata* (<75; 75-150; >150 individuals per hectare, based on Sosinski et al. 2015 and field-validated later; Fig. 1c). The sample points were standardized by a 30m-radius buffer from the geographic coordinate landmark.

Monthly, between November 2019 and March 2020 we searched for focal individuals of *B. odorata* with open inflorescences. In each sampling point we selected 3-9 inflorescences from different individuals, up to two individuals

per month, when available. We considered available those inflorescences that: (1) were accessible (up to 6 meters high); (2) had more than 50% of the male flowers open; (3) were closest to the sample point landmark.

Floral visitors were sampled with entomological nets (30-cm-diameter) in the inflorescences of focal individuals twice in the same day, between 7:00-9:00 a.m. and 10:00 a.m.-12:00 p.m. All insect individuals were classified into morphospecies and identified to the lowest possible taxonomic level (hereafter 'species'). We only included taxa recognized as potential pollinators (hereafter 'pollinators') for the genus *Butia*: Apoidea (Hymenoptera), Syrphidae (Diptera), Muscidae (Diptera), and Nitidulidae (Coleoptera) (Silberbauer-Gottsberger 1990, Silberbauer-Gottsberger et al. 2013), and occasional pollinators that are known to interact with floral resources (e.g., Vespidae - Hymenoptera and Catharidae - Coleoptera). All Collections were deposited in the MCT-PUCRS Scientific Collection.

We measured the following variables of habitat and population structure in each sampling point: (1) distance to the forest edge (mean distance of *B. odorata* focal individuals to the forest), (2) cover percentage of adult trees (based on crown radius within the 30m-radius buffer), (3) density of *B. odorata* individuals (via census of adult individuals within the 30m-radius buffer), and (4) isolation of focal *B. odorata* individuals (mean distance from their four closest conspecifics). The mean distance from each point to the forest edge consisted of a gradient from 19 to 371 meters (175.2 ± 110.5 [mean \pm standard deviation]; Supplementary Material - Fig. S1a). Tree cover ranged from zero to 21.7% (2.63 ± 5.58 ; Fig. S1b). Density of *B. odorata* individuals per point ranged from 3 to 91 (35.26 ± 24.3 ; Fig. S1c). Mean isolation of

focal individuals per point ranged from 3 to 11.9 meters (6.2 ± 2.49 ; Fig. S1d).

Data analyses

We built 15 bipartite interaction networks (one per sampling point) based on A_{ij} matrices, where i are *B. odorata* individuals (male inflorescences) and j are pollinator species, with their abundances describing the interaction frequencies. We performed rarefaction analyses (iNEXT package; Chao et al. 2014, Hsieh et al. 2020) to assess sampling completeness for interactions in each network, where the extrapolation of the number of interactions (pollinators species frequency in each sample point) was weighted by the sample completeness. The sample completeness was higher than 80% for most networks, never lower than 75% (Fig. S2).

We calculated specialization at the network level (H_2' index; Blüthgen et al. 2006) and modularity (Qw index; Beckett 2016). The H_2' index is a measure of the general overlap of interactions between partners, which ranges from zero, when networks have no specialization, to one, when networks are fully specialized, i.e., there is no overlap of partners between nodes of the same trophic level (Blüthgen et al. 2006). For Qw index we used the DIRTLPWb+ algorithm (Beckett 2016), which computes the modularity of quantitative bipartite networks. Modularity indicates to which extent the network is organized into subgroups (modules) of partners that interact more strongly with each other than with other potential partners in the network (Olesen et al. 2007). The Qw index varies between zero and one, with values close to zero representing non-modular networks and one indicating the maximum possible modularity (Dormann & Strauss 2014, Beckett 2016). Considering that each independent calculation of the Qw index can retrieve slightly different values for the same network, we calculated the

metric ten times for each network and used the maximum value (Dormann & Strauss 2014). Once that Qw index is influenced by the size of the networks, we obtained the z-score of their observed values from each network to allow comparisons between them (Beckett 2016). We obtained the network metrics and biplots (Fig. S3) using the “bipartite” package (Dormann et al. 2008, 2009).

Networks were considered more specialized and modular than expected by chance when the observed metrics departed from 95% confidence intervals based on metrics calculated from null models. The null models used to calculate p-values of H_2' and Qw (and z-scores of Qw) were created for each network with the ‘r2dtable’ algorithm (Patefield 1981, Dormann et al. 2009), which keeps marginals totals of the matrices fixed. We used the minimum and maximum values and the standard deviation of the values obtained from the null models (1,000 random networks for each observed network) to calculate the p-value of the observed metrics based on a normal distribution function.

Pollinator diversity was described by mean abundance and richness per focal individual of total, peripheral, and core species. The classification in ‘peripheral’ and ‘core’ was based on the Gc index (Dáttilo et al. 2013) where core species comprise dominant pollinators, accumulating up to 50% of the observed interactions ($G_c > 0$), whereas peripheral species ($G_c < 0$) are rarer and with fewer interactions.

All response variables (network and diversity descriptors) were subjected to normality tests. The variables that did not show normality (mean pollinator abundance, mean core-species abundance, and mean peripheral-species richness) were log-transformed. We also applied log-transformation to tree cover to minimize the effects of the large amplitude of this variable. After running the linear models

(next paragraph) we tested the normality of the residuals for the same variables. All of them met the assumption of normality.

Since habitat structure and population structure variables potentially exhibit complex interactions that can mediate their individual effects on the variables of interest related to the diversity and structure of networks (hereafter referred to as response variables), we opted to use Structural Equation Models (SEM). These models allow us to evaluate the direct and indirect effects between variables of interest (Shipley 2016, Lefcheck 2021). Each of the eight response variables was tested in an independent linear model with identical structure (Fig. 2) created based on the ecological hypotheses

presented (see introduction). We also tested all correlations between exogenous variables. We tested the models assuming the probabilistic thresholds of Fisher's $p > 0.05$ for models and $p < 0.05$ for each causal relationship. The SEM were performed using the "psem" function of the "piecewiseSEM" package (Lefcheck 2016).

All analyses were conducted in R software (R Development Core Team 2023).

RESULTS

We collected 2,155 insects from 98 *B. odorata* individuals (22.0 ± 2.1 insects per plant; mean \pm standard error), distributed in 10 orders and 141 species. Among those, 38 species (26.9%

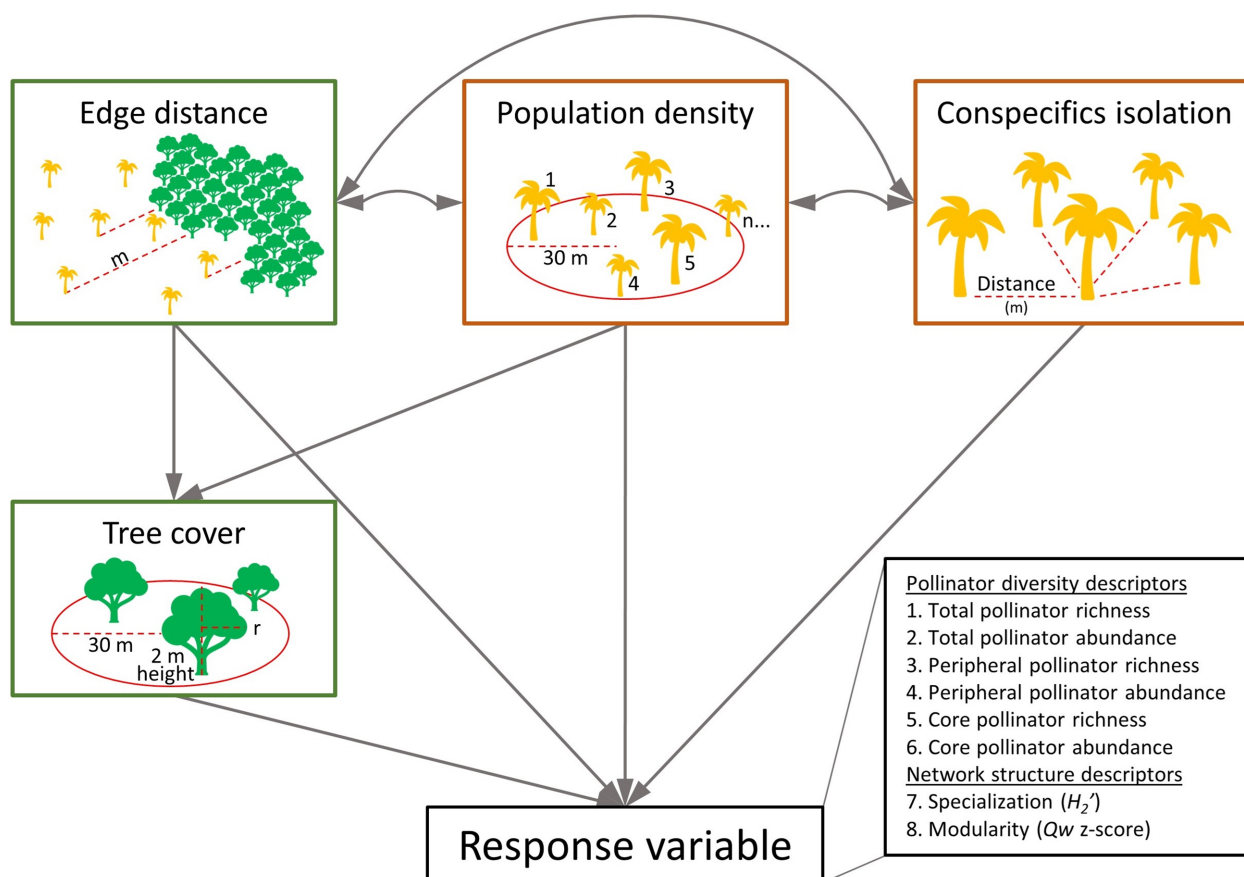


Figure 2. Theoretical model of the causal relationships between predictors (habitat [green boxes] and population structure [brown boxes]) and response variables (pollinator diversity and network descriptors). All eight response variables were tested in an independent model and are represented in the outside box for illustrative purposes. Straight arrows: directional causal relationships; curved arrows: correlation between exogenous variables.

of the total richness) with 664 individuals (30.8%; 6.8 ± 0.6) were recognized as potential pollinators. The most frequent species was *Apis mellifera* (Apinae), with 278 individuals (41.9% of pollinators), followed by the wasp Thynnidae sp., with 101 individuals (15.2%). Family Halictidae (Apoidea, Hymenoptera) was the most frequent, with 10 species and 170 individuals (25.6%; Table SI). Six species were classified as core pollinators in at least one network and *A. mellifera* obtained the highest Gc in most networks (Table I).

Network specialization (H_2') ranged from 0.267 to 0.726 and modularity (Qw) from 0.123 (z-score: 1.218) to 0.513 (z-score: 5.813). Among the 15 networks, 11 showed specialization and 10 showed modularity different from that expected by the null models (Table SII). Mean pollinator

richness ranged from 1.5 to 4.0 (2.8 ± 0.6 [mean \pm standard deviation]; Table SI). Mean richness of peripheral pollinators varied between 0.5 and 2.6 (1.34 ± 0.48). Core pollinator richness ranged from 0.83 to 2.28 (1.46 ± 0.4).

Causal models for all response variables were statistically significant (p-Fisher: 0.092; C-Fisher: 4.4767). Tree cover decreased with distance from forest edge and increased with palm tree population density (R^2 : 0.71; Fig. 3). As would be expected, the correlation between the exogenous variables indicated that sampling points with fewer palm trees showed higher mean distance between individuals (p-value: 0.016). Additionally, isolation between palm trees increased with distance from the forest

Table I. Core pollinator species and their respective Gc index in 15 pollination interaction networks of *Butia odorata* (Arecaceae).

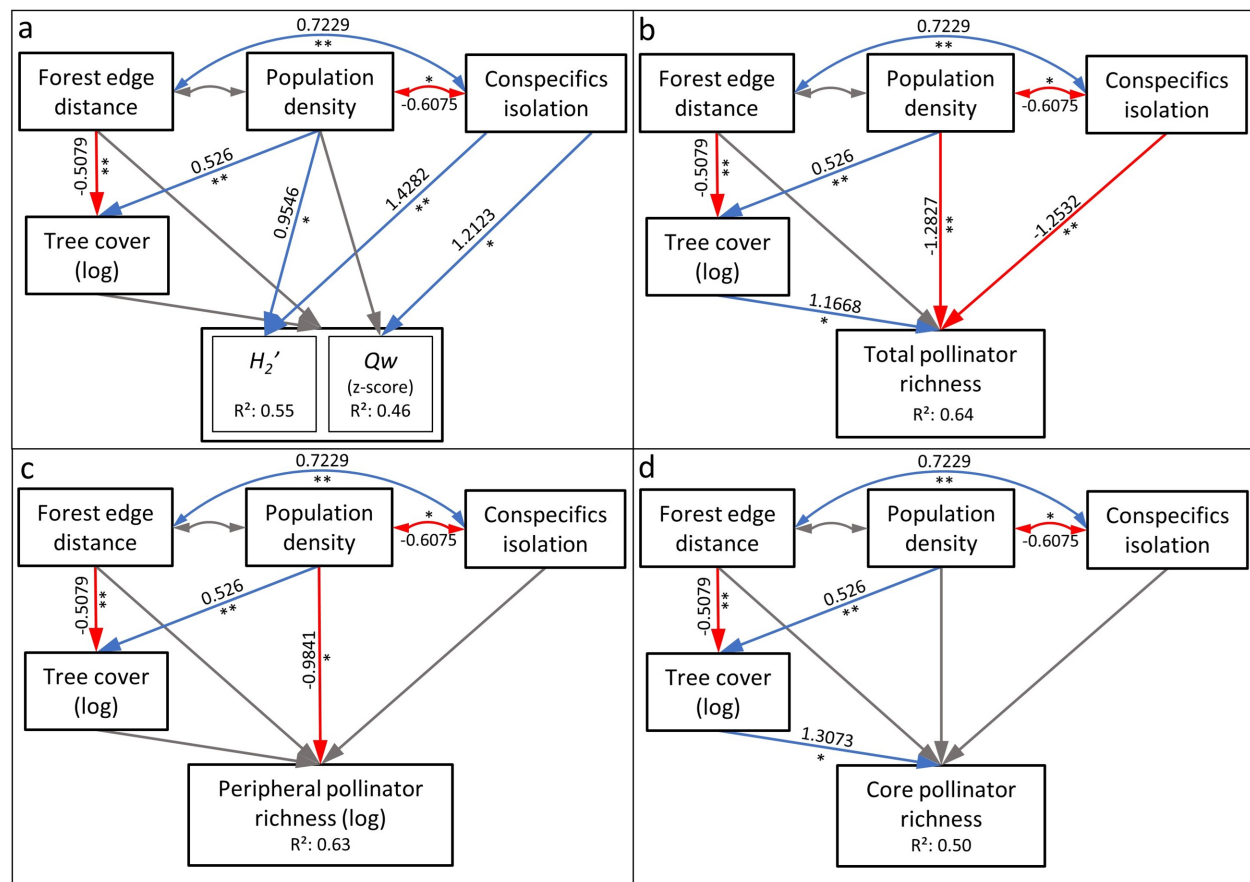
Network/ Species	<i>Apis mellifera</i>	Thynnidae sp.	<i>Augochlorella</i> sp.	<i>Lasioglossum</i> sp.	Nitidulidae sp.	<i>Dialictus</i> sp. 2
Network 1	0.730*	0.243	0.243	0.365	--	0.486
Network 2	1.725*	0.178	-0.297	-0.059	--	-0.297
Network 3	2.564*	0.266	0.036	--	-0.117	-0.271
Network 4	2.058*	-0.311	0.107	0.386	--	--
Network 5	1.738*	0.047	-0.047	-0.235	--	-0.329
Network 6	1.290*	0.157	-0.126	-0.126	--	--
Network 7	0.567*	-0.496	0.567*	--	-0.142	--
Network 8	1.609*	0.079	0.188	-0.358	--	-0.358
Network 9	2.295*	-0.053	-0.329	-0.329	0.499	--
Network 10	1.011*	--	0.046	0.368	-0.368	--
Network 11	1.755*	-0.209	0.536	-0.142	-0.006	--
Network 12	0.193	0.466*	0.011	-0.170	0.375	--
Network 13	1.387*	0.112	0.590	-0.048	-0.207	--
Network 14	0.262	1.312*	0.000	-0.262	-0.262	--
Network 15	0.077	0.981*	-0.310	--	--	--

Positive values (gray): core species; negative values: peripheral species; *: main core species in the network (highest Gc value); "--": species absent in the network. Networks in ascending order of distance from the forest edge.

edge (p-value: 0.002; Fig. 3; see Table SIII for scores of all variables).

Specialization and modularity were higher in sampling points with more isolated palm trees (R^2 : 0.55; 0.46; p-value: 0.007; 0.027 respectively; Fig. 3a). Specialization also responded positively to palm tree density (p-value: 0.047; Fig. 3a). The other predictors did not show a significant causal relationship with network-level descriptors (Table SIII).

Mean pollinator richness was directly explained by three variables: it decreased with increasing densities of *B. odorata* and conspecifics isolation and increased with tree cover (R^2 : 0.64; p-value: 0.007; 0.008; 0.015 respectively; Fig. 3b). Distance to the forest edge and palm population density indirectly influenced pollinator richness, mediated by tree cover (Fig. 3b). Mean richness of peripheral pollinators decreased with the density of *B. odorata* (R^2 : 0.63; p-value: 0.028; Fig. 3c), i.e.,



For a, b, c and d C-Fisher: 4.767; p-Fisher: 0.092.

Figure 3. Structural Equation Models (SEM) and significant causal relationships between metrics of specialization (H_2') and modularity (Qw z-score) (a); and mean pollinator richness (total (b), peripheral (c), and core (d)) of 15 interaction networks of pollinators with *Butia odorata* (Arecaceae) individuals and predictor variables of population and habitat structure. Straight arrows: causal relationships; curved arrows: correlation between exogenous variables; gray arrows: non-significant relationships (p-value ≥ 0.05); blue arrows: positive statistically significant relationship between variables; red arrows: negative statistically significant relationship between variables; *: relationships with p-value < 0.05 ; **: relationships with p-value < 0.01 ; values close to arrows: standardized estimates of statistically significant relationships. H_2' and Qw were tested in independent models and were shown in the same model here for simplification.

more peripheral species were found in sampling points with fewer palm trees. Core pollinator richness increased with the tree cover, being also indirectly influenced by distance from the forest edge and palm population density (R^2 : 0.50; p-value: 0.02; Fig. 3d). Total, peripheral and core pollinator abundance were not statistically significant related to any model variable (Table SIII).

DISCUSSION

In this study, we observed that most of the sampled individual-based networks of *Butia odorata* were significantly modular and specialized, and that networks with more isolated palms were more modular and specialized when compared to those with more grouped palms. Networks with higher density of palm trees (but not necessarily with more grouped individuals) were more specialized and presented fewer pollinating species in comparison with networks with fewer palm individuals. Overall pollinator richness was sensitive to the environmental descriptors across the grassland-forest gradient, showing different responses that were dependent both on habitat context and network pollinator role (i.e., peripheral or core).

The response of network specialization and modularity to conspecific isolation reinforces the known effect of plant population structure on the structure of mutualistic networks (Fortuna et al. 2008, Dupont et al. 2014, Guerra et al. 2017). This observed and consistent pattern for both metrics may be associated with the pollinators' limitations of movement (Burkle & Alarcón 2011) and optimal foraging strategies (Pyke et al. 1977, Bolnick et al. 2003, Hegland 2014). Greater specialization in individual networks is associated with the ability of individuals to access certain resources, directly relating to optimal foraging theory (Bolnick et al. 2003). Pollinators

tend to move more frequently between nearby flowering plants, which affects chances of sharing visitors (Arroyo-Correa et al. 2021). As the distance between conspecifics increases, movement between plants demands more energy and increase foraging costs, especially for pollinators with lower dispersal capacity (Pyke et al. 1977, Chamberlain et al. 2014). In this context, the interactions of the insects with plants tend to concentrate on closer and/or more accessible inflorescences (Pyke et al. 1977), leading to a lower overlap of partner species between more isolated individuals (greater specialization). In networks with greater individual specialization, the formation of more modules is also expected (Bolnick et al. 2003). The formation of modules of preferential interactions in individual networks is expected under conditions where the pollinators community cannot access all plants in the population equally (Dáttilo et al. 2014), making the interactions more frequent between these subgroups of partners (Burkle & Alarcón 2011, Gómez et al. 2011).

Network specialization is directly related to the niche overlap of the involved individuals (Bolnick et al. 2003). Therefore, the difference in specialization along the population gradient of palm trees should result in different scenarios of pollinator overlap among the plants. In situations of high modularity, in turn, the concentration among groups of individuals can lead to spatial segregation in pollen transfer, affecting the local gene flow of the population (Fortuna et al. 2008, Dupont et al. 2014). Plant populations that share more pollinators between individuals tend to have greater gene flow through pollen dispersal than those that share fewer pollinators (Fortuna et al. 2008, Gómez et al. 2011, Gómez & Perfectti 2012). Thus, in more modular and specialized networks we can expect pollinators with pollen load of lower genetic diversity (from fewer donor plant; Fortuna et al. 2008, Gómez et al.

2011, Dupont et al. 2014). Therefore, gene flow within the plant population can be affected by the variation of the structural attributes along the environmental gradient we presented here (Bolnick et al. 2003, Fortuna et al. 2008, Dupont et al. 2014). As observed for other plant species, this variation in gene flow configuration can potentially translate into effects on the fitness of *B. odorata* individuals (Fortuna et al. 2008, Gómez et al. 2011, Gómez & Perfectti 2012, Dupont et al. 2014, Arceo-Gómez et al. 2020), seminal attributes in the conservation of reduced and isolated populations.

Effects of the environmental context on pollinator diversity

Contradicting our hypotheses, higher densities of palm trees also seem to represent a limitation to pollinator displacement, affecting species-level specialization and species richness in a similar way. The lower values of total pollinator richness in networks with greater isolation and density of palm trees reinforce the role of these variables in limiting pollinator movement (Burkle & Alarcón 2011, Gómez et al. 2011). Even sampling points with the lowest pollinator richness were dominated by the two main core species, *Apis mellifera* and Thynnidae sp., suggesting that isolation may be acting as a filter for rare species to access floral resources, while favoring the monopolization of interactions by the most efficient species in locating and exploiting them (Brown & Phillips 2014, Valido et al. 2019, Cruz et al. 2024). Additionally, the effects of palm tree density on pollinators were depended on habitat and species role (peripheral or core species; Hegland 2014, Dáttilo et al. 2015, Arroyo-Correa et al. 2021). As a result, some pollinators may face movement limitations related to the density of palm trees (e.g., difficulty to identify and access available inflorescences due to physical barriers during flight), since most

palm trees do not provide synchronous floral resources in the population, which also implies greater energy expenditure in foraging (Pyke et al. 1977, Burkle & Alarcón 2011).

Pollination systems are often context-dependent, in the sense that different pollinator groups/roles respond differently to habitat characteristics (Chamberlain et al. 2014, Dáttilo et al. 2015, Hegland 2014, Arroyo-Correa et al. 2021). Our data bring novel evidence that supports this context-dependence for pollination networks at the plant individual level. While tree cover was the only variable that predicted the variation of core pollinator richness, peripheral pollinator richness was determined by palm population density. High density of palm trees seemed to reduce species richness by excluding peripheral species. However, core pollinator richness was indirectly influenced by palm density as well, via modulation of tree cover, possibly due to a facilitating role of tree density, which seems to act in decreasing dominance since more species make up the core of the network. The dominance of a community by one or a few species can lead to the exclusion of species with lower competitive potential. However, facilitating effects can promote diversity in this context (Bruno et al. 2003). Greater habitat complexity provides different substrates for nesting, modify factors such as humidity, temperature, wind flow, and potentially increase the overall diversity of floral resources in the palm tree stratum. These effects are recognized as mediators of pollinator composition (Bruno et al. 2003, Janovský et al. 2013, Chamberlain et al. 2014) by making the matrix more accessible and/or permeable to more species.

The lack of response of the pollinator abundance in the models indicates no influence in the number of interactions in the network in these environmental contexts. However, the marginal trends of a negative relationship

between the abundance of peripheral species and the density and isolation of palm trees (Supplementary Material Table II) may reinforce the possible limitation imposed on rare pollinators by these conditions. However, the potential impact of this abundance reduction on the probability of pollen dispersal should be small, if any, since the quality of pollination depends more on species richness than on abundance of individuals (Arceo-Gómez et al. 2020).

Implications of grassland-forest dynamics on *Butia odorata* pollination

The mediation of the variables of habitat (distance from the forest edge) and population (density of palm trees) through tree cover constitutes an interesting pattern from the perspective of the grassland-forest dynamics and in the antagonism of *Butia* with tree species. The effect of forest encroachment on non-forest ecosystems has been recognized for different taxa, generally leading to reduction of the 'invaded' ecosystem resilience by species replacement (Raymundo et al. 2023, Neves et al. 2023). The present climate favors the expansion of forests over the grasslands in the study region (Salgado et al. 2021), that results in the death of the palm trees due to shading and replacement of the herbaceous stratum (Sosinski et al. 2019), which are major concerns for the conservation of this ecosystem (Veldman et al. 2015). Nevertheless, the presence of forest elements seems to be responsible for an increase in the diversity of pollinators in *B. odorata* networks.

The diversity of floral visitors in grasslands can be increased by the presence of (or proximity to) forest environments (Hegland 2014, Pinto et al. 2020). However, the effect of diversity increments on seed productivity and plant fitness can be diverse. A greater diversity of pollinators leads to greater complementarity

and functional redundancy (e.g., different foraging behaviors and physiological responses), generating greater probability/quality of pollination and resilience for the system (Tylianakis et al. 2010). Conversely, depending on the behavior of the pollinators and the amount of interspecific co-flowering, greater diversity of pollinators can lead to greater heterospecific pollen deposition on flowers (Arcéo-Gómez et al. 2020) or to a reduction in visits per plant species (Lara-Romero et al. 2016), impairing pollination. This suggests that intermediate levels of tree cover can avoid possible unwanted effects and generate a positive impact on pollination (Lara-Romero et al. 2016).

CONCLUSIONS

By using an individual-species network approach, our study contributes to the understanding of the role of environmental constraints in structuring the pollination networks of this endangered palm tree in the context of forest-grassland ecotones. Although the advance of forests over non-forest ecosystems, such as palm groves, can be problematic for their conservation, we demonstrate here that the presence of trees and the proximity to forests can promote pollinator diversity in these systems, possibly through facilitation mediated by habitat complexity. Expanding these findings to similar pollination systems in grassland-forest ecotonal conditions, it seems reasonable to assume that an intermediate level of tree species presence should be desirable to maximize conservation results regarding cross pollination and gene flow. Additionally, environmental limitations imposed by palm population structure on the movement of pollinators and the consequent flow of pollen, described by the network's properties, also may have important implications in the fitness and productivity of this ecosystem key species.

However, these expected relationships between metrics of individual pollination networks and plant productivity are still poorly understood, despite their potential theoretical and applied value. Future works that explicitly assess plant productivity parameters and characteristics of individual plants in the pollination networks would help fill this gap.

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SUPPLEMENTARY MATERIAL

Figure S1-S3.
Table SI-SIII.

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All authors contributed to the study conception and sampling design; M.R.-Q. and G.daC.S. conducted field work and data collection; M.R.-Q. conducted material preparation and curation, under supervision of B.B.; M.R.-Q. and P.M.A.F performed data analysis. M.R.-Q led the writing of the manuscript with contributions and comments from all the other authors. All authors read and approved the final manuscript.

