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Habitat and population structure determine patterns of plant-pollinator networks of an endangered palm tree in a grassland-forest ecotone

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Abstract

Pollen transport by insects determines patterns of reproductive encounters between plants with flowers that have spatially or temporally segregated sexes. Pollinators show varied responses to environmental gradients such as those found in grassland-forest ecotones. Individual-based interaction networks are useful yet underexplored tools to understand how interactions vary across these gradients. Interactions between plant individuals and their pollinators directly reflect on plants fitness and genetic structure, seminal attributes for the conservation of endangered species. To test how a grassland-forest ecotone gradient can affect these interactions we studied pollination networks of *Butia odorata* individuals, an extinction-threatened palm tree from remnant palm grove ecosystems in South America. We evaluated how network metrics (specialization and modularity), and pollinator richness respond to gradients of habitat and population structure in a grassland-forest ecotone. Networks with more isolated palm trees showed greater specialization and modularity. Pollinator richness was dependent on the habitat context and pollinator role: peripheral pollinators were negatively affected by palm density, whereas core pollinators were positively affected by tree cover, which in turn was positively associated with palm density and proximity to the forest. Our results indicated that increased tree cover in the grassland matrix can promote pollinator diversity by decreasing the dominance of core species. Palm density may hamper the movement of pollinators pollen transportation, playing a key role for the conservation of *B. odorata*and for palm grove ecosystems. Finally, we emphasize the need of protocols that include traditional grassland management to achieve tree and palm tree density that maximizes conservation results.

Introduction

Insect pollination is essential to gene flow and genetic-diversity structuring of plant populations (Butcher et al. 2020). Pollen transport depends on foraging patterns of pollinators and determines reproductive encounters between cross-pollinating plants (Barrett and Harder 1996; Mitchell et al. 2009). Understanding how environmental gradients can influence patterns of floral visitation is important to predict possible impacts on the genetic structure of plant populations (by gene flow) and their ecological and evolutionary consequences in response to selective pressures (e.g., climate change; Butcher et al. 2020), providing bases for decision-making related to ecosystem management for the conservation of plant genetic heritage.

Investigations on animal-plant interactions related to static environmental gradients (latitudinal and altitudinal) are common in the literature (e.g., Adedoja et al. 2018; López-Segoviano et al. 2021). However, there is limited empirical evidence on the responses of these interactions to dynamic gradients such as directional ecotones (Myster, 2012), as well as the implications of such relationships to plant conservation. 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 their unique 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 these dynamics (mostly focused on plants, e.g., Oliveira and Pillar 2004; 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 contexts (Bascompte and Jordano 2007; Jordano 2016; Tylianakis and 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), the basic units of interactions. At this level, more subtle changes in the structural patterns of networks can be detected in response to environmental variations (Dupont et al. 2014; Rumeu et al. 2018; Guimarães 2020) and individual traits (Bolnick et al. 2003). In mutualism, individual interactions must translate into fitness, which is reflected in demographic and evolutionary patterns (Guimarães 2020) and may reveal emergent properties and patterns undetectable in species-level networks (Tonos et al. 2021). Individual-based network approaches have been originally developed and widely applied to systems of antagonistic interactions (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; Vizentin-Bugoni et al. 2018; Arceo-Gómez et al. 2020; but see Arroyo-Correa et al. 2021).

In these systems, certain network metrics directly describe the sharing of pollinators (upper trophic level) among plant individuals (lower trophic level), revealing patterns related to fitness and genetic structuring of populations (Olesen et al. 2007; Dupont et al. 2014; Tur et al. 2014; Guimarães 2020). For example, specialization at the network level (H2’) describes ‘complementary specialization or selectiveness’ of nodes (i.e., plant individuals and pollinator species) 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 breadth scenarios (Tur et al. 2014), which in pollination networks can indicate how plant individuals share the pool of available pollinators. 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; Dupont et al. 2014). Ultimately, emergent features of individual-based pollination networks may contribute to the understanding of population patterns and dynamics, which could in turn provide a novel perspective to plan for the conservation of these systems.

Butia is a genus of Neotropical palms with 20 species (Soares 2015), many of which are extinction-threatened (Rio Grande do Sul 2014; Brasil 2022; IUCN 2023). They produce unisexual flowers, both sexes occurring in the same inflorescence with protandrous anthesis, thus depending on cross-pollination (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). 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 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) and isolation (distance between palms), producing a density 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 and Miller 2004; Leibold and Chase 2018), 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 and Miller 2004). Therefore, one should expect that these conditions or filters should determine which pollinators have access to flowering individuals along a gradient of woody encroachment over a grassland matrix (Leibold and Miller 2004; Thompson and Gonzalez 2017; Higino and Poisot 2021), affecting the structure of the pollination networks of Butia palms.

In this work, we evaluate how 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 i) network specialization, ii) network modularity, and iii) pollinator diversity.

Materials and Methods

Study site

We conducted this work in a *B. odorata* palm grove of ca. 170 ha, in the municipality of Tapes, Rio Grande do Sul, Brazil (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 sampling 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 occurs mainly from the coastal plains of Rio Grande do Sul, Brazil, to southeastern Uruguay (Soares et al. 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 "EN", Criteria "A4cd": population size with reduction ≥ 50%, due to decline in occupied area, extent of occurrence or quality of habitat and level of potential or current exploitation; IUCN 2012; Rio Grande do Sul 2014). Palm groves in the study 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, providing an important resource for insects (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 under the same anthesis stage.

Sampling design and data collection

We collected data in 15 sample points defined from a stratified random design, from a 50m x 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 inflorescences in each sampling point. We selected 3–9 inflorescences from different individuals per sample point (two per collection campaign, when available), based on: (1) accessibility (up to 6 meters high); (2) more than 50% of the male flowers open; (3) proximity to the sample point landmark.

Floral visitors were sampled by two trained collectors 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 10:00–12:00 a.m. (based on a pilot survey that assessed the activity of floral visitors). 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, Syrphidae, Muscidae, and Nitidulidae (Silberbauer-Gottsberger 1990; Silberbauer-Gottsberger et al. 2013), and occasional pollinators that are known to interact with floral resources (e.g., Vespidae and Catharidae). All collections were carried out under the Permanent Collection License No. 11675 of the Chico Mendes Institute for Biodiversity Conservation, Ministry of the Environment (ICMBio/MMA), Brazil. 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), (3) density of *B. odorata* individuals (via census of adult individuals), 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 ± standard deviation]; Online Resource, Fig. OR1a). Tree cover ranged from zero to 21.7% (2.63 ± 5.58; Fig. OR1b). Density of *B. odorata* individuals per point ranged from 3 to 91 (35.26 ± 24.3; Fig. OR1c). Mean isolation of focal individuals per point ranged from 3 to 11.9 meters (6.2 ± 2.49; Fig. OR1d).

Data analysis

We built 15 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 to assess sampling completeness for interactions. For this, we used the iNEXT package (Chao et al. 2014; Hsieh et al. 2020), where the extrapolation of the number of interactions (pollinators frequency) was weighted by the sample coverage. We observed a sample completeness > 80% for most networks, never lower than 75% (Online Resource, Fig. OR2).

We calculated specialization at the network level using the H_2' index. This 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). Modularity was calculated through *Q* index (DIRTLPAwb + algorithm; Beckett 2016), that 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). This index varies between zero and one, with values close to zero representing non-modular networks and one indicating the maximum possible modularity (Dormann and Strauss 2014; Beckett 2016). Considering that each independent calculation of the *Q* can retrieve slightly different values for the same network, we calculated the metric ten times for each network and used the maximum value (Dormann and Strauss 2014). We also obtained the z-score of observed *Q* values to allow comparisons between the networks of different dimensions (Beckett 2016). To observe if networks were more specialized and modular than expected by chance, we used the minimum, maximum, and the standard deviation of the values obtained from the null models (1,000 random networks for each observed network) to compare with the observed metrics based on a normal distribution function with 95% confidence intervals. Null models were created for each network with the 'r2dtable' algorithm (Patefield 1981; Dormann et al. 2009), which keeps marginals totals of the matrices fixed. We calculated network metrics and plotted the observed networks biplots with “bipartite” package (Dormann et al. 2008, 2009; Online Resource, Fig. OR3)

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 (Gc > 0), whereas peripheral species (Gc < 0) are rarer and with fewer interactions.

All response variables (network and pollinator 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.

We used Structural Equation Models (SEM) to evaluate causal relationships between response and predictor variables (habitat and palm population descriptors). Each response variable was tested in an independent model with identical structure (Fig. 2). In this model, we assumed the direct and/or indirect effect of the predictor variables on the structural and diversity descriptors of the networks. Furthermore, we considered the influence of the distance from the forest edge and the density of *B. odorata* on the increase in the

percentage of tree cover at the sampling points (by facilitating the dispersion and/or establishment of tree species) and the correlation of errors between the exogenous variables of the model. We tested the models assuming the probabilistic thresholds of Fisher's $p > 0.05$ for models and $p < 0.05$ for each causal relationship. For this, we used the "psem" function of package "piecewiseSEM" (Lefcheck 2016). All analyses were carried out 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 ± standard error), distributed in 10 orders and 141 species. Among those, 38 species (26.9% 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*, with 278 individuals (41.9% of pollinators), followed by the wasp Thynnidae sp., with 101 individuals (15.2%; Online Resource, Table OR1). Family Halictidae was the most frequent, with 10 species and 170 individuals (25.6%; Table OR1). Six species were classified as core pollinators in at least one network and *A. mellifera* obtained the highest Gc in most networks (Table 1).

Table 1
Core pollinator species and their respective Gc index in 15 pollination interaction networks of *Butia odorata*

Network/Species	*Apis mellifera*	Thynnidae sp.	*Augochlorella* sp.	*Lasioglossum* sp.	Nitidulidae sp.	*Dialictus* 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.

Causal models for all response variables were statistically significant (p-Fisher = 0.092; C-Fisher = 4.4767). Tree cover decreased with distance to the forest edge and increased with palm tree population density (R^2 = 0.71; Fig. 3). As would be expected, the correlation of errors 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 edge (p-value: 0.002; Fig. 3; see Online Resource, Table OR2 for scores of all variables).

Network specialization (H_2') ranged from 0.267 to 0.726 and modularity (Q) from 0.123 (z-score: 1.218) to 0.513 (z-score: 5.813). Among the 15 networks, 11 were specialized and 10 were modular when compared with null models (Online Resource, Table OR3). 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 OR2).

Mean pollinator richness ranged from 1.5 to 4.0 (2.8 ± 0.6 [mean ± standard deviation]; Table OR1) and 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 varied between 0.5 and 2.6 (1.34 ± 0.48) and decreased with the density of *B. odorata* (R^2: 0.63; p-value: 0.028; Fig. 3c), i.e., more peripheral species were found in sampling points with fewer palm trees. Core pollinator richness ranged from 0.83 to 2.28 (1.46 ± 0.4), it 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 OR2).

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. Interestingly, 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 plant isolation may be associated with the pollinators' limitations of movement (Burkle and Alarcón 2011) and optimal foraging strategies (Charnov 1976; Pyke et al. 1977; Hegland 2014). 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) and to more frequent interactions between these subgroups of partners, ultimately increasing network modularity (Burkle and Alarcón 2011; Gómez et al. 2011).

Plant populations that share more pollinators between individuals tend to have greater gene flow through pollen dispersal than those that share fewer pollinators (Gómez et al. 2011). Thus, in more modular and specialized networks we can expect pollinators with pollen load of lower genetic diversity (from fewer donor plant; Gómez et al. 2011). Therefore, gene flow within the plant population will be affected by the variation of the structural attributes along the environmental gradient we presented here, which can potentially translate into effects on the fitness of *B. odorata* individuals (Gómez et al. 2011; Arceo-Gómez et al. 2020), seminal attributes in the conservation of reduced and isolated populations.

Effects of the environmental context on pollinator diversity

As seen for network-level metrics, higher densities of palm trees 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 and 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. Additionally, the effects of palm tree density on pollinators were depended on habitat and species role (peripheral or core species; Hegland 2014; 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 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 and 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; 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 independent 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 (Stachowicz 2001; 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 a larger species pool.

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 (Table OR2) may reinforce the limitation that these conditions may pose to rare pollinators. 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).

Finally, *A. mellifera* was the most abundant (41% of interactions) and stable (central role in most networks) species in our sampled networks. Although it is an alien species with well-known invasive behavior, it is difficult to assess its impacts on this pollination system (due the absence of data prior to the invasion). Biological invasions in pollination networks, especially by this species, usually imply the monopolization of floral resources (Hung et al. 2019; Valido et al. 2019), decrease in native species richness, changes in behavior of native pollinators due to competition (Hung et al. 2019; Valido et al. 2019), and changes in the structure and functionality of pollination networks (Valido et al. 2019). Nevertheless, it is recognized that the presence of *A. mellifera* can represent an effective increase in the pollination rates for native plants, especially in situations in which the populations of native pollinators are reduced (Hung et al. 2019), even though this reduction seen in the present might have been caused by the invasion in the first place. Thus, the repercussion in terms of pollination process/service does not seem to be as drastic as the displacement of pollinator populations from their original space in the networks. Based on the current picture of the system, *A. mellifera* plays a central role in networks of *B. odorata* pollen transport due to its abundance, temporal stability, and high flight range, which should enhance cross pollination, especially considering the context of high habitat fragmentation common to most natural areas in the region and, specifically, in palm groves.

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; Ye 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). These intermediate levels of ligneous species in non-forest ecosystems, such as *Butia* palm groves, could be maintained with management practices that include fire and grazing (Ferreira et al. 2020; Ferreira et al. 2021), since both disturbances are evolutionary linked to these ecosystems worldwide and are a sustainable land use, compatible with species conservation (Coughenour 1985; Pillar and Quadros 1997; Bond 2021).

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 an 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 influence emergent network properties and 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 contribution of tree species 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 also has important implications for the conservation of these systems. Optimal tree species and palm tree density could be achieved by applying traditional grassland management techniques that include fire and grazing, although the basic knowledge to choose the adequate intensity and frequency of these disturbances is still limited. We suggest that future works should be focused on these land management aspects, as well as on individual plant traits that could (i) influence pollinator attraction and network structure, and (ii) describe in more detail the effects of the patterns presented here on plant fitness.

Declarations

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

Data Availability The datasets and R scripts of this work are available at: https://zenodo.org/badge/latestdoi/626070087. Raw data are available from the corresponding author on reasonable request.

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Figures

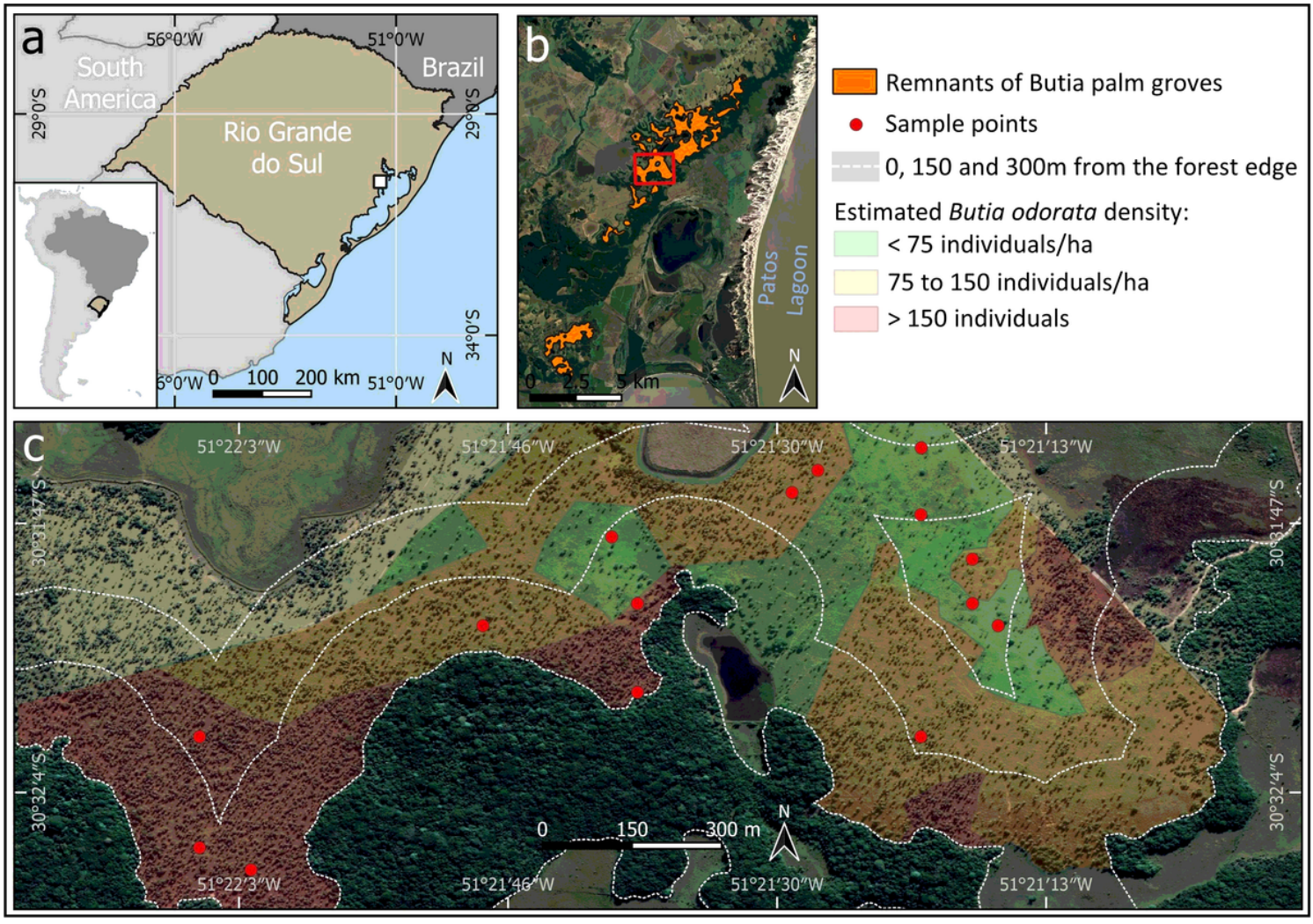


Figure 1

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.

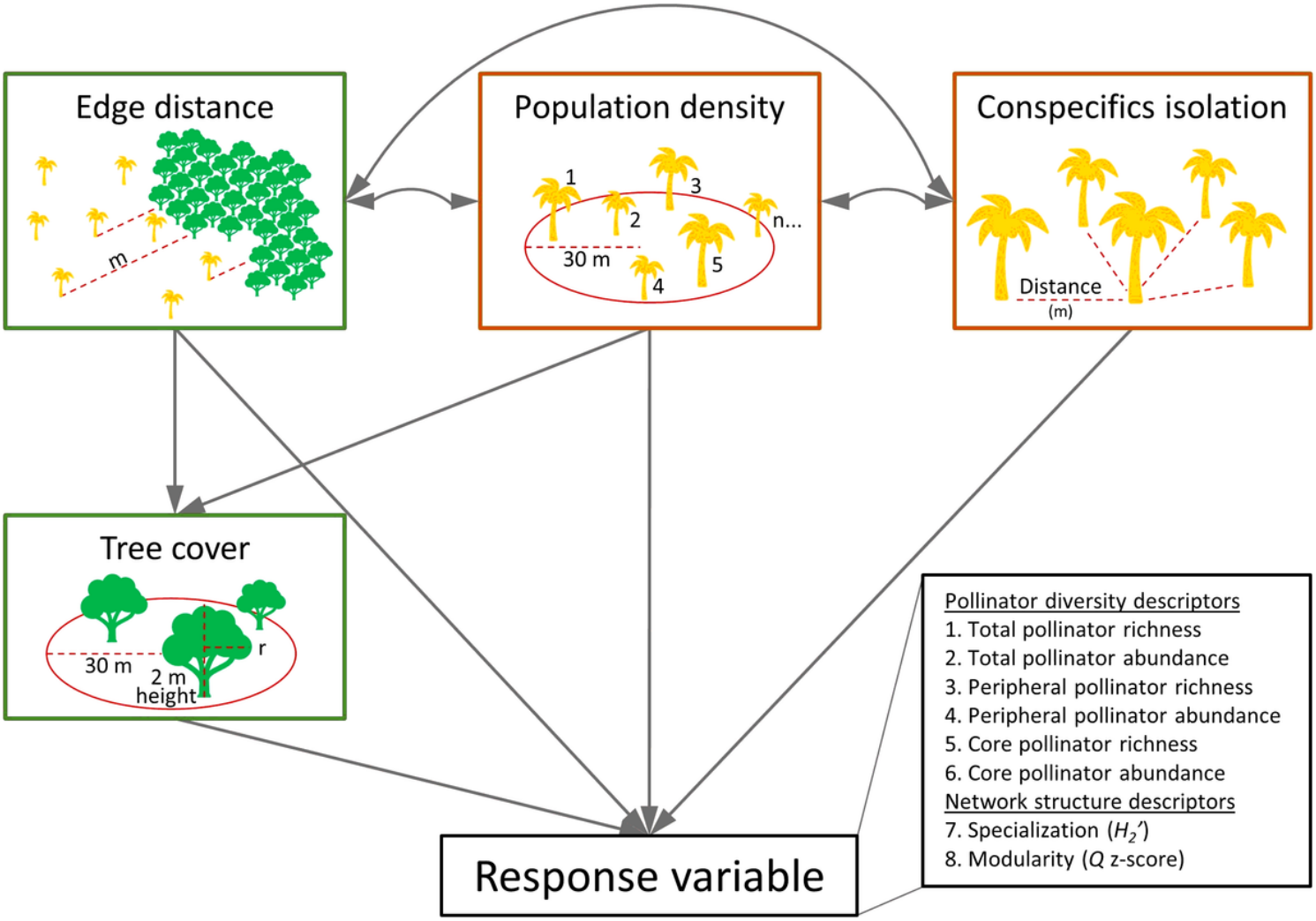
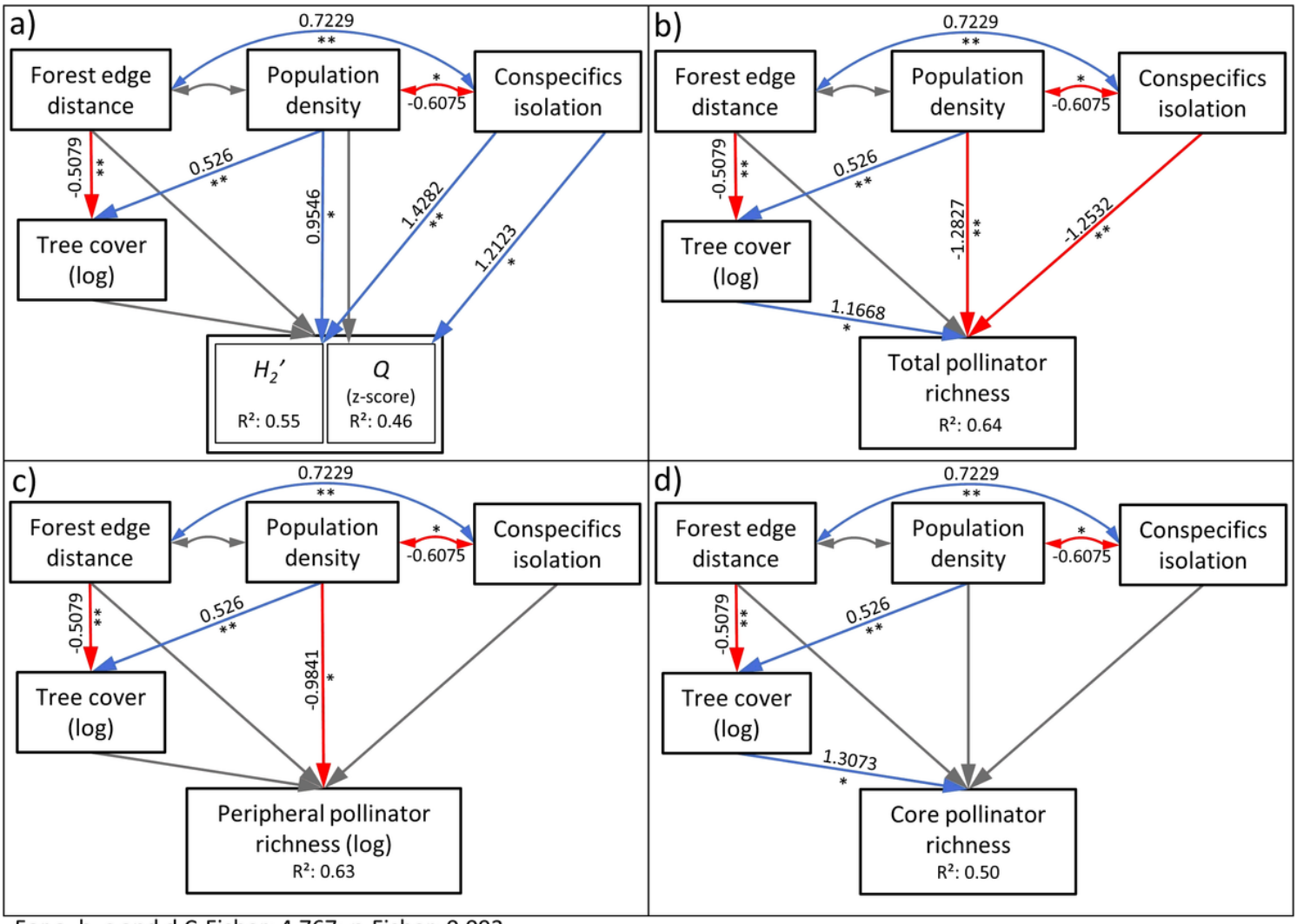


Figure 2

Theoretical model of the causal relationships between predictors (habitat [green boxes] and population structure [brown boxes]) and response variables (network and pollinator descriptors). Straight arrows: directional causal relationships; curved arrows: correlation of errors between exogenous variables.



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 (Q z-score) (a); and mean pollinator richness (total (b), peripheral (c), and core (d)) of 15 interaction networks of pollinators with *Butia odorata* individuals and predictor variables of population and habitat structure. Straight arrows: causal relationships; curved arrows: correlation of errors between exogenous variables; gray arrows: non-significant relationships (p-value > 0.05); *: 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 Q were tested in independent models and were shown in the same model here for simplification

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