

Plant-centred sampling estimates higher dissimilarity of interactions than pollinator-based sampling across habitats

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Abstract

When describing plant-animal networks, sampling can be performed using plant- or animal-centred approaches. Importantly, while the method affects the characterisation of network structure, how it may affect estimates of interaction dissimilarity across networks is still unknown. Here, we investigated how sampling affects the characterization of pollination networks and their dissimilarities across habitats in a heterogeneous tropical landscape. We also asked whether plant traits influence the difference in interaction specialization according to sampling. Plant-centred networks reported higher interaction and species dissimilarity in space, mainly due to interaction rewiring, while animal-centred networks showed higher specialization and modularity. Floral type and pollination systems affected how specialization was influenced by the sampling method. Combining animal- and plant-centred approaches returned intermediate values for dissimilarity and network metrics, indicating that complementary methods should be used for a better characterization of interaction networks, especially those including groups with distinct mobilities, such as plant and pollinators.

Introduction

Biodiversity is heterogeneously distributed, and the underlying factors that causes its variation across space and time has attracted much attention (Soininen et al. 2018). The biodiversity component that expresses this heterogeneity, beta diversity, captures the dynamic nature of species' distribution patterns (Whittaker 1960). Beta diversity patterns across gradients are used to understand the underlying factors that causes variation in diversity, i.e. the causality between process and patterns (Baselga 2010). It is thus crucial to understand sampling effects on beta diversity assessments, especially in plant-pollinator interactions that can be sampled by many different methods (Vizentin-Bugoni et al. 2018). Moreover, not only species but their interactions are known to vary across ecological gradients (Poisot et al. 2012; Trøjelsgaard et al. 2015; Tylianakis & Morris 2017), including for plants and their pollinators (Burkle & Alarcon 2011; Carstensen et al. 2014; CaraDonna et al. 2017). Nevertheless, while environmental gradients often predict dissimilarity in species composition (turnover component), the dissimilarities of interactions between the shared species among communities (rewiring component) is poorly understood (Burkle & Alarcon 2011; Carstensen et al. 2014; Trøjelsgaard et al. 2015). A better comprehension of interaction beta diversity would help better predict pairwise interactions, and ultimately contribute to conservation of interactions and associated ecosystem services (Trøjelsgaard et al. 2015; Burkle et al. 2016).

Plant-pollinator interactions may be sampled either using a plant-centred (phytcentric) approach, which

consists in identifying and quantifying interactions observing focal flowering plants, or an animal-centred (zoocentric) one, in which interactions are inferred through the identification of pollen grains attached to pollinators' bodies (Bosch et al. 2009; Jordano 2016; Zhao et al. 2019). The plant-centred approach is by far the most common one in the existing literature on pollination networks (Vizentin-Bugoni et al. 2018) probably because it is the most efficacious method and pollen sampling from example vertebrates is not straightforward. This overrepresentation of one method in relation to another may be especially relevant, as indices derived from the two methods sometimes lead to distinct estimates of network structure (Bosch et al. 2009; Ramirez-Burbano et al. 2017; Zhao et al. 2019). For instance, the addition of pollen load to visitation data recovered higher species degree and connectance in a Mediterranean pollination network (Bosch et al. 2009).

Although there is rich literature on the structure of pollination networks, we are not aware of studies evaluating the dissimilarities of networks according to sampling methods. While the plant-centred method concentrates on static organisms, the animal-centred method focuses on highly mobile organisms, usually with flight abilities. This fundamental difference in mobility between the two focal groups also means that while there is a delimited spatial sampling unit in the plant-centred method, this is not the case in the animal-centred approach which captures pollinator species with distinct mobility (Jordano 2016; Ramirez-Burbano et al. 2017). Since flying organisms tend to show lower beta diversity across communities than nonmobile organisms such as plants (Kessler et al. 2009; Carstensen et al. 2014), it is likely that sampling method affects the estimates of beta-diversity of interactions.

Here we analysed plant-pollinator interaction networks from the largest continuous floodplain in the world, the Pantanal. We ask: (1) how sampling methods (animal and plant-centred approaches) influences the patterns of beta diversity of interactions, network metrics and species level indices; (2) if possible differences in species level indices are related to plant traits (e.g. pollinator group, resource offered, size and type of flowers). We expect to find higher specialization and modularity in animal- than plant-centred networks due to the greater refinement of interactions obtained through the analysis of pollen grains attached to the pollinators' body (Bosch et al. 2009; Zhao et al. 2019). Furthermore, pollen grains may adhere more or less to the bodies of these animals, or pollinators may have different behaviours during the visit to the flowers, affecting estimates of specialization (Ballantyne et al. 2015; Zhao et al. 2019). In addition, plants with non-specialized morphology and generalist pollination system will possibly be associated to higher differences on species level metrics between the two sampling methods. Regarding beta diversity, due to differences in vegetation structure and composition in the Pantanal vegetation, we expect considerable turnover of species and interactions across the landscape (Maruyama et al. 2014; Tylianakis & Morris 2017). We also expect to find lower interaction dissimilarity in animal-centred networks, owed to higher mobility of pollinators which would blur the spatial limits of plant-centred approach and yield lower variability on interacting partners across space.

We show that the sampling methods influenced the assessment of species dissimilarity, beta diversity of interactions and network patterns. Plant-centred methods yield higher species and interaction dissimilarities across space and lower levels of modularity and specialization. Furthermore, pollination systems and floral type were related to differences on estimates of species level specialization between methods. Specifically, plants pollinated by oil-bees showed higher difference in specialization than plants pollinated by small bees. In addition, flag and gullet flowers had higher difference in specialization between methods than dish type flowers. These findings help to fill in a key gap in the understanding of how dissimilarities across interaction networks are characterized according to the sampling approach used, hence on the inferences on spatial variation of ecological networks.

Material and methods

Study site and periods

We collected data on plants and their potential insect pollinators in Southern Pantanal, located in the Central region of Brazil. The Pantanal is the largest continuous floodplain in the world and spans 140.000 km². Its

location, surrounded by the main Brazilian vegetation domains, results in a biota with representatives of these neighbouring provinces. It is influenced by the Amazon in the north; by Chaco to the west; by Atlantic Forest in the southeast and by the Cerrado, which covers the plateaux surrounding the Pantanal, in its eastern portion (Alho 2008). The geographical location, the interaction between soil and biogeographic factors, as well as the occurrence of seasonal flooding, are factors that explain the mosaic of vegetation types that characterize the Pantanal (Alho 2008). This heterogenous landscape, as well as differences in flooding regimes, result in a variety of phytophysionomies such as riparian forests, monodominant pioneer plant formations, ponds with aquatic plants and savannas (Nunes da Cunha & Junk 2009; Pott et al. 2011).

The study area is characterized by a marked seasonality, presenting a warm and rainy season from October to March, and a relatively colder and dry period from April to September (see Souza et al. 2018). It is characterized by the presence of a high and threatened biodiversity (Ramsar & MMA 2010). Studied plots included three distinct and representative habitat types in the region: 1) “Paratudal” mono-dominant stands of flooded savannah dominated by *Tabebuia aurea* (Bignoniaceae); 2) “Canjiqueiral” mono-dominant stands of *Byrsonima cydoniifolia* (Malpighiaceae); and 3) Riparian forest (Nunes da Cunha & Junk 2009; Fig. S1). Despite the monodominance of distinct plant species in Canjiqueiral and Paratudal, other plant species occurring in the three studied habitat types show considerable similarity (Pott et al. 2011).

Plant-pollinator interactions data were sampled monthly for two years, from October 2014 to September 2016, nearby the Pantanal Field Station of the Federal University of Mato Grosso do Sul (57°02'22.80" W; 19°34'52.24" S), in the Miranda sub region of Southern Pantanal (Alho 2008). We selected 36 areas (12 for each habitat), and in each of these areas we established ten 10x10m plots, at least 50 meters apart, with a total of 3600m² and 750 hours of sampling (Fig. S1).

Plant-pollinator interaction sampling

For the plant-centred sampling, we carried out observations of pollinators on flowers between 0700h and 1700h. We did ten minutes of focal observations on each individual flowering plant within the plots (usually four/five days of data collection per month). In order to include time-dependent variations on plant-pollinator interactions, we recorded all plots at different periods of the day during the sampling period. In addition, we conducted focal observations on a given plant species both in the morning and in the afternoon. Sampling effort depended on species occurrence, phenology, and abundance, resulting in a variable observation time for each species (range, mean \pm standard deviation): 10-1780min, $x=177\pm 293$ min per plant species. We recorded the frequency of visitation to flowers and only included in the analysis the legitimate interactions (i.e. when the floral visitor contacted the reproductive structures of the flowers). Hereafter, these legitimate visitors are referred as “pollinators”. We collected vouchers for all plant species that were identified and deposited in the CGMS Herbarium. The family names followed the Angiosperm Phylogeny Group (APG IV 2016), and species names were confirmed in the Plant List database (<http://www.theplantlist.org/>) and updated/corrected whenever necessary.

For the animal-centred sampling, after a visit was recorded, we captured the insect visitors and placed them in separate clean kill-vials for exterior pollen sampling and identification in the laboratory. For pollen sampling, we rubbed small cubes of fuchsine-stained gelatine on the insect’s body, and then placed the gelatine on glass slides (Dafni 1992; Bosch et al. 2009). We avoided pollen storage areas such as pollen baskets of bumblebees because these contain pollen unlikely to be available for pollination. We identified pollen grains mainly by comparison to a reference palynological collection we assembled for the study region, and with the aid of literature (e.g. Erdtman 1952). We built the reference collection using the same technique employed for pollen on pollinators, collecting pollen grains from all flowering plants we found in the study area. We deposited the collected insects at the Zoological Collection of Federal University of Mato Grosso do Sul (ZUFMS), and the pollen slides at the CGMS Herbarium. For the animal-centred pollen transport network we considered the number of pollinator individuals carrying a given pollen morphotype as the estimate of interaction frequency (Fig. 1). This was done instead of counting the number of pollen grains on pollinators’ body as different plant species vary greatly in the amount of pollen produced and on how the pollen grains stick to the body of the flower visitor (e.g. presence of pollenkitt), rendering such counts less informative (Maglianesi et al.

2015; Sazatornil et al. 2016; Ramírez-Burbano et al. 2017; Manincor et al. 2020).

Plant-pollinator interaction networks

We built a total of 36 networks of interactions for each method, 12 for each habitat type (Paratudal, Canjiqueiral and Riparian forest). Thus, there were 36 networks for the plant-centred method, 36 matrices for the animal-centred method and 36 for the combined method (plant + animal centred method). In addition, we built a general matrix for each method including all sampled habitats (3), totalling 111 matrices constructed.

We evaluated the sampling completeness of the plant-centred, animal-centred and the combined networks (Chacoff et al. 2012; Vzentin-Bugoni et al. 2016). For this purpose, each combination of a plant and a pollinator species is taken as “species” and the frequency of each pairwise interaction represents its “abundance” (Vzentin-Bugoni et al. 2016). We estimated the diversity of interactions using Chao 1 estimator of species richness (Chao 1984; Colwell & Coddington 1994). Then, we calculated sampling completeness as the ratio of the observed and estimated richness of interactions (Chacoff et al. 2012). The Chao 1 estimator was computed using the “iNEXT” package (Hsieh et al. 2014) in R (R Development Core Team 2019). Sampling completeness of the studied networks are shown in the supplementary material (Table S1).

We evaluated how species partition their interactions using two quantitative metrics for combined, plant and animal-centred networks. Network-wide specialization was estimated by the H_2' index that describes whether species restrict their interactions from those randomly expected based on partner’s availability (Blüthgen et al. 2006). Modularity indices quantify the prevalence of interactions within subsets of species in the community and was calculated using the LPAwb+ algorithm (Beckett 2016). Besides these two network-level indices, we also calculated two species-level indices that capture distinct topological properties of a species in the network: 1) degree, expressing the number of interaction partners that each species is linked to in the network; and 2) species-level specialization d' , which quantifies how interaction frequencies of a given species deviate in relation to the availability of interaction partners in the network, with higher values indicating higher specialization (Blüthgen et al. 2006). We calculated all network-related indices with the “bipartite” package version 2.14 (Dormann et al. 2008) in R (R Development Core Team 2019).

Beta diversity of interactions

To evaluate how sampling methods (plant- or animal- centred) capture the variation of species and interactions across habitats, we calculated the β -diversity between all pairwise combinations (a total of 1296 pairwise combinations). Specifically, we calculated the β -diversity of plants and pollinator species separately (β -plants and β -pollinators), the dissimilarity of all plants and pollinators (β_{ST}), the dissimilarity of interactions between shared species, i.e. rewiring (β_{OS}), and the dissimilarity of all interactions in the network ($\beta_{WN} = \beta_{ST} + \beta_{OS}$). All these measures were calculated using the framework proposed by Poisot et al. (2012) and the quantitative extension from Magrath et al. (2017), which employs quantitative measures of β -diversity based on the Ruzicka distance coefficient (Legendre 2014). This metric takes values of 0 when the interaction composition is the same, whereas values of 1 indicate a completely different composition. These analyses were conducted in R (R Development Core Team 2019), using the code at github (https://github.com/ibartomeus/betalink/tree/new_features). In addition, we calculated the β -diversity measures within the same habitat (Canjiqueiral, Riparian forest and Paratudal) and between habitats in order to verify whether the beta diversity would be greater between the different sampled habitats than within the same sampled habitats.

Statistical analysis

We contrasted plant–pollinator network indices according to sampling method using linear mixed-effects models in the “lme4” package in R (Bates et al. 2015). We took network indices (modularity and specialization) as response variables, network type (plant- or animal-centred) as fixed- and plot identity as random-factors in the models. The statistical significance was tested using a likelihood ratio test comparing the model with and without the fixed factor using the R package “car” (Fox & Weisberg 2011). Similar models were constructed considering β -diversity of plants and pollinators, total species turnover (β_{ST}), in-

teractions rewiring (β_{OS}) and all interactions in the network (β_{WN}) as response variables. We contrasted the species level indices (degree and specialization) between the two sampling methods for both plants and pollinators. We considered species level indices as response variables, network type as fixed factor and identity of species and plots as random factors in the models. We log10 transformed species degree and beta diversity of species and interactions within and between the three different habitats studied to improve the distribution of the residuals. After detecting significant factors in the models, we conducted multiple comparisons (post hoc Tukey test) using the function `glht` in the package “multcomp” (Hothorn et al. 2008). In addition, we compared the contribution of rewiring (β_{OS}) and turnover (β_{ST}) to the total beta diversity of interactions (β_{WN}) and compared plants and pollinators dissimilarity between sites using a `t` test.

In order to understand how plant species level indices (degree and specialization) are determined by plant traits, we performed linear models including plant species traits: 1) floral type (flag, gullet, dish, inconspicuous, brush and tube); 2) flower size (width and length); and 3) pollination system: small bees, large bees, oil bees, butterflies (including moths), beetles and “dsi” (diverse small insects) (modified from Frankie et al. 1983; Danieli-Silva et al. 2012; Table S2). Since we only sampled one species with a beetle pollination system (*Annona nutans*), we removed it from the analysis. We included all plant traits as fixed effects, and species level indices as response variables. This analysis was done only for the combined network and we log10 transformed species degree prior to the analysis to improve the distribution of the residuals.

We also built linear models using the absolute difference of degree and specialization between the plant-centred and combined network as response variables ([?]specialization d' and [?]degree), to evaluate how plant traits modulated the effect of adding pollen data on estimates of species specialization. Then, for significant fixed effects we conducted multiple comparisons with posthoc Tukey test using the function `glht` in the package “multcomp” (Hothorn et al. 2008). All statistical analyses were conducted in R (R Development Core Team 2019).

Results

In total, we recorded 99 pollinator species (33 bees, 14 flies, 13 lepidopterans; 18 wasps and 22 diverse small insects) and 125 plant species in our field surveys, resulting in 6614 interactions (Fig. 1). The plant-centred network from Pantanal comprised 4609 interactions among 99 pollinators and 106 plant species, while the animal-centred network comprised 2006 interactions and a total of 47 pollinators and 95 plants species. Among plants, 78 species were recovered by both sampling methods, 31 species only with the plant- and 19 species only in the animal-centred networks. Most of pollen samples (89%) were identified to species level using the reference collection. In relation to pollinators, 44 species occurred in both networks, 55 species occurred only in the plant-centred network and three species were unique to the animal-centred network.

Network structure, species level indices and their relationship with floral traits

The 36 animal-centred networks showed higher levels of interaction partitioning than the plant-centred ones considering both specialization (animal-centred: $x = 0.77 \pm 0.08$, plant-centred: $x = 0.67 \pm 0.17$; $\chi^2 = 9.24$, $P < 0.0001$) and modularity (animal-centred: $x = 0.74 \pm 0.08$, plant-centred: $x = 0.35 \pm 0.18$; $\chi^2 = 85.78$, $P < 0.0001$; Fig. 2; Table S3). When the animal-centred network was used as a complement to the plant-centred network, the levels of specialization ($x = 0.70 \pm 0.14$) and modularity ($x = 0.52 \pm 0.14$) resulted in intermediate values (Fig. 2).

Considering species level indices, degree (animal-centred: $x = 3.94 \pm 5.29$, plant-centred: $x = 3.78 \pm 5.80$; $\chi^2 = 75.146$, $P < 0.001$) and specialization (animal-centred: $x = 0.47 \pm 0.22$, plant-centred: $x = 0.41 \pm 0.26$; $\chi^2 = 49.45$, $P < 0.0001$) were higher in animal-than plant-centred networks (Table S3). Aggregating animal- and plant-centred networks resulted in higher degree than in each of the two methods separately ($x = 4.38 \pm 6.37$), while specialization ($x = 0.45 \pm 0.24$) resulted in intermediate values.

Plants with distinct pollination systems differed in species degree ($F = 4.29$; $P < 0.01$; Fig 3a) and specialization ($F = 6.60$; $P < 0.0001$; Table S4) in the combined network. Specifically, plant species with oil-bee pollination had a higher species and specialisation degree than plants pollinated by large bees and

dsi (diverse small insects). This result was consistent even after removing from the analysis the outlier mono-dominant *Byrsonima cydoniifolia* (Malpighiaceae), a highly connected oil-producing species. For specialization, plants pollinated by oil-bees showed higher specialization than plants pollinated by small bees and dsi (Fig. 3b; Tukey test $P < 0.05$). None of the evaluated factors explained [?] degree estimated between the plant-centred and the combined network ($F = 1.44$; $df = 4$; $P = 0.18$). On the other hand, oil-bee pollinated plants showed higher differences on specialization between sampling methods than the observed for small-bees pollinated plants. In addition, there was a large difference on the specialization of flag and gullet flowers between sampling methods compared to dish flowers (Fig. 3d).

Beta diversity of interactions and beta diversity indices

We recorded a high turnover on interactions across both sampling methods and in the combined networks. Regardless of the method used, interaction turnover was mostly due to rewiring (β_{OS}) in comparison to species turnover (β_{ST}) (Fig. 4a, in the combined network $\beta_{OS} = 0.65 \pm 0.28$ and $\beta_{ST} = 0.18 \pm 0.22$; $T = 31.89$; $P < 0.0001$). Plants showed greater dissimilarity between networks than animals (β -plants: $x = 0.76 \pm 0.15$, β -animals: $x = 0.65 \pm 0.22$; $P < 0.0001$, Fig. 4b). Considering the different sampling methods, the plant-centred method presented the highest values of beta diversity for both plants and pollinators ($\chi^2 = 29.27$, $P < 0.0001$ and $\chi^2 = 76.61$, $P < 0.0001$, respectively; Fig. 4b; Table S3). Following the same trend, the plant-centred method returned the highest values of interaction beta diversity. The rewiring component of interaction beta diversity differed between methods ($\chi^2 = 11.58$, $P < 0.0001$), with the plant-centred network showing higher values than the animal-centred network, but no differences were recorded between the combined network and the other two (Fig. 4a). In contrast, species turnover differed among the three approaches ($\beta_{ST} - \chi^2 = 45.74$, $P < 0.0001$, Tukey test $P < 0.05$). Thus, the total beta diversity of interactions (β_{WN}) also resulted in higher values in the plant-centred network compared to both animal-centred and combined networks ($\chi^2 = 57.90$, $P < 0.0001$; Fig. 4a; Table S3).

The rewiring of interactions did not differ within and between habitats ($\chi^2 = 1.55$, $P = 0.21$, Fig 5a), and the interaction turnover was significantly higher between than within habitats ($\chi^2 = 15.68$, $P < 0.001$, Fig 5b). The total dissimilarity in the landscape considering the combined network was generally high, but did not differ between and within habitats (β_{WN} within habitats: $x = 0.83 \pm 0.20$; β_{WN} between habitats: $x = 0.85 \pm 0.19$; $\chi^2 = 58.49$, $P = 0.07$; Fig. 5c; Table S5). When considered separately, beta diversity of plants was significantly higher between than within habitats ($\chi^2 = 6.93$, $P < 0.001$, Fig 5d), but this was not the case for the animals ($\chi^2 = 1.21$, $P = 0.27$; Fig. 5e; Table S5e).

Discussion

We show, for the first time, that the sampling method significantly affects how interaction dissimilarity across space is characterized in a heterogeneous tropical landscape. This finding is likely caused by differences in the mobility of organisms embedded in the ecological web, since across all analyses animals showed lower dissimilarity than plants. Importantly, some differences between methods may be linked to plant's floral traits, especially the type of flowers and the pollination systems of plant species. These outcomes have important consequences on how plant-pollinator networks are described and that organism groups are affected differently by the complementary sampling methods.

Network structure, species level indices and their relationship with floral traits

Although pollen data do not always lead to higher estimates of interaction partitioning in pollination networks (Ramirez-Burbano et al. 2017; Manincor et al. 2020), there is a trend that subsequently calibrating interaction data with “better” estimates of potential pollination, i.e. pollen loads, pollen deposition on stigma and fruit set, will increasingly render only the subsets of interactions constrained by specific co-adaptations and higher estimates of interaction specialization (Bosch et al. 2009; Santiago-Hernandez et al. 2019; Zhao et al. 2019). Considering only the visitors that contact the reproductive structures of flowers is a first step in including only potential pollination interactions, but not all such visitors actually carry and deposit pollen (e.g. Mesquita-Neto et al. 2018; Hernandez et al. 2019), leading to distinct estimates of specialization according to the currency used to estimate interactions. Although pollen in the body of

pollinators may have other fates than deposition on stigmas (Bosch et al. 2009; Ballantyne et al. 2015), calibrating the interaction networks with currencies that better approximates pollination will likely better reflect the most effective pollinators that drive convergent evolution of floral forms (Rosas-Guerrero et al. 2014). A refinement of pollination networks with better estimates of interactions is certainly important to better understand the community level evolutionary drivers of plant-pollinator associations (Phillips et al. 2020), including for interaction dissimilarities (Trøjelsgaard et al. 2015).

The differences according to method observed at the network level were partially reflected at the species level, and importantly it was related with some species traits. Oil flowers had both higher degree and higher specialization than some other pollination systems such as non-oil bee pollinated plants and species associated with small diverse insects that characterize more opportunistic pollinator groups (Lima et al. 2020). Besides that, oil rewarding plants also showed greater differences on specialization values depending on the method when compared to plants pollinated by small bees. Hence, oil pollination had more bee species as pollinators than some other systems, but these are a specialized subset of bees not observed in other plants. Floral oil is a crucial resource for solitary bees that feed the larvae with a mixture of oil and pollen (Simpson & Neff 1981), and many interactions of oil flowers were only detected through pollen loads. The more specialized nature of this resource as well as the often short flowering period of oil plants (Barônio et al. 2017; Souza et al. 2018) may explain the difficulty in detecting these interactions by the plant-centred method, and pollen based method could be especially interesting for detecting interactions for this group of plants (Dorado et al. 2011; Rabelo et al. 2015). Gullet and flag floral types were associated with higher differences in specialization levels between methods than others, which may be caused by the fact that these floral types show floral morphologies that are often more restrictive to floral visitors (Westerkamp & Claßen-Bockhoff 2007, Olesen et al. 2007) and hence less well-detected.

Beta diversity of species and interactions

Plant-centred sampling indicated higher interaction and species beta-diversity in space. Beta diversity of interactions is driven by both changes in species composition, which depends largely on the environmental tolerances, phenology and dispersal ability of interacting partners (Trøjelsgaard et al. 2015; Graham & Weinstein 2018; Noreika et al. 2019), and rewiring, when co-occurring species interact differently over gradients owed to changes in the costs and benefits of interactions (CaraDonna et al. 2017). While plant-centred sampling is limited in space by the chosen sampling unit, animal-centred networks vary in spatial coverage according to the mobility of pollinators. Reflecting that, with the plant-centred method around half of plants (54%) and pollinator (51%) species were found in more than three sampled networks, while in the animal-centred sampling most plants (85%) and pollinator (96%) species occurred in more than three networks. Therefore, the higher mobility of pollinators compared to plants indicate that pollinators rewire their interactions across space, with a resulting decrease on the dissimilarity of interactions. This is also reinforced by the higher species turnover detected between habitats for plants than for animals.

It has been argued that animal-centred sampling could help unveil rare interactions not detected by direct observations for both rare and common plant species (Dorado et al. 2011). Such additional detection blurs the “artificial” separation of different networks imposed by sampling units, revealing greater uniformity in interaction across space, and a lower dissimilarity of interactions. However, distance-decay is an expected pattern for network similarities if the spatial scale is sufficiently large, suggesting a threshold where there are shifts and delimitation in the coevolutionary dynamics of local plant-pollinator communities (Trøjelsgaard et al. 2015). Furthermore, interaction rewiring, rather than species turnover, was the dominant driver of interaction turnover. This pattern was consistent across both methods and the combined networks, providing evidence that rewiring plays an important role on the dynamics of ecological networks (CaraDonna et al. 2017), which indicates that species show high flexibility/plasticity in their interactions across the heterogeneous landscape.

Conclusions

In summary, sampling method affects not only how plant-pollinator network structures are characterized,

but also the dissimilarity among networks. Moreover, we also show that some plant traits contributed to method-related differences on estimates of interaction specialization, including pollination systems and floral morphology. Importantly, our results highlight that plant-centred sampling reflects the spatial limits defined by the researcher, which is usually smaller than the flight capacity of many of the interacting organisms. In fact, because distinct pollinator species and groups will have distinct mobilities, choosing a unique “correct” sampling unit may not be possible. In this regard, even when considering distinct habitat types in the studied heterogeneous landscape, animal-based sampling still contributes to overall lower levels of dissimilarity among networks. Hence, flying pollinators show considerable flexibility to rewire their interactions in space. Since understanding the variation in architecture of interaction networks and flexibility of species to rewire interactions across environmental gradients has important implication for conservation (Burkle et al. 2016) and coevolutionary dynamics (Tylianakis & Morris 2017) of plant-pollinator communities, we advocate for using distinct and complementary methods to estimate interaction network structure and dissimilarity. This is likely most relevant in interactions involving organisms with considerable differences in mobility, such as plants and their pollinators.

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Figures legends

Figure 1. Interaction networks and their sampling methods. We used the frequency of visits for plant-centred network and the frequency of pollinator individuals with pollen grains of a given plant species for the animal-centred network as currencies of interactions. The two methods were aggregated to form the combined networks.

Figure 2. Network metrics describing the structure of interaction networks between plants and pollinators in the Pantanal. We used visitation (plant-centred) and pollen load (animal-centred) to sample interactions. The differences in specialization (H_2'), and modularity (Q_w) of the networks using the different methods and their combination are shown in figures (a) and (b).

Figure 3. (a) Differences on plant species degree and (b) specialization d' among the different pollination systems (combined network); pollination systems (c) and the type of flowers (d) were associated to absolute differences in species level specialization according to sampling method ([?] d' specialization). Pollinators images were obtained from www.divulgare.net.

Figure 4. β diversity of species and interactions according to sampling methods (animal-centred, plant-centred and combined method). a) Interaction β diversity, β_{OS} - rewiring, β_{ST} - turnover and β_{WN} - total beta diversity of interactions; b) species β diversity for animals and plants.

Figure 5. β diversity of interactions and β diversity of species measures within and between the three different studied habitats (Canjiqueiral, Riparian forest and Paratudal), considering the combined networks: a) rewiring of interactions - β_{OS} ; b) turnover of interactions - β_{ST} ; c); total β diversity of interactions - β_{WN} ; and species turnover for d) animals and e) plants.

Figure 1

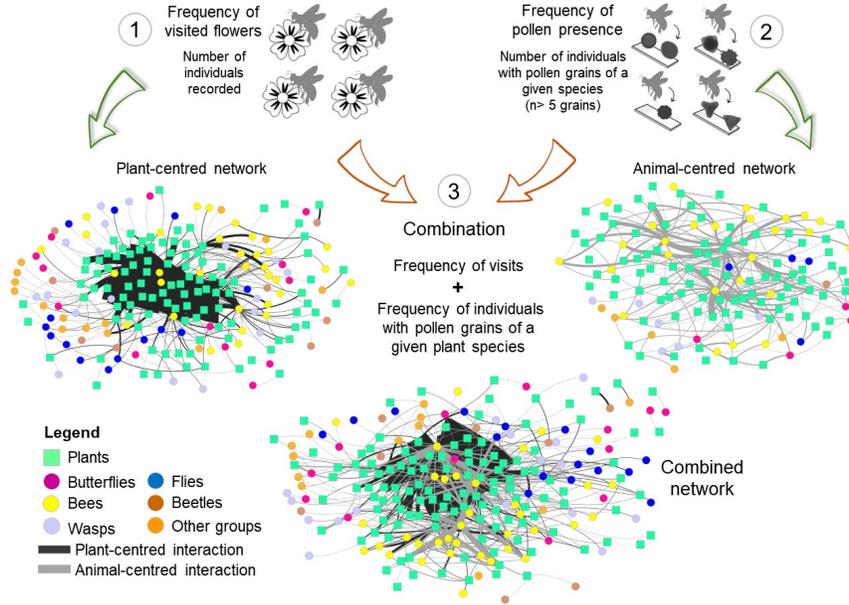


Figure 2

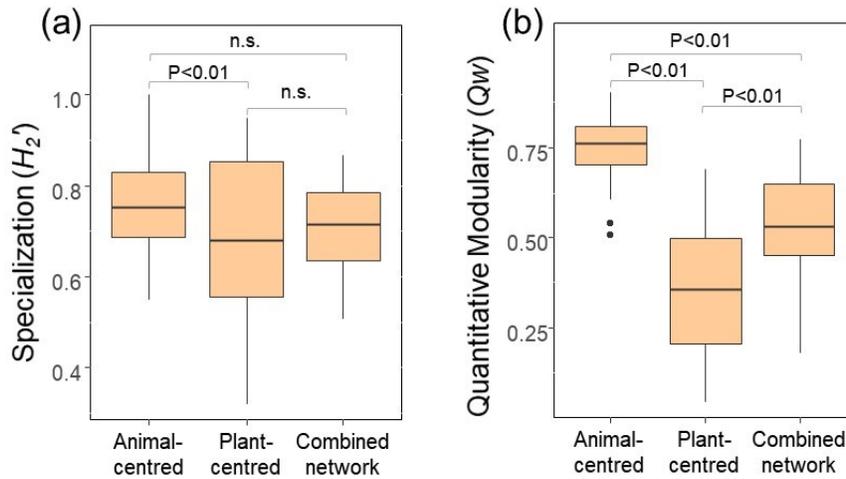


Figure 3.

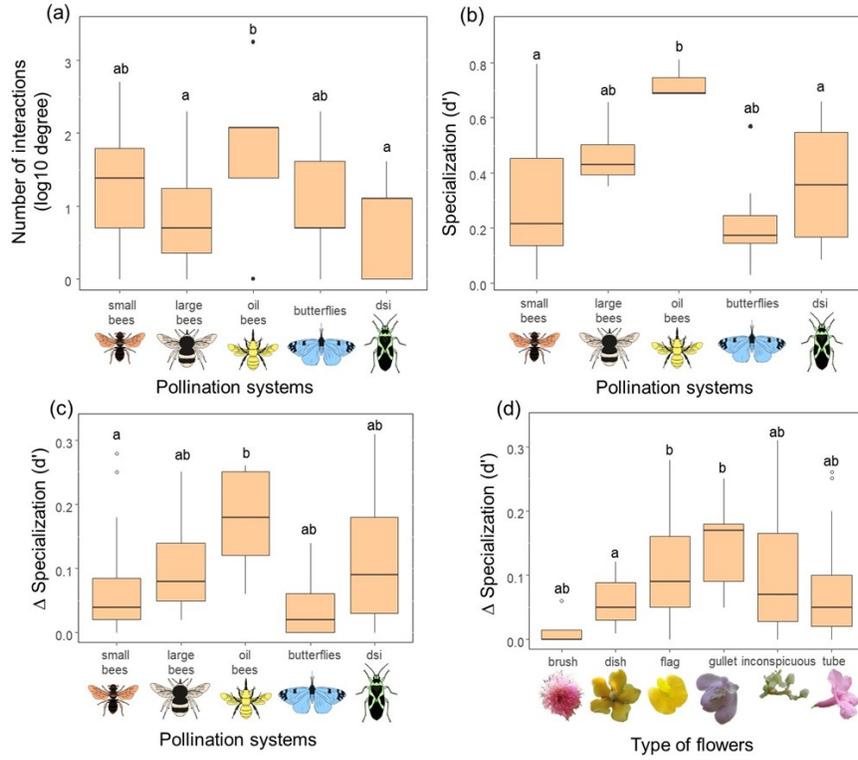


Figure 4

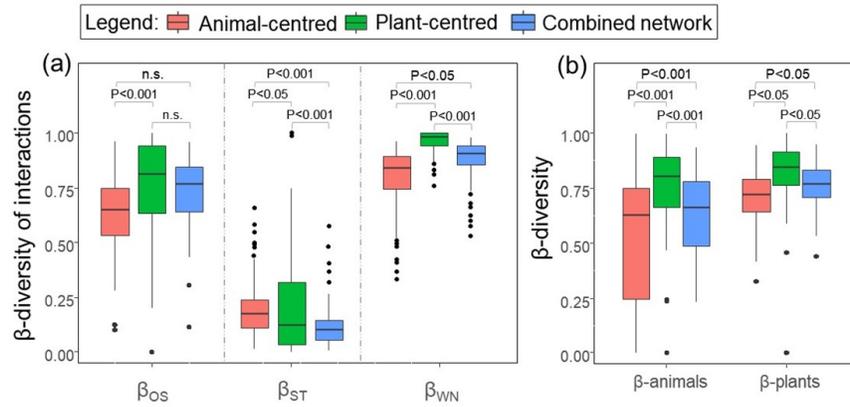


Figure 5

