UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

A invasão de um ecossistema campestre Neotropical por uma gramínea exótica altera a estrutura e o funcionamento das interações planta-abelha

Exotic grass invasion alters the structure and functioning of plant-bee interactions in a Neotropical grassland ecosystem

Leandro Hachuy Filho

Dissertação apresentada ao Instituto de Biociências, câmpus de Botucatu, UNESP, para obtenção do título de Mestre no Programa de Pós-Graduação em Ciências Biológicas (Botânica), Área de concentração Ecologia Vegetal.

BOTUCATU – SP

2019

Instituto de Biociências, UNESP, Câmpus de Botucatu
Seção Técnica de Pós-Graduação
Rua Professor Doutor Antonio Celso Wagner Zanin, s/nº, Botucatu, SP – CEP 18618-689
Telefone (14) 3880-0780 posgraduacao@ibb.unesp.br

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

UNIVERSIDADE ESTADUAL PAULISTA
"Júlio de Mesquita Filho"
INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

A invasão de um ecossistema campestre Neotropical por uma gramínea exótica altera a estrutura e o funcionamento das interações planta-abelha

Exotic grass invasion alters the structure and functioning of plant-bee interactions in a Neotropical grassland ecosystem

Leandro Hachuy Filho

Felipe Wanderley de Amorim
Orientador

Dissertação apresentada ao Instituto de Biociências, câmpus de Botucatu, UNESP, para obtenção do título de Mestre no Programa de Pós-Graduação em Ciências Biológicas (Botânica), Área de concentração Ecologia Vegetal.

BOTUCATU – SP

2019

Instituto de Biociências, UNESP, Câmpus de Botucatu
Seção Técnica de Pós-Graduação
Rua Professor Doutor Antonio Celso Wagner Zanin, s/nº, Botucatu, SP – CEP 18618-689
Telefone (14) 3880-0780 posgraduacao@ibb.unesp.br

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSANGELA APARECIDA LOBO-CRB 8/7500

Hachuy Filho, Leandro.
Exotic grass invasion alters the structure and functioning of plant-bee interactions in a Neotropical grassland ecosystem / Leandro Hachuy Filho. - Botucatu, 2019

Dissertação (mestrado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências de Botucatu
Orientador: Felipe Wanderley Amorim
Capes: 20502001

1. Pollinators. 2. Fertilization of plants. 3. Ecosystem. 4. Grassland. 5. Biodiversity.

Palavras-chave: Functional diversity; Interaction turnover; Plant-pollinator interaction network.

Agradecimentos

Agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pois sem o seu auxilio, a realização deste trabalho não seria possível.

Ao meu orientador Prof. Dr. Felipe W. Amorim por ter aceitado trabalhar comigo e me orientar durante o mestrado, depositando confiança no meu trabalho.

Aos colegas Professores Pietro Maryuama e Jeferson Vizentin-Bugoni pelas considerações que ajudaram no desenvolvimento do trabalho.

Aos professores Ana Paula Fortuna e Eduardo Almeida pela ajuda com a identificação de plantas e abelhas, respectivamente.

Ao amigo e colega de laboratório Caio Simões Ballarin pela ajuda na articulação das ideias e redação do manuscrito.

Aos colegas e amigos de laboratório Diego Polizello, Murilo Massufaru Giffu, João Vitor Alcantara, Maria Júlia Walermarin Doria, Guilherme Alcaras, Julyana Izquierdo e Julia Almeida Monteiro que ajudaram na execução do trabalho em geral, sendo com apoio no campo ou com ideias posteriores.

À minha namorada Adriana Tanigawa Bitiati pela ajuda com a revisão do manuscrito e pelo apoio durante as fases mais cansativas do trabalho.

Não poderia deixar de agradecer a toda a minha família pelo apoio e principalmente pelos exemplos, especialmente minha mãe, Cecilia Helena Muniz Cirne e meu avô Bario Raymundo Cirne, que compartilhou comigo durante toda a vida suas experiências com a pesquisa e ensino.

Obrigado a todos!

Summary

Resumo

As mudanças globais mediadas pela ação antrópica estão alterando a biodiversidade e os ecossistemas em um ritmo acelerado. Um dos principais impulsionadores dessas mudanças é a introdução de espécies exóticas em ecossistemas nativos. Entre os grupos de organismos afetados por este processo, o das plantas é reconhecido um dos mais preocupantes, uma vez que a produção primária limita o tamanho e a composição das comunidades e participa através de efeitos em cascata em interações multi-tróficas. Uma das principais relações ecológicas influenciada por esse efeito é a relação entre plantas e polinizadores, cujo papel é importante para estrutura e funcionamento das comunidades biológicas, não apenas porque as plantas fornecem recursos alimentares essenciais para muitos grupos de animais que visitam flores, mas também porque o sucesso reprodutivo da maioria das plantas com flores depende dos serviços bióticos fornecidos por estes animais. Neste contexto, a introdução de espécies de plantas exóticas invasoras pode ter impactos críticos nas interações planta-polinizador ao nível da comunidade, principalmente através da competição com espécies nativas. Como as interações planta-polinizador são cruciais para determinar a estrutura da comunidade, nesse estudo nós testamos como o crescimento rápido de uma gramínea invasora altera a composição das espécies de plantas nativas em um campo cerrado, juntamente com os impactos deste processo sobre a estrutura das interações planta-polinizador. Para isso, analisamos as mudanças na composição da comunidade e nas interações planta-polinizador ao longo do tempo, através da mudança temporal (diversidade β ou *turnover*) das espécies de plantas e polinizadores, das interações planta-polinizador e dos atributos funcionais das plantas. Nossos resultados mostram que o crescimento da gramínea invasora *Brachiaria decumbens* alterou a composição da comunidade de plantas, que por sua vez também afetou as interações entre plantas e abelhas. Tais mudanças foram acompanhadas por um alto *turnover* das interações entre as espécies, juntamente com mudanças no papel dos polinizadores dentro da rede de interação planta-polinzador. Dessa forma, nosso estudo mostrou que o rápido crescimento de uma espécie de gramínea exótica e invasora em um ecossistema campestre tem um grande impacto nas interações planta-polinizador através de efeitos bottom-up, que marcadamente mudam a estrutura e o funcionamento da comunidade.

Palavras-chave: Diversidade funcional, turnover de interações, rede de interação planta-polinizador

Abstract

The global change mediated by anthropic action is altering biodiversity and ecosystems in a fast pace. One major driver of these changes is the introduction of alien species in native ecosystems. Among the groups of organisms that are affected by this process, plants are recognized to be one of the most concern, since primary production limit global communities' sizes and composition, and participate through cascade effects on multi-trophic interactions. One crucial type of interaction that is influenced by this effect is the plant-pollinator relationship, which have an important role in the structure and functioning of biological communities, not only because plants provide essential food resources for many groups of animals that visit flowers, but also because the reproductive success of most flowering plants depends on the biotic services provided by animals. In this context, the introduction of invasive alien plant species may have critical impacts on plant-pollinator interactions at community level, mainly through competition with native species. Since plant-pollinator interactions are determinants of community structure, here we evaluated how the rising of a fast-growing invasive alien grass species changes plant species composition of a Neotropical grassland community along with its impacts on the structure of plant-pollinator interactions. For this, we analyzed the changes in community composition and plant-pollinator interactions over time, through the temporal turnover (β-diversity) of plants and pollinators species, plant-pollinator interactions and plant functional traits. Our results show that the rising of the invasive alien grass, *Brachiaria decumbens*, changed the composition of plant community, which impacted plant-bee interactions. Such changes were followed by a high turnover of species interactions along with shifts in pollinators' roles within the plant-pollinator interaction network. In conclusion, our study showed that the fast growth of an invasive alien species of grass in a grassland ecosystem has a great impact on plant-pollinator interactions via bottom-up effects which markedly changes community structure and functioning.

Keywords: Functional diversity, interaction turnover, plant-pollinator interaction network

Introduction

The global change mediated by anthropic action is altering biodiversity and ecosystems in a fast pace (Tilman et al. 2001). These changes are drove by many factors that interact in a complex way (Fischer and Lindenmayer 2007, Brook et al. 2008). One major driver of these changes is the introduction of alien species in native ecosystems, whose abundance influence community's biodiversity, that leads to impacts in multi-trophic interactions (Mack et al. 2000). Among the groups of organisms that are affected by this process, plants are recognized to be one of the most concern, since primary production limit global communities' sizes and composition (Wilkinson and Sherratt 2016). We know by many empirical studies that the impacts of invasive species are related to cascade effects that primarily alter the food chain. However, these effects can also be extended to other type of interactions within community, for example, the mutualism established by plants and pollinators, which is yet not well investigated.

Community-level interactions between plants and their pollinators have a crucial role in the structure and functioning of biological communities (Memmott and Waser 2002, Bergamo et al. 2018), not only because plants provide essential food resources for many groups of animals that visit flowers, but also because the reproductive success of most flowering plants depends on the biotic services provided by animals (Ashman et al. 2004, Aguilar et al. 2006, Ollerton et al. 2011). The visitation rates of pollinators, and consequently, plants reproduction depend on community trophic interactions controlled by both the highest and the lowest levels (i.e. "top-down" and "bottom-up" control; Croll et al 2005, Heithaus et al. 2008, Burkle and Irwin 2009, Baum and Worm 2009, Estes et al. 2011, Scherber et al. 2010, Shurin et al. 2012). Therefore, the introduction of invasive alien species may affect not only plants and their consumers (herbivores) but also, the plant reproduction itself through changes in the visitation patterns of pollinators (Karron et al. 2009, Bergamo et al. 2018). At community level, this process can cause several changes on the structure of plant-pollinator interactions, leading to shifts on pollinator roles and variation in their resource using preferences (Hiraiwa and Ushimaru, 2017), which can influence intra and inter-specific ecological processes such as competition and facilitation (Waser 1978, Ratchke, 1983; Bergamo et al. 2018).

The extension of these impacts depends on how native plants interact with the invader. A key ecological process that mediate this interaction, is the competition of plants for the availability of biotic and abiotic resources (Casper and Jackson 1997, Craine and Dybzinski 2013). In grassland communities such as the cerrado vegetation (Neotropical savannas of Brazil) dominated by herbaceous plants, the individuals are more susceptible to an intensified competition for light, since a slight difference in a plant height and leaf area can confer a significant advantage, limiting the growth and development of neighboring shorter plants (Newman 1973, Silvertown 2004, Martin et al. 2009). Therefore, the competition generated by invasive plants can affect the establishment of native plants by limiting their growth and decreasing their abundance within a given community, which in the end, may decrease the overall community diversity and reduce the availability of floral resources to pollinators (Hendrickx et al. 2007).

The introduction of invasive alien grass plants is particularly reported in the Brazilian savannas, where many species of fast-growing African grasses have been introduced along the last century (Williams and Baruch 2000). Among those species, some of the most common and widely distributed in grassland ecosystems in Brazil belongs to genus *Brachiaria* spp., an aggressive group of competitors that grow fast and spread easily (Pivello et al. 1999). Regards of the wide distribution of these African grasses in Brazil, the ecological effects of their presence is not well known. Thus, it's important to know how species such as *Brachiaria* spp. can modify the structure and the functioning of plant communities, primarily through changes in the abundance of native species, especially those of the herbaceous layer, which may ultimately alter plant-pollinator interactions at community level (Schweiger et al. 2010).

Since plant diversity is known to affect the structure of the above ground multitrophic interactions through bottom-up control (see Scherber et al. 2010), in this study, we intend to understand how an invasive fast-growing species of the genus *Brachiaria* affects the structure of plant-pollinator interaction network, through its impact on the abundance, composition, and diversity of herbaceous plants in a grassland community. Hence, we analyzed the temporal changes in plant-pollinator interaction networks and hypothesized that the rising of a population of *Brachiaria decumbens* will change the abundance pattern and decrease species diversity of herbaceous plants, therefore, decreasing resource availability and the functional diversity of flowers (Figure 1). We also hypothesized that such changes may lead to a decrease in the network level specialization, through the mitigation of interaction strength generated by the limitation of floral resource availability, that will make the network less modular due to an increase in niche overlap between pollinator species competing for floral resources (Figure 1). For this, we analyzed the turnover (β-diversity) in plants and pollinators' species composition over time, and temporal changes in plant-pollinator relationship through β-diversity of interactions and plant functional traits.

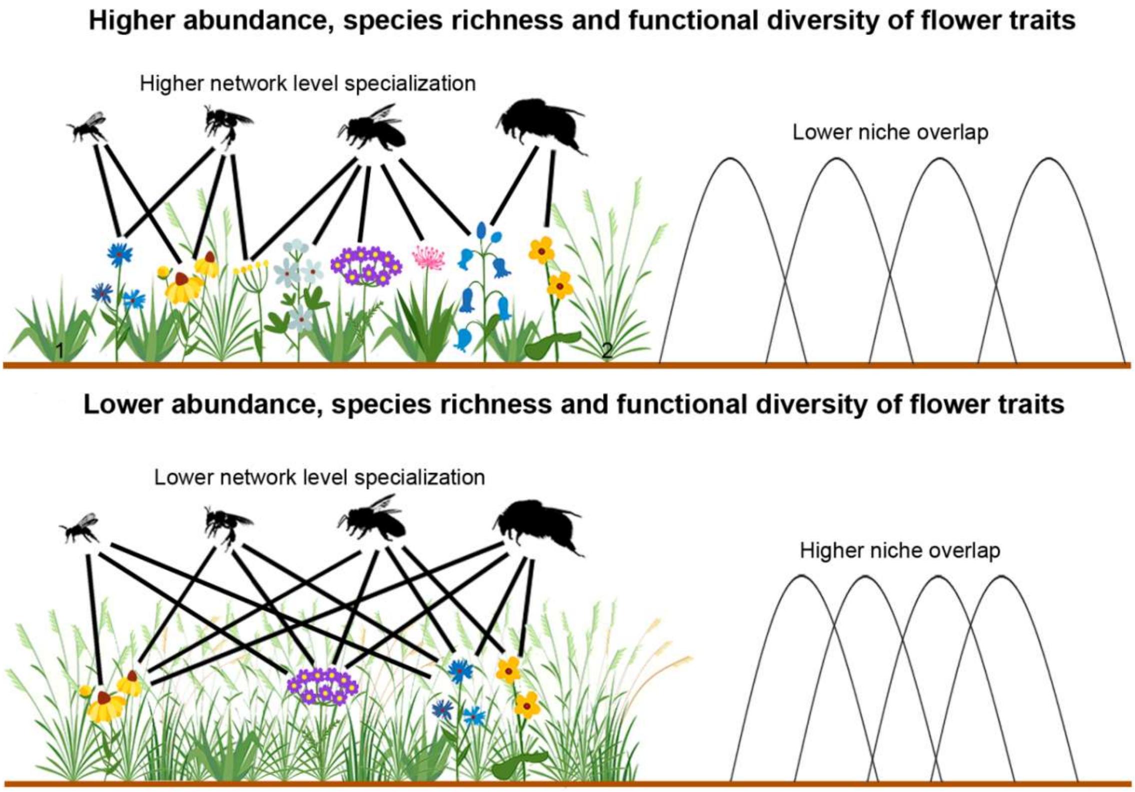

Figure 1. Representation of the hypothesized effects of the alien invasive grass, *Brachiaria decumbens*, on the structure and functioning of a grassland community. Increasing in abundance of *B. decumbens* (bottom image) is expected to reduce abundance, species richness and functional diversity of bee-pollinated herbaceous plants, consequently, making plant-bee interactions less specialized and increasing niche overlap between bee species. 1 and 2 on the top image represent native and invasive grass, respectively.

Methods

Study site

The data was collected in a Neotropical grassland field known as *campo cerrado* (sensu Oliveira-Filho and Ratter 2002), located in the municipality of Botucatu, São Paulo, southeastern of Brazil. The vegetation is characterized by having few scattered trees and shrubs with a dominant grassy herbaceous layer (see Oliveira-Filho and Ratter 2002). The total area of the study site comprehends two hectares and is located at the highest point in the region (about 900 m a.s.l.), locally known as "morro da Igreja de Rubião Júnior" (; 22°53'35.98"S 48°29'25.78"W). The climate is warm temperate (Cwa according to Köppen classification, see Kottek et al. 2006) with two seasons well marked: a cold and dry winter from March to September, and a hot and wet summer from October to April. The study was conducted during the years of 2016 to 2018, and the data was collected during the flowering peak from November to January of each year. For convenience, we will refer to both periods along the text as 2016 (November 2016 to January 2017) and 2017 (November 2017 to January 2018). In order to prevent the risk of fire, in most years, during the driest period (June-July) the area that comprehends the study site is submitted to an annual grass control by mowing. However, in 2017 the area was not mowed, allowing the growth of the alien invasive grass *Brachiaria decumbens* (see *A1* in supplementary appendices). Hence, we could compare the structure and functioning of this grassland ecosystem before and after the rising of the invasive alien grass.

Community composition and interactions sampling

To sample the community composition and interactions between plants and pollinators five transects (50m x 2m) were drawn uniformly in order to cover the entire study area. During the period of the study, interactions between plants and pollinators were sampled on a weekly basis for three to four days at each week. To include the daily time-dependent variations, these interactions were recorded at different periods of the day. Thus, on sampling days, we recorded interactions from 07:00h to 10:00h in the morning, and from 15:00h to 18:00h in the afternoon. During the sampling days, interactions from every focal species were recorded for thirty minutes. We recorded only legitimate interactions, defined as when the visitor touched the reproductive structures of the plant, indicating a potential for pollination. Since our study site was composed manly by herbaceous plants and bees were the only group of visitors that made significant numbers of legitimate interactions, we referred bees as "pollinators". Interactions were sampled at night (19:00h to 23:00h) if any plant with nocturnal flowers were blooming. On rainy days the sampling was shifted to the following day. Since the flowering period varies according to the species phenology, the sampling effort depended on the duration of the species flowering season across the sampling period, which resulted in a variable observation time for each species (range, means ± standard deviation) 2016: Total = 4970 minutes, $\bar{x}$ = 191 ± 165 minutes per plant species; 2017: Total = 6500 minutes, $\bar{x}$ = 342 ± 272 minutes per plant species.

We calculated the plant species richness inside transects and, to estimate the abundance, we counted the number of individuals and the total number of flowers per plant. Plants were collected and taken to the herbarium of São Paulo State University (herbarium BOTU) for further identification. Bees were collected during the plant visitation with entomological nets and euthanized with a killing jar using a 10% ammonia solution. They were mounted and identified using identification keys (Silveira et al. 2002) and with aid of specialists. We identified most plants at the species level, but some plants were identified only at the genus level and four of them at the family level. Bees were identified at least at the genus level, but most bees were identified at species level.

Plant-pollinator interaction networks and metrics

To compare both years, we constructed two quantitative interactions matrices using the visitation frequency of bees on plant flowers. Each plant or bee species was regarded as a node in the interaction networks and the interaction frequency as links. To illustrate the temporal structural differences between each network, we calculated several network metrics prioritizing the quantitative indices, which are less influenced by sampling effort (Fründ et al. 2016, Vizentin-Bugoni et al. 2016). The metrics calculated were the following: Weighted nestedness, which quantifies whether specialized species interactions represent subsets of the more generalist species interactions, evaluated by the wNODF index (Almeida-Neto and Ulrich 2011). Higher values of wNODF demonstrate whether the cores species of the network also establish the higher frequencies of interactions (Almeida-Neto and Ulrich 2011). Network wide-specialization, represented by the H_2’ index, which describes how a species restrict their interactions from those expected based only in partner’s availability (Blüthgen 2006), thus, demonstrate the degree of interaction exclusiveness. Niche Overlap (R_0), which measures the similarity of interaction patterns between species of the same level, calculated by the Horn’s index (Horn 1966). Lower values of Niche Overlap indicate that species are partitioning one or more of their niche’s axis (Hutchinson 1957). Modularity (Q’), an index that quantifies how interactions are structured within networks modules (which are a subset of interactions), relative to interactions occurring between modules. Higher values of Q’ demonstrate the existence of pollinator interaction’s preferences for a particular subset of plant species, indicating a niche specialization. We estimated the modularity of the networks using the QuanBiMo algorithm (Dormann and Strauss 2014), which calculate modularity in an iterative approach, thus, the value of Q' might have a slight variation between the algorithm runs, therefore we ran the algorithm 10 times to find the optimal module conformation with the highest value of Q, and the number of Markov Chain Monte Carlo (MCMC) moves was set to 10^9 steps (as in Maruyama 2014, Maruyama 2015, Araujo et al. 2018, but see also Dormann and Strauss 2014). All the network-level metrics were calculated using the package *bipartite* in R (Dormann et al. 2008).

To assess the significance of the network metrics, we compared the observed values to those generated by null-models. To generate the simulated matrices, we used the Patefield algorithm (Patefield 1981), which maintain the total number of interactions by fixing the marginal totals, hence this algorithm keeps the network size and species richness. In order to assess the significance, we estimated 95% confidence interval (CI) for each metric from the simulated values.

Species roles of bees in the interaction networks

Since we wanted to know how bee roles within network changes between periods, during the difference in resource availability and plant species composition, we evaluated several species level indices that characterize distinct topological properties of the species: species strength (*ss'*), which represents the sum of the proportions of interactions established by a given species across all its interaction partners. Higher values illustrate how plants depend on a specific bee species, and vice-versa (Bascompte et al. 2006). Species-level specialization (*d'*), which describes how the frequencies of interactions of a given bee species diverge relative to the availability of interaction partners in the network, represented by plants' marginal totals, hence, higher values denote high specialization (Blüthgen et al. 2006). We performed paired t-tests to test whether *d'* and *ss*' differed between years. For these analyses, we considered only the shared bee species (10 from 13 species) in both periods. Also in order to characterize the importance of bee species across both periods to the structure the modules, we also calculated two indices based on the modular webs: the within-module degree (*z*), an index that represents the number of connections that a species has within its own module relative the other species in that module. Among-module connectivity (*c*), which informs how well a given species is connected to species from other modules (Olesen 2007). Thereafter, following Olesen (2007) we classified species in four categories according to their "*c"* and "*z"* values: (1) peripherals, represented by low values of both *c* and *z*; (2) connectors, represented by high *c* and low *z*; (3) module hubs, represented by high *z* and low *c*; (4)

network hubs, represented by high values of both *c* and *z*. The threshold values for *c* and *z* were 0.5, that represent 50% of the range for both axes, which we understand to be parsimonious, since there is an equiprobability of a species to rely on one of these four categories, avoiding biases caused by an unequal, unproportioned threshold. (Guimera and Amaral 2005, Olesen 2007).

Floral resources availability and floral trait diversity

The data for resource availability and floral trait diversity was sampled on a fortnightly basis in all transects. As we are interested to know how resource availability and floral diversity changes between years, we used the richness of the blooming species as well as their floral abundance as a proxy for resource availability. To measure the functional diversity of plant species we recorded floral traits from four distinct measures (both qualitative and quantitative): i) the flower morphology, classified as poricide, tubular, dish, bell-funnel, brush, flag, and gullet (according to Souza et al. 2018, modified from Machado and Lopes 2004); ii) operative flower length, measured from the ovary to the highest floral whorl; iii) floral width, measured from the most peripheral floral structure to the opposite extremity; and iv) floral reward, defined as pollen, nectar, pollen/nectar, and oil.

Changes in community structure and functioning over time

To assess whether community structure and functioning have changed over time after the growth of *Brachiaria decumbens*, we assessed β-diversity in plant and bee communities, as well as the changes in the structure of bee-plant interaction network. Also, to analyze how such changes may affect community functioning, we first used Hill numbers to assess the changes in pairwise interaction pattern over time using rarefaction and extrapolation analyses. Second, we assessed β-diversity in plant-pollinator interactions over time (Poisot et al. 2012, Carstensen et al. 2014) and β-diversity of plant functional traits (Anderson et al. 2006).

In order to assess species turnover (β-diversity) of plants and bees over time, we used the uncorrected probability version of the abundance-based Chao-Sørensen index, i.e., $\beta_{\text{Chao-Sørensen}}$ (see Chao et al. 2006, but see also Barwell et al. 2015). The index varies from 0 to 1, where values close to 1 indicate high species turnover between years and were computed using *CommEcol* and *vegan* packages in R (Dixon 2003, Melo and Melo 2017).

To calculate individual-based rarefaction and extrapolation curves of interactions (see Chao et al. 2014), we used Hill numbers of the diversity orders of $q = 0$ (richness of pairwise interactions), $q = 1$ (Shannon diversity of interactions) and $q = 2$ (Simpson diversity of interactions). To make these comparisons, we extrapolated the smallest reference sample (period of 2017) up to the same number of interactions recorded in the largest reference sample (period of 2016). Then, we obtained 95% confidence intervals for all rarefied and extrapolated curves by using a bootstrap method with 200 replications (for details see Hsieh et al. 2016). Rarefaction and extrapolation analyses were performed using the package *iNEXT* in R (Hsieh et al. 2016).

Moreover, to calculate the β-diversity of interactions, we first used a model proposed by Magrach et al. (2017), which applies a quantitative measure of β-diversity using the Ruzicka distance coefficient. This coefficient is based on the shared pairwise interactions between the communities and is weighted by the interaction frequencies. Two components of the β-diversity were calculated: i) the interaction turnover, which describes the portion of the interaction β-diversity that occurred due to the loss of pairwise interactions, and ii) the interaction rewiring (βOs), which describes the portion that occurred due to the rewiring of the interactions (CaraDonna et al. 2017). The metric value ranges from 0 to 1, where 0 is when the interaction composition is exactly the same, and 1 when it's completely different.

However, despite the robustness of these metrics, it is widely known that plant-pollinator interaction networks may be affected by sampling (Vázquez et al. 2009, Vizentin-Bugoni et al. 2016), since not all interactions are actually recorded, especially the rarer ones. Hence, considering that rarer interactions are less likely to be sampled, β-diversity analyzes of interaction networks may suffer from important biases due to an uneven sample probability of pairwise interactions which may lead to wrong interpretations. In this sense, in order to calculate β-diversity of interactions and to avoid sampling biases, we used the corrected version of the abundance-based Chao-Jaccard and -Sørensen indices, which takes into account the occurrence of "unseen" shared species, considered in our analysis as "unseen" pairwise interactions (see Chao et al. 2005, Chao et al. 2006). For this, we decomposed the communities' interaction matrices into pairwise interactions and considered each pair of interaction as a single species. We used the version "*rare*" of the function *dis.chao* in *CommEcol* R package to calculate Chao-Jaccard and -Sørensen β-diversity indices considering the effect of "unseen" plant-pollinator interactions. Furthermore, in order to assess the significance of the corrected Chao-Jaccard and -Sørensen β-diversity indices, we used the R package *vegan* to produce a null distribution of both β-diversity indices using the Patefield null model (Patefield 1981). The Chao-Jaccard and -Sørensen β-diversity indices also range from 0 to 1, with 1 representing a higher temporal turnover on plant-pollinator interactions.

Finally, in order to calculate the temporal β-diversity of plant functional traits in the community, we used the model proposed by Anderson et al. (2006), as follow: first, we made a functional trait matrix for each year, and then we computed the distance matrices using the Gower dissimilarity coefficient (Gower, 1971). Each distance matrix was submitted to a principal coordinate analysis (PCoA), and then, using the function *betadisper* in the *vegan* package, we performed an analysis of multivariate homogeneity of group dispersions to calculate the average trait distance of species from each period to a group centroid in the principal coordinate trait space (Anderson et al. 2006). To assess the significance of the distance difference between each group (period of collection) we performed a permutation test for homogeneity of multivariate dispersions using the function *permutest* in *vegan* (Legendre 2011). We also used the combination of plant functional traits to calculate two metrics of functional diversity: functional evenness (FEve, Villéger et al. 2008) and functional dispersion (FDis, Laliberté and Legendre 2010). FEve ranges between 0 and 1 and measures the regular distribution of individual species abundances in the trait space. Higher values of FEve demonstrate a more equally spaced distance between species, which can be interpreted as a low functional diversity (Villéger et al. 2008). FDis, is calculated by projecting one community centroid in a trait space, and then, calculating the mean distance of species to the community centroid. High values indicate the presence of functionally distinct species (Laliberté and Legendre 2010). Both of these metrics evaluate the difference in species abundance and weight for species relative abundances, represented here, by the total number of flowers. We calculated these metrics using the package *FD* in R (Laliberté and Legendre 2010).

Results

Community composition and interactions sampling

A total of 399 interactions were recorded in 33 species of plants, distributed in 16 families, and 13 species of bees distributed in 9 tribes during the two years of collection. The period of 2016 presented a greater number of plant species (26) and flower abundance (8830 flowers), along with 11 species of bees. In 2017 the number of plant species that bloomed was lower (19) with also, lower flower abundance (4950 flowers). The number of bee species, on the other hand, was higher in the second period (12 species, Table 1, Figure 2, see supplementary appendices, *A2*). Among the plant families with recorded interactions, Asteraceae was the family with the highest frequency of visits (28% of total interactions), followed by Myrtaceae (24%) and Rubiaceae (8%). The tribe of bees with the highest frequency of interactions was Apini (41% of interactions represented by one species), Meliponini (25% of interactions represented by four species) and Augochlorini (14% of interactions represented by three species). The exótic bee *Apis mellifera* was the most frequent flower visitor in both years (Figure 2).

Plant-pollinator interaction networks and metrics

Specialization (H2’) were higher in 2016 (Table 1, Figure 2). Nestedness (wNODF) were higher in 2017 (Table 1, Figure 2). The modularity (Q’) was higher in 2016, however, the Niche Overlap (R0) were higher in 2017 (Table 1, Figure 1, Figure 3). All network metrics were higher than the intervals of the null model (Table 1).

Table 1. Changes in community composition and structure over the two periods of the study. * represents significant values based on a null distribution using Patefield null model.

	Period	
Community Composition	**2016**	**2017**
Plant richness	26	19
Flower availability	8830	4950
$\beta_{\text{Chao-Sørensen}}$-diversity of plants	0.19	
Bee richness	11	12
$\beta_{\text{Chao-Sørensen}}$-diversity of bees	0.02	
Network metrics	Observed value (95% Confidence Interval)	
Nestdness (wNODF)	4.14 (12.37 - 18.94)	11.8 (0.76 - 0.79)*
Specialization (H_2’)	0.80 (0.16 - 0.26)*	0.66 (0.07 - 0.13)*
Niche Overlap (R_0)	0.05 (0.22 - 0.32)*	0.23 (0.39 - 0.62)*
Modularity (Q)	0.69 (0.25 - 0.33)*	0.46 (0.12 - 0.17)*

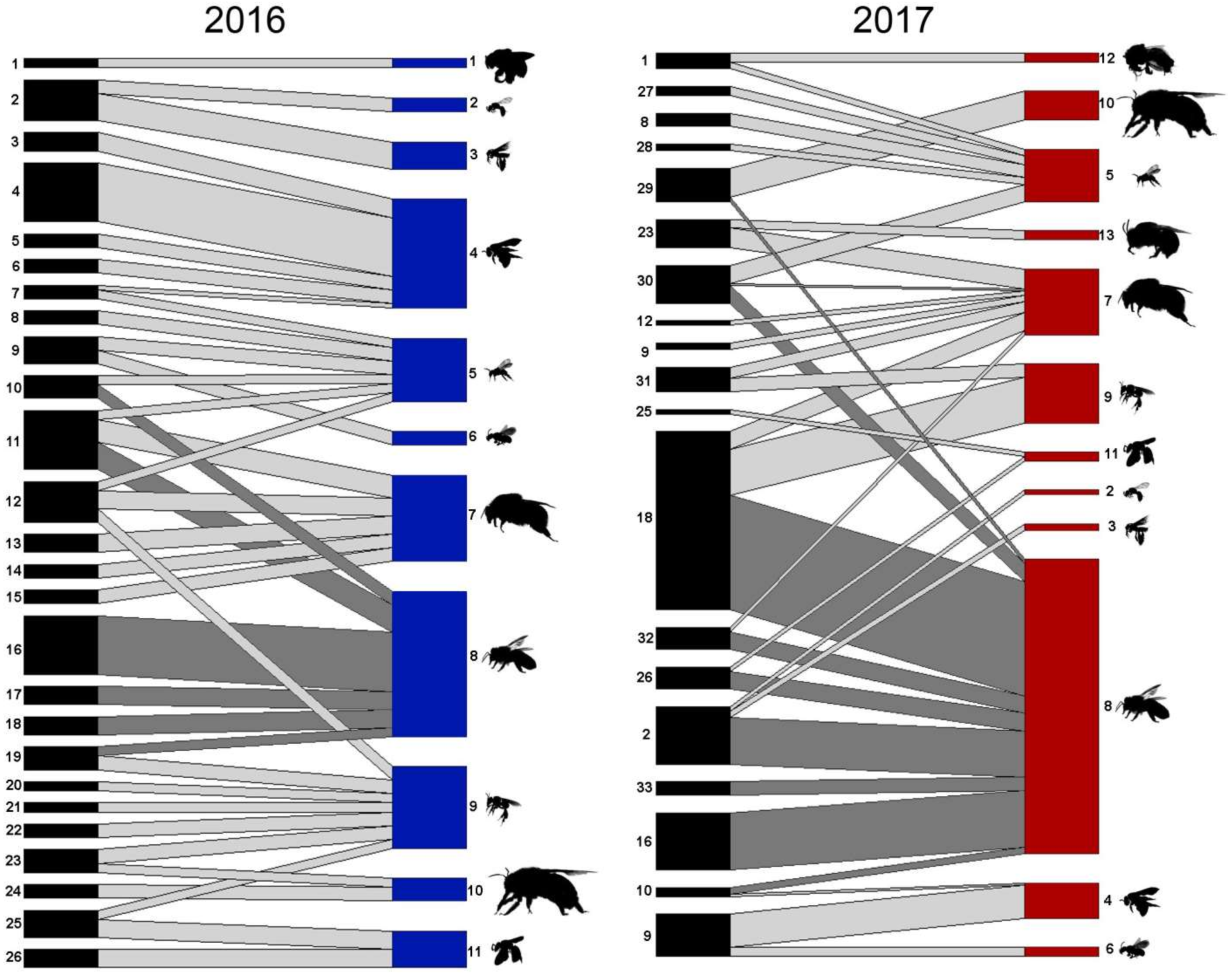

Figure 2. Plant-bee interactions over periods. Bar thickness represents the interaction frequencies for each pairwise interaction. Interactions involving *Apis mellifera*, the bee with most interactions, are highlighted in dark grey. Numbers represent species identities, respectively for plants and bees, as follow: 1 - *Banisteriopsis campestris*, 2 - *Chromolaena laevigata*, 3 - *Richardia grandiflora*, 4 - *Psidium grandifolium*, 5 - *Trimezia juncifolia*, 6 - *Waltheria communis*, 7 - *Cambessedesia espora*, 8 - *Byrsonima intermedia*, 9 - *Acisanthera quadrata*, 10 - *Trichogonia attenuata*, 11 - *Borreria poaya*, 12 - *Solanum* sp., 13 - *Mimosa debilis*, 14 - *Mimosa dolens*, 15 - *Polygala violacea*, 16 - *Cosmos sulphureus*, 17 - *Hyptis* sp., 18 - *Myrcia bella*, 19 – Fabaceae sp. 2, 20 - Asteraceae sp. 1, 21 - Fabaceae sp. 1, 22 - Asteraceae sp. 2, 23 - *Caryocar brasiliense*, 24 - *Mandevilla emarginata*, 25 - *Commelina erecta*, 26 - *Borreria capitata*, 27 - *Byrsonima pachyphylla*, 28 - *Eriope macrostachya*, 29 - *Kielmeyera variabilis*, 30 - *Gazania* sp. , 31 - *Palicourea rigida*, 32 - *Lessingianthus grandiflorus*, 33 - *Erythroxylum cuneifolium*. 1 - *Centris* sp., 2 - *Augochlora* sp., 3 - *Tetrapedia* sp., 4 - *Augochloropsis* sp., 5 - *Plebeia* sp., 6 - *Nannotrigona testaceicornis,* 7 - *Bombus morio,* 8 - *Apis mellifera,* 9 - *Trigona spinipes,* 10 - *Xylocopa brasilianorum,* 11 - *Augochlorella* sp., 12 - *Monoeca* sp*.*, 13 - *Ptiloglossa* sp.

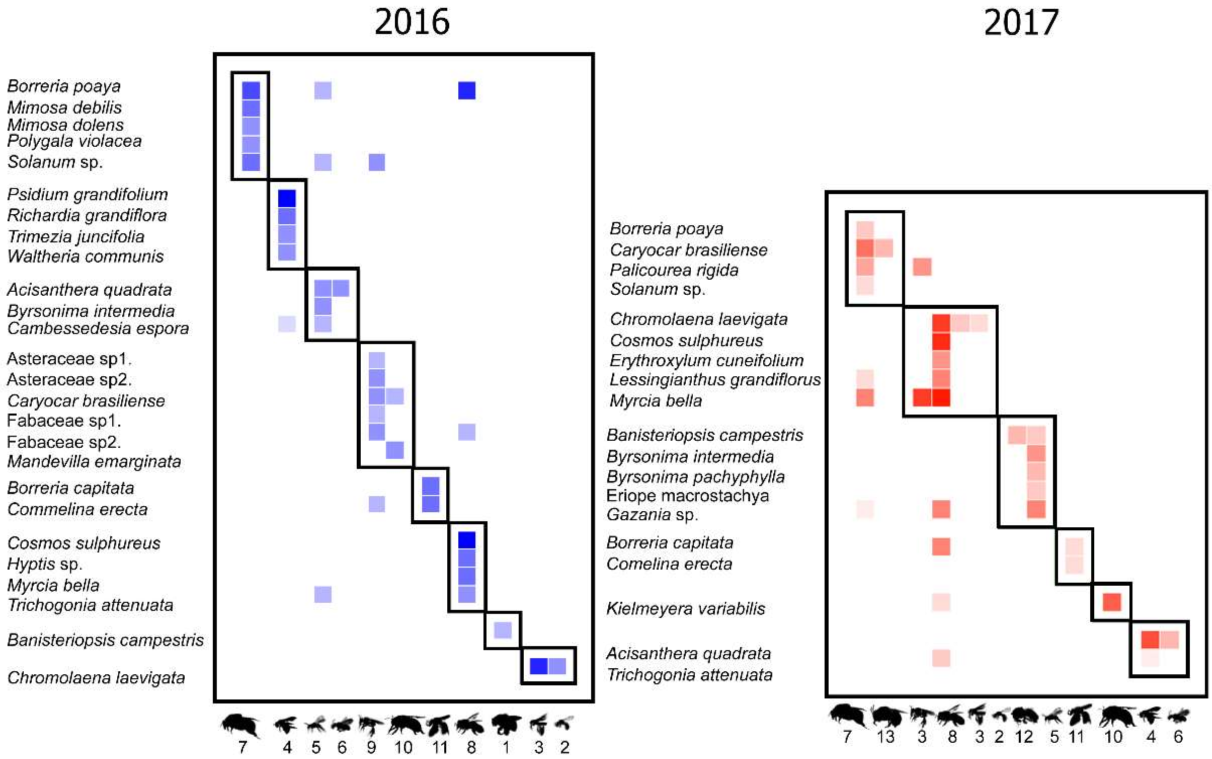

Figure 3. Modular webs over periods. Numbers represent bee species as in Figure 2.

Bee roles in networks

We found no difference between bee species strength (ss') indices between periods (Table 2, t = 1.37, p = 0.19), however, bee species specialization (d') differed across years, with an overall decrease in bee species specialization from 2016 to 2017 (Table 2, t = 2.69, p = 0.02, Figure 4). According to bee c and z values (Figure 5), in 2016, eight bees (73%), were classified as "peripherals" and the remaining three, *Trigona spinipes*, *Apis mellifera* and *Plebeia* sp. were classified respectively as "module hub" (9%), "connector" (9%) and "network hub" (9%). Nevertheless, in 2017, seven bees (58%), were classified as "peripherals", two bees (17%), *Plebeia* sp. and *Augochloropsis* sp. were classified as "module hubs", one bee (8%), *Trigona spinipes* were classified as "connector" and two bees (17%), *Bombus morio* and *Apis mellifera* were classified as "network hubs" (Figure 5).

Table 2: Bee species level specialization (*d'*) and strength (*ss'*) index over periods.

	d'			*ss'*		
Species	2016	2017	Trends	2016	2017	Trends
Plebeia sp.	0.56	0.84	+	2.94	3.90	+
Bombus morio	0.75	0.50	-	3.83	3.50	-
Trigona spinipes	0.72	0.46	-	4.87	0.80	-
Augochlorella sp.	0.90	0.77	-	1.67	1.20	-
Xylocopa brasilianorum	0.85	0.95	+	1.40	0.87	-
Apis mellifera	0.82	0.54	-	4.46	6.40	+
Augochloropsis sp.	0.97	0.88	-	4.33	1.03	-
Tetrapedia sp.	0.82	0.40	-	0.67	0.11	-
Augochlora sp.	0.57	0.33	-	0.33	0.08	-
Nannotrigona testaceicornis	0.73	0.55	-	0.50	0.21	-
Centris sp.	1.00			1.00		
Monoeca sp.	0.84			0.57		
Ptiloglossa		0.66			0.31	

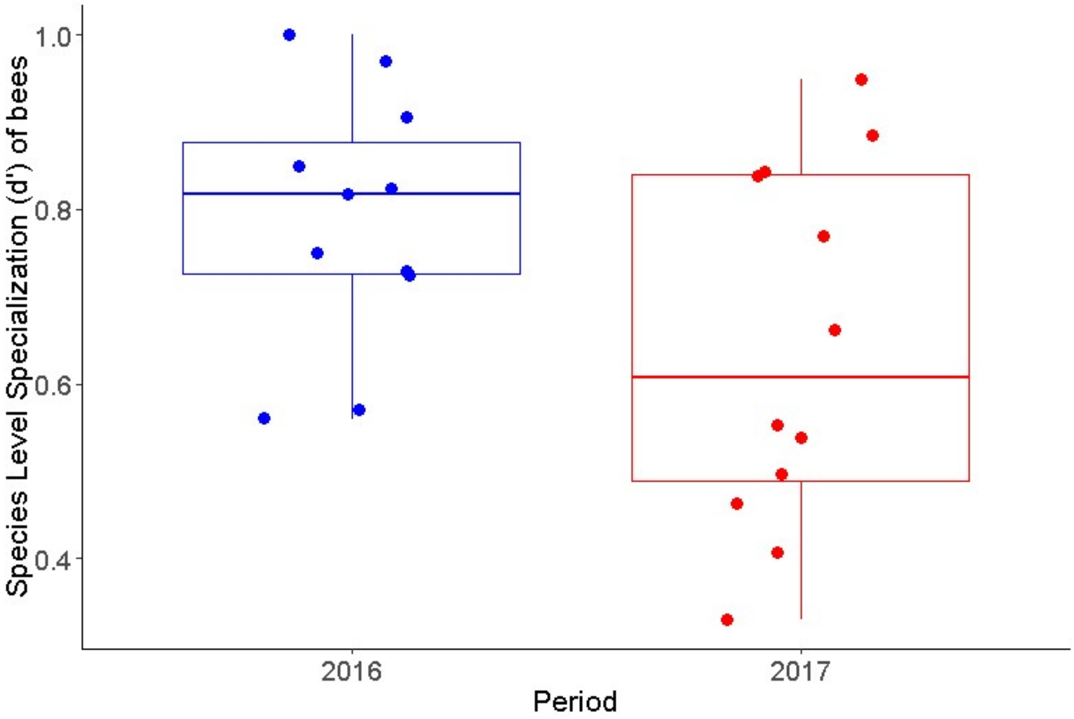

Figure 4. Species-level specialization (*d*') of bees over periods. Blue boxplot represents 2016 bee *d*' values, red boxplot represents 2017 *d*' values. The bee community at 2016 showed a higher overall specialization than in 2017 (t = 2.69, p = 0.02).

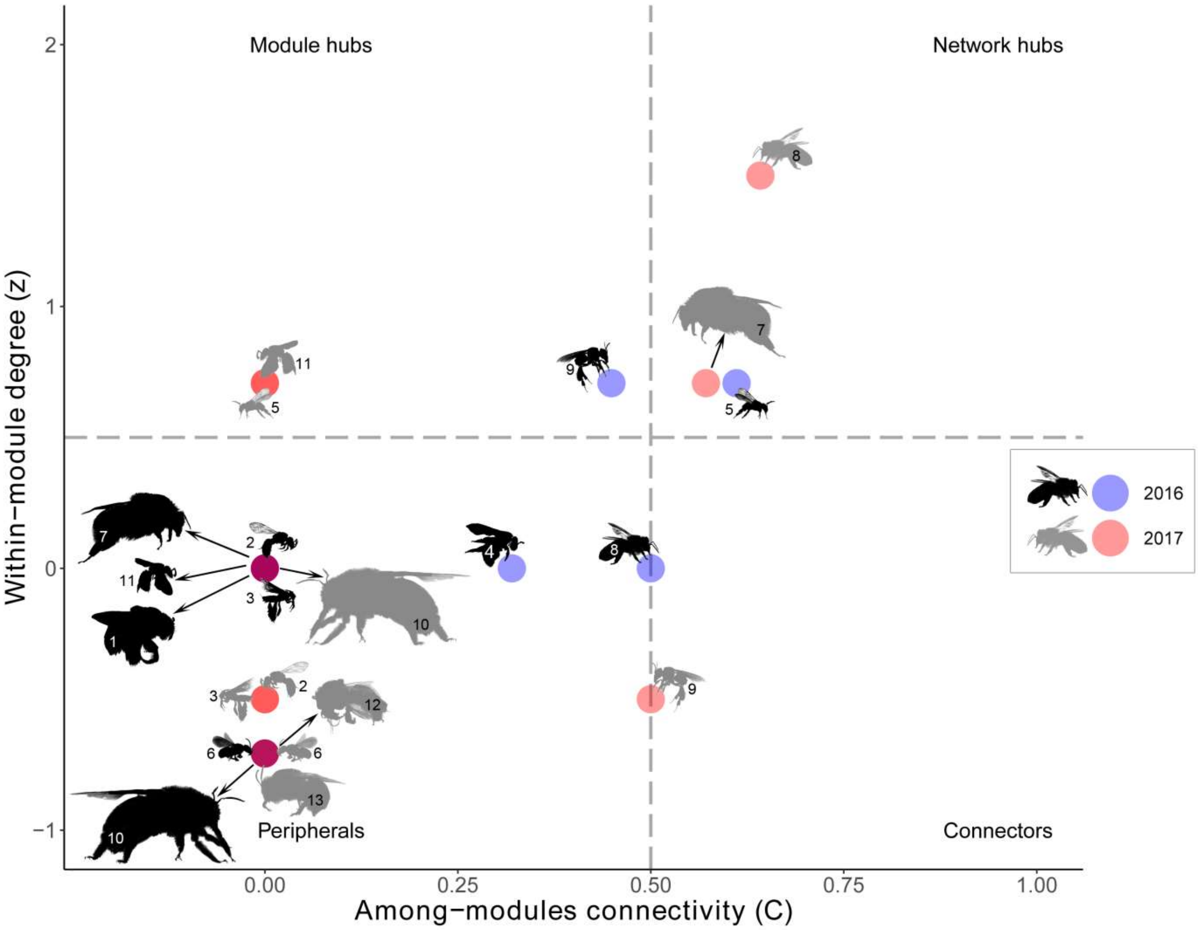

Figure 5. Distribution of bee species according to their *c*- and *z-scores* over the periods. 2016 is represented by blue circles and black silhouettes, and 2017 by red circles and gray silhouettes. Circle colors have 30% opacity to highlight the overlying values of *c*- and *z-scores*. Bee species are represented here by the same numbers as in Figure 2.

Changes in community interactions and functional traits over time

Rarefaction analysis showed higher interaction richness, Shannon and Simpson interaction pairwise diversity in 2016 before the rising of *B. decumbens* (Figure 6). However, extrapolation analysis showed no difference in interaction richness between years, but Shannon and Simpson interaction diversity were higher in 2016 even considering the extrapolation analysis (Figure 6). The Ruzicka interaction β-diversity was 0.88, from which 64% of it (βOs = 0.64) was due to the rewiring of bee-plant interactions. β-diversity in plant-bee interactions over time was still high even when we considered the effect of “unseen” shared interactions, since the corrected version of Chao-Jaccard and -Sørensen β-diversity indices were as high as 0.76 and 0.61, respectively. The observed values of both β-diversity indices were significantly higher ($p < 0.001$) than expected from random pairwise interactions generated by Patefield null model (Figure 7). The β-diversity of plant functional traits did not differ between periods (Figure 8, pseudo-F = 0.41, p = 0.52), and presented similar values of functional evenness and dispersion for both periods (2016, FEve = 0.46, FDis = 0.44; 2017, FEve = 0.46, FDis = 0.52).

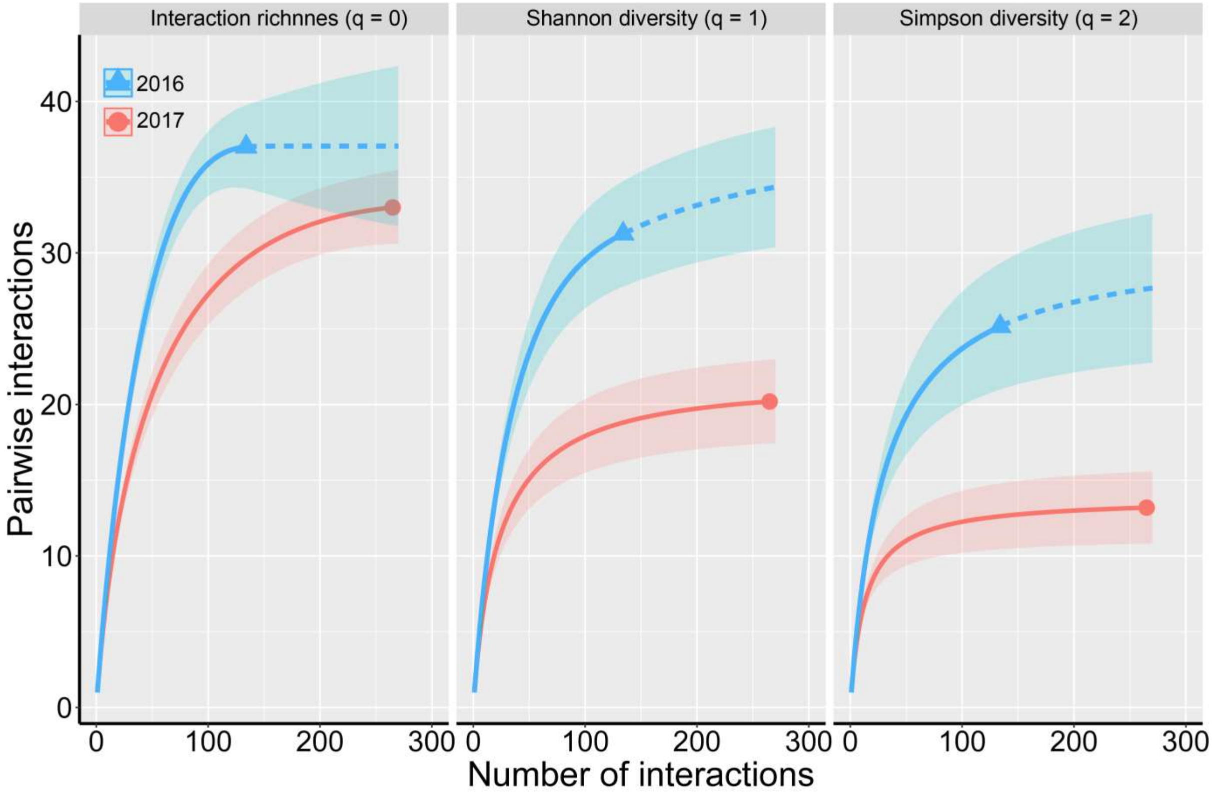

Figure 6. Rarefaction and extrapolation curves with Hill numbers of the diversity order of q = 0, 1 and 2 of pairwise interactions over periods. 2016 is represented by blue curves, and 2017 the red ones. The smallest reference sample (2017) was extrapolated up to the same number of interactions as the reference sample (2016). Shaded areas represent 95% confidence intervals for each sample after 200 bootstraps.

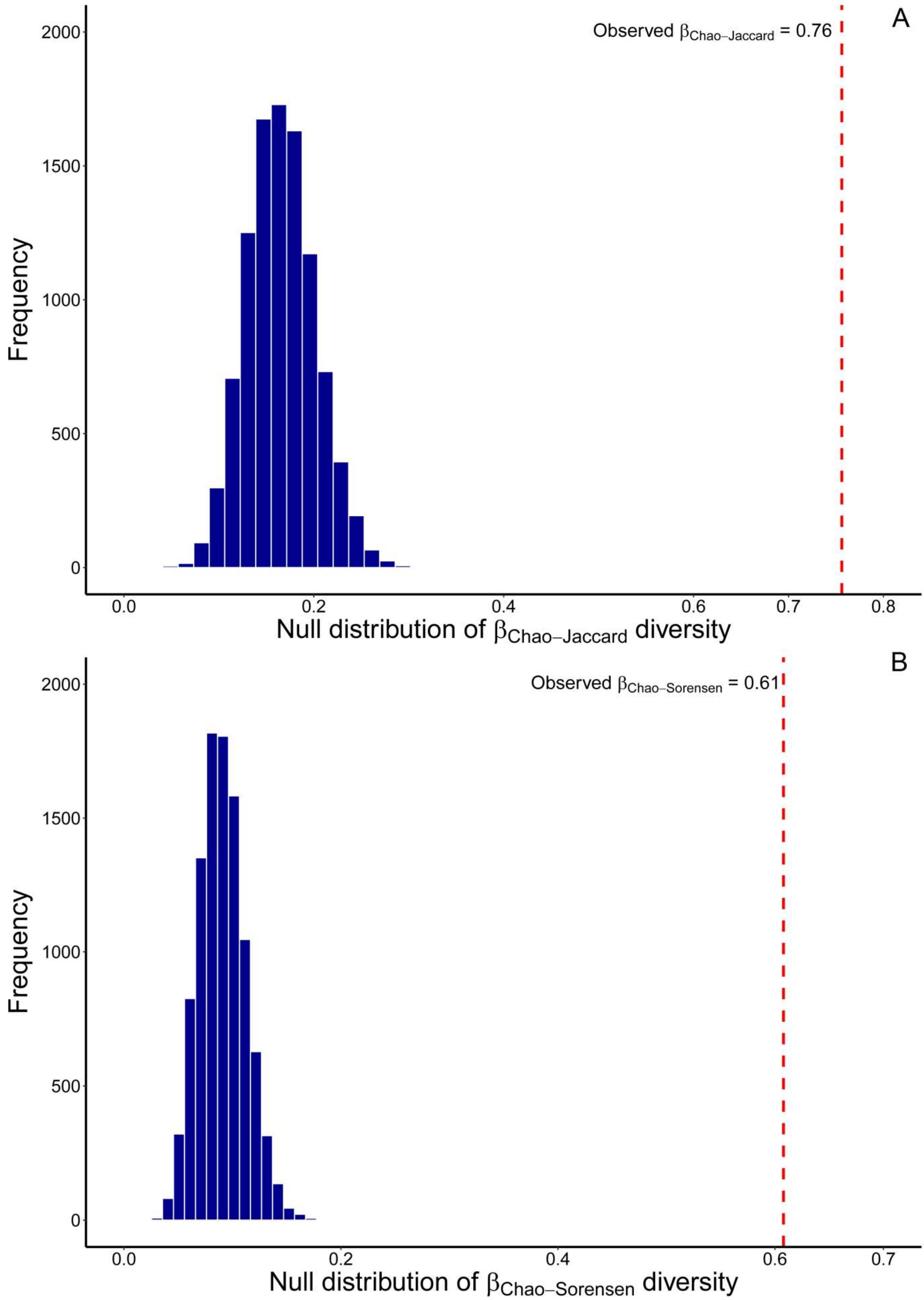

Figure 7. Null distribution (histograms) and observed values of temporal species interaction turnover based on a) $\beta_{\text{Chao-Jaccard}}$- and b) $\beta_{\text{Chao-Sørensen}}$-diversity indices

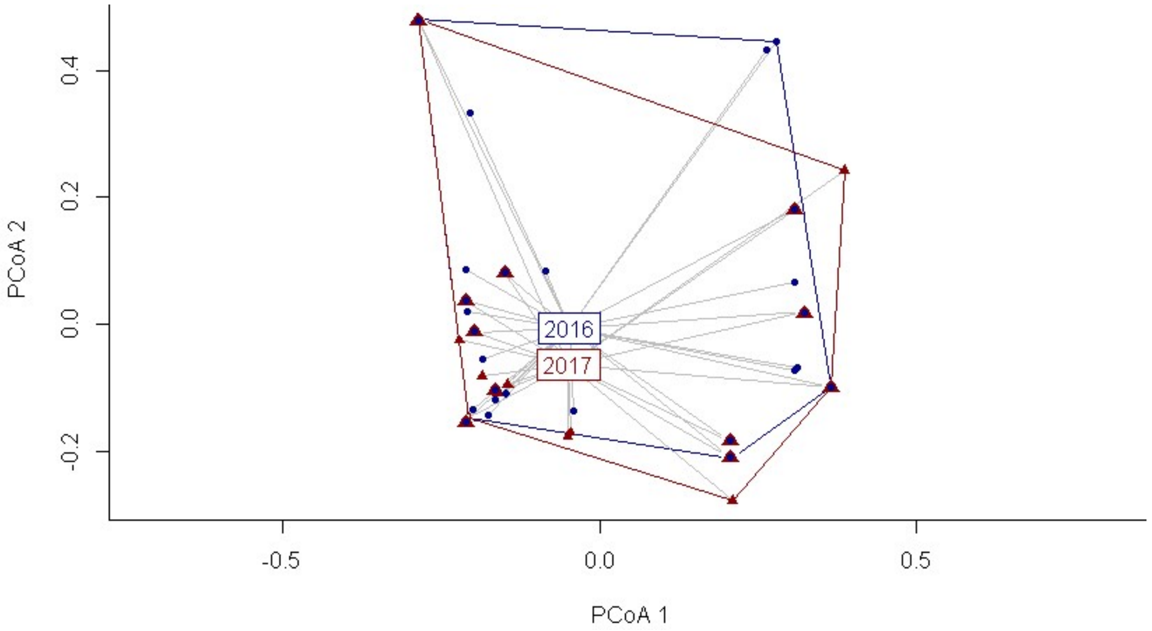

Figure 8. β-diversity of plant functional traits represented in the principal coordinate analysis (PCoA) space. Blue dots represent functional traits of plants in 2016 and red triangles represent functional traits of plants in 2017. Red triangles with blue dots inside represent species with equal functional traits. Polygons represent the functional space occupied by overlapping species (pseudo-F = 0.41, p = 0.52).

Discussion

Our results show that the rising of an invasive fast-growing alien grass species in a grassland ecosystem changed plant community composition by decreasing plant richness, which impacted plant-pollinator interactions via bottom-up effects (Cox and Elmqvist 2000, Munoz et al. 2005, Scherber et al. 2010). Such changes were followed by a decrease in plant-bee interaction diversity along with a high turnover in community pairwise interactions. Despite the short temporal and small geographic scale of our study, these results should be interposed within higher spatiotemporal context, since bees are the most common group of pollinators worldwide, also being one of the first groups to feel the impacts of a sudden change in community structure (Potts et al. 2010). In addition, herbaceous plants compose an important component of savanna and grassland ecosystems over the world, whose diversity severely limit the overall primary production, which in turn, direct impact lower trophic levels and mediates indirect effects on higher trophic levels (Gilliam 2007, Scherber et al. 2010, Peet et al. 2014).

We evidenced that the decrease in plant's composition diversity had an impact on the structure of bee-plant interactions. Thus, across the periods, plant-bee interaction network became less modular and specialized, showing a higher niche overlap. These changes are related to the rewiring of plant-pollinator interactions due to shifts in bee resource usage, together with a potential intensified bee-bee competition (Burkle and Alarcón 2011). Therefore, it seems that the rising of *B. decumbens* provided a novel community scenario where, in short-term, exacerbated i) bee-bee exploitative competition, through dominance of the reduced floral resource by the more competitive bees (Steiner et al. 2007), and ii) apparent competition, through the capacity of the less competitive bees in use the remaining floral resources (Magrach et al. 2017). These processes culminated in a formation of new bee topological roles within the community that resulted in a more nested conformation of the interaction network, which means that some bees occupied a larger portion of the feeding niche axis, i.e., being more generalists (Dáttilo et al. 2014).

These shifts in bee resource usage across periods were highlighted by the decrease in overall bee specialization within the network, which mitigated interaction strength between bees and plants (Guimaraes et al. 2007). In fact, bee species that compose the community of our study system are polylectic, i.e., they use a wide variety of plants as food sources (Michener 2000). Hence, in a scenario of reduced resource availability, polylectic bees may easily change their pattern of flower resource usage (Waser et al. 1996, Souza et al. 2018), therefore, changing their roles in the plant-bee interaction network. Especially more dominant bees, such as *Apis mellifera* and *Bombus morio*, tend to dominate the majority of available plants (Magrach et al. 2017), then acting as network hubs, as we observed in 2017. On the other hand, from the plants perspective, a community-wide decrease in pollinator specialization means higher risk of interspecific pollen deposition, potentially impairing plant reproduction by decreasing fruit and seed formation (Galen and Gregory 1989).

The decrease in plant species richness mediated by the invasive grass did not imply in a reduced functional diversity of floral traits, similarly as what was observed in other cerrado/grassland ecosystems in Brazil where natural changes in plant species richness occur due to seasonality (Souza et al. 2018). Hence, our results indicate that the change in plant-pollinator network structure and functioning occurred due to the reassembling of interactions mediated by the decline in floral resource availability and not by the turnover of plant functional traits. In addition, although the short-term resilience of our community in terms of functional diversity of floral traits, the persistence of invasive species in native communities may lead to cumulative changes (Sakai et al. 2001, Strayer et al. 2006). In long-term, such changes can drive greater impacts on community structure, reducing even more species richness and abundance, which invariably will reduce the functional diversity and convert an originally diverse grassland community in a simplified pasture dominated by few species (Strayer et al. 2006).

Notwithstanding, the interaction richness was not affected by the reduction in plant species richness, the interaction diversity decreased about 50%. This reduction in interaction diversity highlights the indirect effect of the invasive grass on the structure and functioning of plant-bee interaction network since the reduction in plant abundance and richness led the best bee competitors to dominate the resources. In this sense, it's a remarkable finding that an another exotic African invasive species (*Apis mellifera*), had, by its fast learning ability and great labile preference (i.e., the behavior to use the most rewarding flowers), contributed in a synergic way with *B. decumbens* to impoverish the diversity of interactions (Waser 1986, Goulson 1999, Seifan et al. 2014). In addition, besides the overall decrease in the species-level specialization of bees, most bees, in particular, the less competitive ones also decreased interaction strength, which demonstrates that these interactions were more susceptible to be disrupted and lastly, extinct. Indeed, the extinction of ecological interactions occurs much faster than species extinctions per se and may affect ecosystem functioning in a quicker way through cascading effects (Janzen 1974, Aizen et al. 2012, Valiente-Banuet et al. 2015). In this sense, our results highlight how multi-trophic interactions can be affected by one of the major causes of biodiversity changing mediated by anthropic disturbance, which is the introduction of exotic species (Chapin III et al. 2000).

References

Aguilar, R. et al. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. - Ecology Letters 9: 968-980.

Aizen, M. A. et al. 2012. Specialization and rarity predict nonrandom loss of interactions from mutualist networks. - Science 335: 1486-1489.

Almeida-Neto, M. and Ulrich, W. 2011. A straightforward computational approach for measuring nestedness using quantitative matrices. - Environmental Modelling & Software 26: 173-178.

Anderson, M. J. et al. 2006. Multivariate dispersion as a measure of beta diversity. - Ecology Letters 9: 683-693.

Araujo, A. C. et al. 2018. Spatial distance and climate determine modularity in a cross-biomes plant–hummingbird interaction network in Brazil. - Journal of Biogeography 00:1–13.

Ashman, T. L. et al. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. - Ecology 85: 2408-2421.

Barwell, L. J. et al. 2015. Measuring β-diversity with species abundance data. - Journal of Animal Ecology 84: 1112-1122.

Bascompte, J. et al. 2006. Asymmetric coevolutionary networks facilitate biodiversity maintenance. - Science 312: 431-433.

Baum, J. K. and Worm, B. 2009. Cascading top-down effects of changing oceanic predator abundances. - Journal of Animal Ecology 78: 699-714.

Bergamo, P. J. et al. 2018. Trait patterns across space and time suggest an interplay of facilitation and competition acting on Neotropical hummingbird-pollinated plant communities. Oikos. Accepted Author Manuscript. doi:10.1111/oik.05571.

Blüthgen, N. et al. 2006. Measuring specialization in species interaction networks. - BMC Ecology 6: 9.

Brook, B. W. et al. 2008. Synergies among extinction drivers under global change. - Trends in ecology & evolution 23: 453-460.

Burkle, L. A., and Alarcón, R. 2011. The future of plant-pollinator diversity: understanding interaction networks across time, space, and global change. - American Journal of Botany 98: 528-538.

Burkle, L., and Irwin, R. 2009. The importance of interannual variation and bottom-up nitrogen enrichment for plant-pollinator networks. - Oikos 118: 1816-1829.7

CaraDonna, P. J. et al. 2017. Interaction rewiring and the rapid turnover of plant-pollinator networks. - Ecology Letters 20: 385-394.

Carstensen, D. W. et al. 2014. Beta diversity of plant-pollinator networks and the spatial turnover of pairwise interactions. - PLoS One 9: e112903.

Casper, B. B. and Jackson, R. B. 1997. Plant competition underground. - Annual Review of Ecology and Systematics 28: 545-570.

Chao, A. et al. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. - Ecology Letters 8: 148-159.

Chao, A. et al. 2006. Abundance-based similarity indices and their estimation when there are unseen species in samples. - Biometrics 62: 361-371.

Chao, A. et al. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. - Ecological Monographs 84: 45-67.

Chapin III, F. S. et al.2000. Consequences of changing biodiversity. - Nature 405: 234.

Cox, P. A., and Elmqvist, T. 2000. Pollinator extinction in the Pacific Islands. - Conservation Biology 14: 1237-1239.

Craine J. M. and Dybzinski R. 2013. Mechanisms of plant competition for nutrients, water and light. - Functional Ecology 27: 833-840.

Croll, D. A. et al. 2005. Introduced predators transform subarctic islands from grassland to tundra. - Science 307: 1959-1961.

Dáttilo, W. et al. 2014. Ant dominance hierarchy determines the nested pattern in ant-plant networks. - Biological Journal of the Linnean Society 113: 405-414.

Dixon, P. 2003. VEGAN, a package of R functions for community ecology. - Journal of Vegetation Science 14: 927-930.

Dormann, C. F. and Strauss, R. 2014. A method for detecting modules in quantitative bipartite networks. - Methods in Ecology and Evolution 5: 90-98.

Dormann, C. F. et al. 2008. Introducing the bipartite package: analysing ecological networks. - Interaction 1: 0-2413793.

Estes, J. A. et al. 2011. Trophic downgrading of planet Earth. - Sience 333: 301-306.

Fischer, J. and Lindenmayer, D. B. 2007. Landscape modification and habitat fragmentation: a synthesis. - Global ecology and biogeography 16: 265-280.

Fründ, J. et al. 2016. Sampling bias is a challenge for quantifying specialization and network structure: lessons from a quantitative niche model. - Oikos 125: 502-513.

Galen, C., and Gregory, T. 1989. Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, Polemonium viscosum. - Oecologia 81:120-123.

Gilliam, F. S. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. - AIBS Bulletin, 57: 845-858.

Goulson, D. 1999. Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. - Perspectives in plant ecology, evolution and systematics 2: 185-209.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. - Biometrics 857-871.

Guimaraes Jr, P. R. et al. 2007. Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. - Current Biology 17: 1797-1803.

Guimera, R., and Amaral, L. A. N. 2005. Functional cartography of complex metabolic networks. - Nature 433: 895.

Heithaus, M. R. et al. 2008. Predicting ecological consequences of marine top predator declines. - Trends in ecology & evolution 23: 202-210.

Hendrickx, F. et al. 2007. How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. - Journal of Applied Ecology 44: 340-351.

Hiraiwa, M. K. and Ushimaru A. 2017. Low functional diversity promotes niche changes in natural island pollinator communities. - Proc. R. Soc. B 284: 11.

Horn, H. S. 1966. Measurement of "overlap" in comparative ecological studies. - The American Naturalist 100: 419-424.

Hsieh, T. C. et al. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). - Methods in Ecology and Evolution 7: 1451-1456.

Hutchinson, G.E. 1957. Concluding remarks. - Cold Spring Harbor Symposia on Quantitative Biology 22: 415–427.

Janzen, D. H. 1974. The deflowering of central America. - Nat Hist 83: 48-53.

Karron, J. D. et al. 2009. Pollinator visitation patterns strongly influence among-flower variation in selfing rate. - Annals of Botany 103: 1379-1383.

Kottek, M. et al. 2006. World map of the Köppen-Geiger climate classification updated. - Meteorologische Zeitschrift 15: 259-263.

Laliberté, E. and Legendre, P. 2010. A distance-based framework for measuring functional diversity from multiple traits. - Ecology 91: 299–305.

Legendre, P. et al. 2011. Testing the significance of canonical axes in redundancy analysis. - Methods in Ecology and Evolution 2: 269-277.

Machado, I. C. and Lopes, A. V. 2004. Floral traits and pollination systems in the Caatinga, a Brazilian tropical dry forest. - Annals of Botany 94: 365-376.

Magrach, A. et al. 2017. Honeybee spillover reshuffles pollinator diets and affects plant reproductive success. - Nature ecology & evolution 1: 1299.

Martin, P. H. et al. 2009. Why forests appear resistant to exotic plant invasions: intentional introductions, stand dynamics, and the role of shade tolerance. - Frontiers in Ecology and the Environment 7:142-149.

Maruyama, P. K. et al. 2014. Morphological and spatio-temporal mismatches shape a neotropical savanna plant-hummingbird network. - Biotropica 46: 740-747.

Maruyama, P. K. et al. 2015. Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. - Oecologia 178: 783-793.

Melo, A. S. and Melo, M. A. S. 2017. Package ‘CommEcol’. - japi 14: 1.

Memmott, J. and Waser, N. M. 2002. Integration of alien plants into a native flower–pollinator visitation web. - Proceedings of the Royal Society of London B: Biological Sciences 269: 2395-2399.

Michener, C. D. 2000. The bees of the world. - JHU Press. 1.

Munoz, A. A. et al. 2005. Bottom-up effects of nutrient availability on flower production, pollinator visitation, and seed output in a high-Andean shrub. - Oecologia 143: 126-135.

Newman, E. I. 1973. Competition and diversity in herbaceous vegetation. Nature 244: 310.

Mack, R. N. et al. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. - Ecological applications 10: 689-710.

Olesen, J. M. 2007. The modularity of pollination networks. - Proceedings of the National Academy of Sciences of the United States of America 104: 19891–19896.

Oliveira-Filho, A. T. and Ratter, J. A. 2002. Vegetation physiognomies and woody flora of the cerrado biome. - The cerrados of Brazil: ecology and natural history of a neotropical savanna 91-120.

Ollerton, J. et al. 2011. How many flowering plants are pollinated by animals? - Oikos 120: 321-326.

Patefield, W. M. 1981. Algorithm AS 159: An efficient method of generating random R x C tables with given row and column totals. - Journal of the Royal Statistical Society: Series C (Applied Statistics) 30: 91–97.

Peet, R. K. et al. 2014. Herbaceous layer species richness of southeastern forests and woodlands: patterns and causes. In: The herbaceous layer in forests of eastern North America. - Oxford University Press.

Pivello, V. R. et al. 1999. Alien grasses in Brazilian savannas: a threat to the biodiversity. - Biodiversity & Conservation. 8:1281-1294.

Poisot, T. et al. 2012. The dissimilarity of species interaction networks. - Ecology Letters 15: 1353-1361.

Potts, S. G. et al. 2010. Global pollinator declines: trends, impacts and drivers. - Trends in ecology & evolution 25: 345-353.

Rathcke, B. 1983. Competition and facilitation among plants for pollinators. – In: Real, L. (ed.), Pollination biology. Academic Press, pp. 309–329.

Sakai, A. K. et al. 2001. The population biology of invasive species. - Annual review of ecology and systematics 32: 305-332.

Scherber, C. et al. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. - Nature 468: 553.

Schweiger, O. et al. 2010. Multiple stressors on biotic interactions: how climate change and alien species interact to affect pollination. - Biological Reviews 85: 777-795.

Seifan, M. et al. 2014. The outcome of shared pollination services is affected by the density and spatial pattern of an attractive neighbour. - Journal of Ecology 102: 953-962.

Shurin, J. B. et al. 2012. Warming shifts top-down and bottom-up control of pond food web structure and function. - Philosophical Transactions of the Royal Society of London B: Biological Sciences 367: 3008-3017.

Silveira, F. A. et al. 2002. Abelhas brasileiras: sistemática e identificação. - Belo Horizonte: Min. Meio Ambiente/Fund. Araucária 253.

Silvertown, J. 2004. Plant coexistence and the niche. - Trends in Ecology & Evolution 19: 605-611.

Souza, C. S. et al. 2018. Temporal variation in plant-pollinator networks from seasonal tropical environments: Higher specialization when resources are scarce. - Journal of Ecology 00: 1-12

Steiner, C. F. et al. 2007. Resurrecting the ghost of competition past with dormant zooplankton eggs. - The American Naturalist 169: 416-422.

Strayer, D. L. et al. 2006. Understanding the long-term effects of species invasions. - Trends in ecology & evolution 21: 645-651.

Tilman, D. et al. 2001. Forecasting agriculturally driven global environmental change. - Science 292: 281-284.

Valiente-Banuet, A. et al. 2015. Beyond species loss: the extinction of ecological interactions in a changing world. - Functional Ecology 29: 299-307.

Vázquez, D. P. et al. 2009. Uniting pattern and process in plant-animal mutualistic networks: a review. - Annals of Botany 103: 1445-1457.

Villéger, S. et al. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. - Ecology. 89: 2290–2301.

Vizentin-Bugoni, J. et al. 2016. Influences of sampling effort on detected patterns and structuring processes of a Neotropical plant–hummingbird network. - Journal of Animal Ecology 85: 262-272.

Waser, N. M. 1978. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. - Ecology 59: 934-944.

Waser, N. M. 1986. Flower constancy: definition, cause, and measurement. - The American Naturalist 127: 593-603.

Waser, N. M. et al. 1996. Generalization in pollination systems, and why it matters. - Ecology 77(4): 1043-1060.

Wilkinson, D. M. and Sherratt, T. N. 2016. Why is the world green? The interactions of top-down and bottom-up processes in terrestrial vegetation ecology. - Plant Ecology & Diversity 9: 127-140.

Williams, D. G. and Baruch, Z. 2000. African grass invasion in the Americas: ecosystem consequences and the role of ecophysiology. - Biological Invasions 2: 123-140.

Supplementary material appendices

Appendix 1*(A1)*. Campo cerrado of the hill of Rubião Júnior church in 2016 (top image) and 2017 (bottom image). Note the difference in vegetation coverage between both periods, due to the rising of *Brachiaria decumbens* in 2017, when this invasive alien grass was not controlled by mowing.

Appendix 2*(A2)*. Plant species, floral abundance and functional traits from both studied periods.

Species	Floral abundance		Floral width	Flower length	Flower type	Floral resource
	2016	2017				
Acisanthera quadrata	64	13	5.00	5.67	poricide	polen
Asteraceae sp.1	128	-	1.67	4.67	tube	nectar
Asteraceae sp.2	29	-	9.67	6.00	tube	nectar
Banisteriopsis campestres	34	1102	4.67	8.00	dish	oil
Borreria capitata	560	28	2.67	4.67	bell-funnel	nectar
Borreria poaya	646	9	3.00	5.33	bellfunnel	nectar
Byrsonima intermedia	64	27	4.33	6.67	dish	oil
Byrsonima pachyphylla	-	36	1.00	6.33	dish	oil
Cambessedesia espora	72	-	3.33	10.67	poricide	polen
Caryocar brasiliense	38	17	16.00	24.33	brush	nectar
Chromolaena laevigata	66	411	5.67	10.00	tube	nectar
Comelina erecta	22	3	5.00	3.00	dish	polen
Cosmos sulphureus	107	360	7.33	11.33	tube	nectar
Eriope macrostachya	-	120	3.33	5.67	gullet	nectar
Erythroxylum cuneifolium	-	30	3.67	3.67	dish	nectar
Fabaceae sp.1	21	-	2.33	2.33	flag	polen
Fabaceae sp.2	6	-	1.67	3.67	flag	polen
Gazania sp.	-	192	1.67	5.67	dish	nectar
Hyptis sp.	36	-	2.67	5.33	gullet	nectar
Kielmeyera variabilis	-	227	13.00	17.33	dish	polen
Lessingianthus grandiflorus	-	266	3.67	7.33	tube	nectar
Mandevilla emarginata	1840	-	4.67	8.33	tube	nectar
Mimosa debilis	43	-	17.67	7.00	brush	polen
Mimosa dolens anisitsii	27	-	20.67	6.00	brush	polen
Myrcia bella	3912	2043	4.33	2.33	brush	nectar
Palicourea rigida	-	34	3.33	12.67	tube	nectar
Polygala violacea	9	-	4.33	5.67	dish	nectar
Psidium grandifolium	78	-	13.33	13.00	brush	nectar
Richardia grandiflora	396	-	9.00	12.67	bellfunnel	nectar
Solanum sp.	36	24	6.33	13.67	poricide	polen
Trichogonia attenuata	578	8	1.33	3.67	tube	nectar
Trimezia juncifolia	2	-	13.67	12.33	dish	nectar
Waltheria communis	16	-	1.33	4.00	bellfunnel	nectar