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THE ROLE OF FRUIT CROP SIZE, DIVERSITY OF AVIAN FRUGIVORES AND LONG-TERM TEMPORAL VARIATION OVER SEED DISPERSAL EFFECTIVENESS IN A NEOTROPICAL SAVANNA

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The role of fruit crop size, diversity of avian frugivores and long-term temporal variation over seed dispersal effectiveness in a Neotropical savanna

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Resumo: A quantidade de frutos que uma planta produz é uma característica chave para a atração de uma assembleia de dispersores abundante e diversa, o que pode ter impactos positivos sobre a efetividade de dispersão de sementes (SDE). Mudanças temporais na disponibilidade de recursos e abundância de dispersores também são fatores que podem influenciar a SDE. No entanto, ainda não compreendemos como as dinâmicas de dispersão funcionam em amplas escalas de tempo. Esse conhecimento é crucial nos cenários atuais, marcados por crescentes impactos antrópicos, que muitas vezes levam a extinção e declínios nas populações de frugívoros (inclusive dispersores-chave). Nós selecionamos duas plantas zoocóricas abundantes, nativas de Cerrado, *Miconia rubiginosa*, rica em carboidratos, e *Xylopia aromatica*, rica em lipídeos. Avaliamos como a produção de frutos influencia a diversidade de visitantes, o componente quantitativo da SDE (efetividade quantitativa), a frequência de visitas por aves especialistas e generalistas e como a diversidade de visitantes influencia a efetividade quantitativa (EQ). Também comparamos a EQ provida por assembleias de dispersores (aves e formigas) separadas temporalmente por 15 anos. A produção de frutos pode ter um papel importante na atração de dispersores mais diversos, com impactos positivos sobre a EQ. Possivelmente, o serviço de dispersão de sementes é mantido ao longo do tempo por um núcleo de espécies. No entanto, também existem flutuações nas contribuições de dispersores para a EQ ao longo do tempo, o que ocasiona variações na vulnerabilidade dos sistemas à perda de espécies. A dispersão de sementes é uma função ecossistêmica chave para a diversidade e regeneração das comunidades de plantas, influenciando na provisão de serviços ecossistêmicos. Determinar os fatores que influenciam a dispersão de sementes, além de como os sistemas de dispersão variam a longo-prazo, revela quão frágeis esses sistemas podem ser, quão problemáticos são os casos de declínios populacionais e extinção de espécies para a regeneração das plantas, e o quão crucial é a preservação desse mutualismo.

Palavras-chave: Cerrado; efetividade quantitativa; *fruit crop size hypothesis*; mutualismo; recrutamento de plantas, SDE; seleção mediada por aves.

Abstract: Fruit crop size may be a key-trait for attracting an abundant and diverse assemblage of dispersers, possibly leading to positive outcomes for seed dispersal effectiveness (SDE). Temporal changes in resources or frugivore abundance can also influence SDE, but it is not yet clear how seed dispersal dynamics work when we consider broad time scales. Current scenarios are marked by increasing anthropogenic impacts leading to population decline and loss of frugivores (including key-dispersers), and thus it is fundamental for us to understand seed dispersal dynamics. We chose two abundant zoochoric plant species native from Cerrado, a Neotropical savanna hotspot, the sugar-rich *Miconia rubiginosa* and the lipid-rich *Xylopia aromatica*. We evaluated how fruit crop size affected the diversity of visitors, the quantity component of SDE (quantitative effectiveness), frequency of visits by specialist and generalist birds, and how diversity of visitors affected quantitative effectiveness (QE). Also, we compared QE provided by disperser assemblages (birds and ants) temporally separated by 15 years. We found that fruit crop size may have a role in attracting diverse assemblages of dispersers, with positive outcomes for QE. Also, seed dispersal services may be maintained over time by a core of species. There are also some fluctuations in seed dispersers contributions to QE over time, causing systems to vary in time in their vulnerability to species loss. Seed dispersal is a key ecosystem function for plant community diversity and regeneration, influencing the provision of ecosystem services. Accessing the factors influencing seed dispersal, as well as how seed dispersal systems vary along temporal scales, reveal how fragile dispersal systems could be, how problematic are population declines and species loss to plant regeneration and conservation, and how crucial it is to preserve this mutualism.

Key words: Cerrado; bird-mediated selection; fruit crop size hypothesis; mutualism; plant recruitment; quantitative effectiveness; SDE.

Introduction

Seed dispersal, i.e. the transport of seeds away from the parent plant, is one of the most important ecological processes influencing distribution, abundance and genetic diversity of plant populations (Wang and Smith 2002), as well as diversity of plant communities (Christian 2001). Diaspores (i.e. the unity of dispersal) dispersed away avoid high rates of mortality due to the presence of predators, pathogens and seedling competition around the parent plant (Janzen 1970, Connell 1971). Also dispersed seeds can colonize new areas (Escribano-Avila et al. 2012), increase gene flow and diversity of plant populations (Howe and Smallwood 1982, Jordano et al. 2007), as well as facilitate coexistence between species at a community scale (Stoll and Prati 2001, Rejmánek 2002).

The Neotropics have the highest diversity of frugivores, i.e. animals that depend on fruits for at least part of their lives. In addition, almost 90% of Neotropical trees and shrubs depend on animals to disperse its seeds (Jordano 2000). Animals can perform primary dispersal, removing the diaspore from the canopy of the parent plant to a certain distance (phase I), or secondary dispersal, providing a subsequent transport of the seed that has already reached the ground (phase II). There is an increasing number of studies unraveling the complexity of seed dispersal systems in the tropics, frequently involving these two subsequent dispersal phases, performed by different dispersal agents, such as birds and ants (i.e. diplochory; see Christianini and Oliveira 2009, 2010, Vander Wall and Longland 2004). Diplochory can lead to increases in plant recruitment because seeds that reach the floor naturally, inside animals feces or during fruit manipulation in the canopy, can be rescued by secondary dispersers (Camargo et al. 2016). The fate of seeds depends rescued primarily on which ant species interacts with it, and two extreme outcomes are being deposited in an appropriate site for germination or being destroyed (Penn and Crist 2018).

One way to evaluate how efficient is the seed dispersal service provided by a disperser assemblage and what are the implications over plant regeneration is by calculating seed dispersal effectiveness (SDE, Schupp 1993, Schupp et al. 2010, Figure 1). SDE involves quantity and quality components. The quantity component estimates the number of seeds dispersed away and involves both the number of visits a frugivore does to the plant and the number of seeds dispersed per visit. The quantity component can be addressed as quantitative effectiveness (QE) and refers to the quantitative aspect of the seed dispersal service provided. However, plant recruitment also depends on a qualitative component, which consists in estimating the probability that a dispersed seed will produce an adult plant (Schupp 1993). Quality of seed dispersal involves both the treatment a seed will receive by the disperser and the site where it will be deposited (Nathan and Muller-landau 2000).

The quality component is difficult to measure because ideally it would be necessary to follow seed fate (Schupp et al. 2010). Novel approaches have been efficient in measuring directly the quality component, such as tracking the seeds by marking them with stable isotopes (Carlo et al. 2013) and using genetic markers (Jordano et al. 2007). Other techniques consist in indirect extrapolations, such as registering which type of habitat the disperser goes after feeding (Pizo and Camargo 2018) or estimating locomotion distances (Christianini and Oliveira 2009, 2010), but those provided a limited view of the quality component. Despite the great potential of novel approaches, using standardized variables to measure the quantitative effectiveness could be useful to compare seed dispersal services between years or provided by different visitors (Calviño-Cancela and Martín-Herrero 2009).

SDE provided by a disperser assemblage depends on characteristics of interacting animals. For example, the number of visits to a plant could vary accordingly to dispersers local abundance (Herrera 1998), diet and degree of dependence on fruits (Katusic- Malmborg and Willson 1988). The number of seeds dispersed per visit depends on some morphological characteristics of the disperser, such as body size (Wotton and Kelly 2011), weight or beak width (Jordano 2000). Quality of treatment depends on feeding behavior, i.e. if the seed survives or not after manipulation (Levey 1987, Penn and Crist 2018), and quality of deposition depends on movement patterns after feeding, for example associated to post-visit locomotion distances (Ness et al. 2004, Jordano et al. 2007) or provision of safe sites to germination (Levey and Byrne 1993, Farji-Brener et al. 2004, Christianini and Oliveira 2009, 2010). Thus, animals vary in their level of reliability as seed dispersers: those who don't damage the seeds while feeding, remove them from the vicinity of the parent plant, deposit them in an adequate site for germination, and visit the plant regularly, are considered more reliable than those who don't (Howe and Estabrook 1977).

Also, the number of diaspores removed from a plant can vary accordingly to their fruit crop production, which can vary between plant individuals and over time. Plants producing more fruits could attract a more abundant and diverse disperser assemblage (fruit-crop size hypothesis; e.g. Ortiz-Pulido & Rico-gray, 2000), which could bring positive outcomes to QE, depending on which species are attracted. For example, larger fruit crops could attract a great number of reliable and unreliable dispersers, possibly resulting in no variation of QE compared to smaller crops attracting only a few reliable dispersers (Jordano and Schupp 2000). However, there is a couple of scenarios in which a diverse assemblage of dispersers could be beneficial to seed dispersal. For example, in case higher quality dispersers are satiated, seeds that otherwise would be wasted can be dispersed by lower quality dispersers, such as generalists (Schupp et al. 2010). Also, multiple disperser agents permit complementary dispersal, i.e. transport of seeds to a variety of distances and microhabitats, increasing the quality component of SDE (Spiegel and Nathan 2007).

Because SDE depends on the assemblage of visitors and the fruit crop production, interannual variation in these components could imply variation in QE provided by dispersers and number of diaspores removed from plant individuals over time (Pizo and Camargo 2018), indicating QE depends on a temporal context (Schupp et al. 2010). Yet, we don’t know how exactly seed dispersal dynamics work when we consider broad time scales. For example, in some seed dispersal systems one or few species can dominate contribution to QE, providing a better service (Blendinger 2017 and references therein). However, we are not sure how recurrent are those interactions over time and what are the consequences over systems susceptibility to impacts. Systems dominated by a core of species (i.e., specialized) are more likely to suffer a collapse in seed dispersal services (Rumeu et al. 2017). On the other hand, more generalized systems would have functionally equivalent species (i.e., species providing similar seed dispersal services), being less susceptible to anthropic perturbations, species loss or temporal fluctuations (Zamora 2000). Lack of long-term studies impede our understanding about how these systems work on a broad scale.

Unfortunately, frugivores have been suffering from population declines caused by anthropogenic impacts all over the world (Dirzo et al. 2014). Species richness and abundance are essential to the maintenance of seed dispersal services (Rumeu et al. 2017) and population declines could provoke decreases or even loss of this ecological function (McConkey and O’Farrill 2016). In fact, seed dispersal is one of the most threatened mutualisms in a global scale (Neuschulz et al. 2016), which reflects changes in regeneration, distribution (Beckman and Rogers 2013) and genetic diversity of plant populations (Giombini et al. 2017). With time, these alterations could have effects in a broader scale, affecting also community and ecosystem structure in unexpected ways (Culot et al. 2017, Egerer et al. 2018).

The current scenario marked by increasing anthropogenic impacts leading to species loss, including key-dispersers, makes it fundamental for us to understand seed dispersal dynamics. Accessing the role of plant traits (such as fruit crop size), dispersers abundance and diversity, as well as long-term temporal variation, over seed dispersal systems, could help us comprehend how these systems would function after perturbations and what would be the implications of the loss of seed dispersers to plant regeneration and conservation. Our goal was to access seed dispersal dynamics of two native species of Cerrado. In the first chapter we investigated how fruit crop size affected diversity of visitors, the frequency of visits by specialists and generalists, and QE received by plants, along with how diversity of visitors affects QE. The second chapter covered how long-term temporal variation influence dispersers' (primary and secondary) contributions to QE, considering common, uncommon, specialist and generalist species, as well as implications the number of diaspores removed from plants.

The Cerrado is highlighted as one of the most endangered phytogeographic domains, threatened by land use changes (Strassburg et al. 2017). Besides being a hotspot of biodiversity, the Cerrado provides a variety of ecosystem services to human populations, such as carbon stocking (Grace et al. 2006), water provision (Lima et al. 2017), ecotourism (Murphy et al. 2016), among others. Despite its value, the conservation of Cerrado, as well as other tropical savannas, has been neglected (Parr et al. 2014 and references therein). The role of seed dispersal in plant regeneration makes information about these mutualistic systems valuable to Cerrado conservation and for the maintenance of ecosystem services it provides.

Study area

This study was carried out in Estação Experimental de Itirapina (22º 15’ S - 47º 51’ W, Figure 1), a Station located in Southeast Brazil, where there are still some fragments of Cerrado, a Neotropical savanna. The Station is located inside the Bacia Sedimentar do Paraná and it is drained by Jacaré-Guaçú river basin. The soil is formed by sandstone and basalt, having a high capacity of water retention (Troppmair 2000), making the area important for the provision of water to the Guarani aquifer (Zanchetta 2006). There is a dry and cold season concentrated in April to November, and a warm and wet season occurring in December to March. Mean annual pluviosity and temperature are 1.459 mm and 21.9ºC, respectively. The area is mainly covered by timber plantations with exotic trees such as *Pinus* spp. and *Eucalyptus* spp., coming from its history of use for silviculture. The physiognomy is predominantly dense with a continuous cover of shrubs and trees, the most abundant species being *Xylopia aromatica* (Lam.) Mart., *Miconia albicans* (Sw.) Triana, and *M. rubiginosa* (Bonpl.) DC., which represent almost 75% of the individuals (Mariano et al. 2019). Low strata exhibit predominantly grass species (mostly *Urochloa decumbens* (Stapf) R.D.Webster near fragment edges), but also some “gravatás” (*Bromelia balansae* Mez) and “indaiás” (*Attalea geraensis* Barb.Rodr.). Although fragmented, the study site (known as “cerrado do Valério”) is classified as top priority for Cerrado conservation (Bitencourt and Mendonça 2004). More information about the floristics of study site can be found in Mariano et al. (2019).

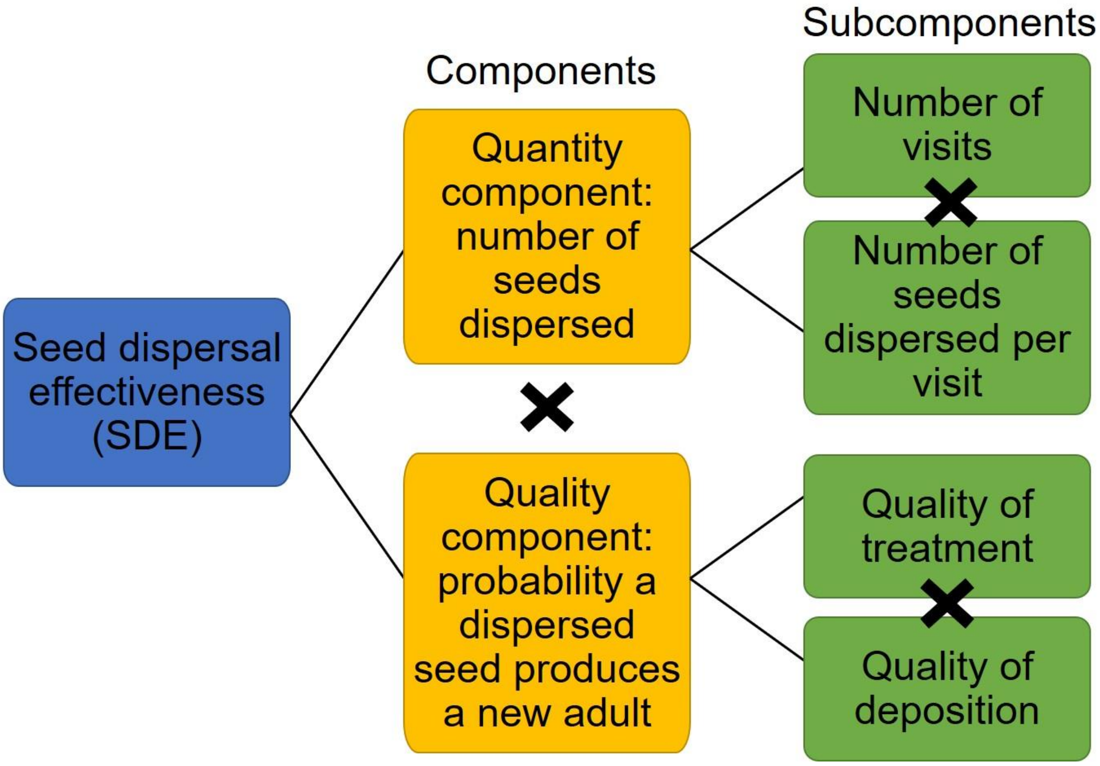

Figure 1. Framework created by Schupp et al (2010) representing the determinants of seed dispersal effectiveness (SDE). 'Components' and 'subcomponents' provide the major organizing framework for developing studies and calculating SDE. The boxes are multiplicative, e.g. the quantitative effectiveness is calculated by multiplying the number of visits and the number of seeds dispersed per visit.

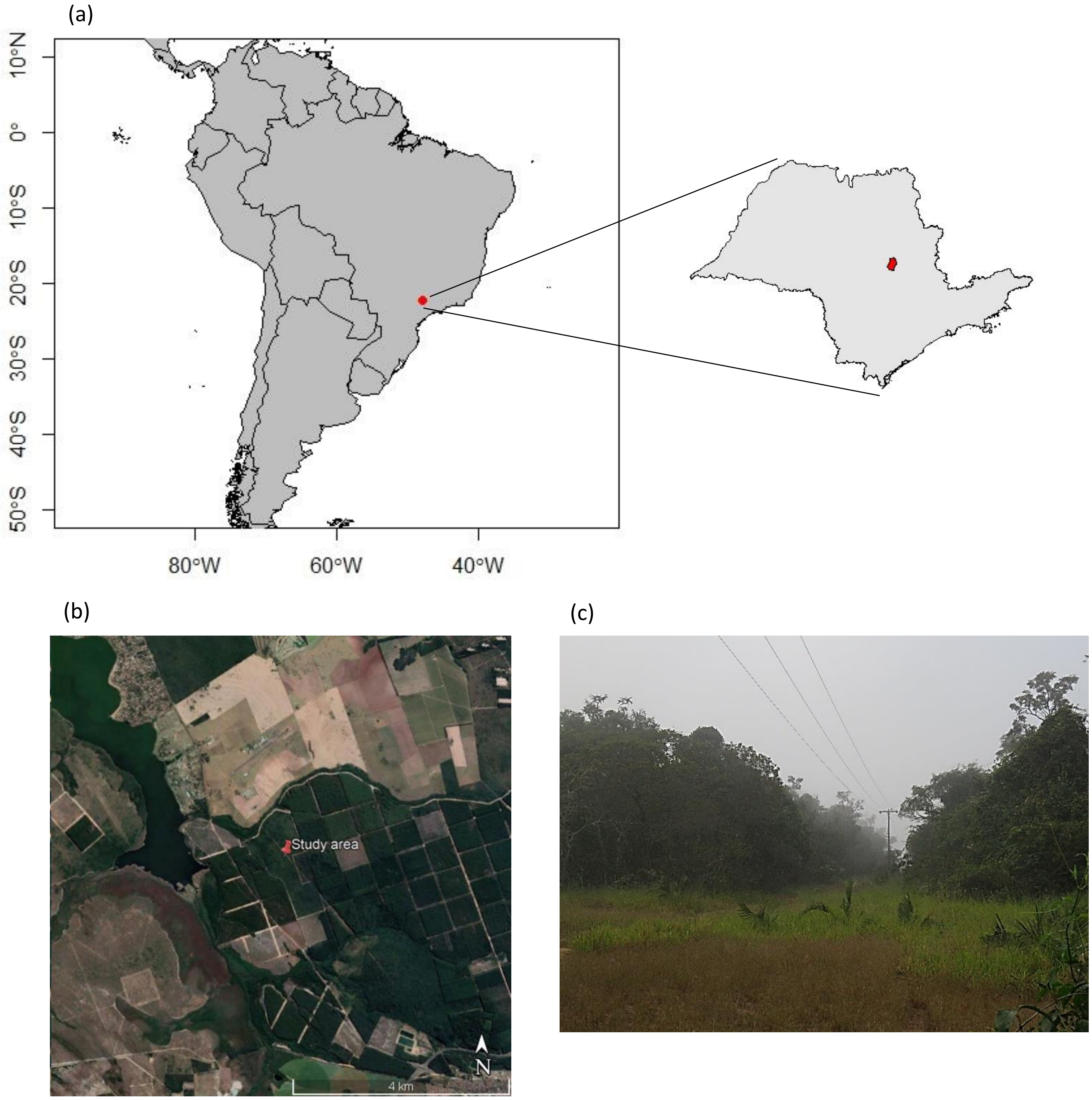

Figure 2. Location of study site in state of São Paulo, Southeastern Brazil (a), satellite image of study site and its surroundings (b) and photo of the study site (c).

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Chapter 1

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Fruit crop size and diversity of visiting birds enhance quantitative effectiveness of seed dispersal

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Highlights

- Fruit crop size may enhance the quantity component of SDE.
- This pattern is more likely to occur for plant species that produce larger crops.
- Larger crops attract a higher diversity of dispersers, possibly enhancing QE.
- There may be a positive link between diversity of visitors and plant regeneration.

Abstract. The fruit crop size hypothesis states that plants producing large crops are likely to attract a greater abundance and diversity of frugivores than conspecifics producing small crops, which could lead to positive outcomes for seed dispersal effectiveness (SDE). SDE can be estimated from the quantity component (QE), tied to the number of seeds dispersed away, and quality component, which accounts for the likelihood of per capita seed recruitment. We evaluated how fruit crop size affected the diversity of visitors and QE, frequency of visits by specialist and generalist birds, and if the diversity of visitors affected QE. We estimated crop size and performed focal observations for birds visiting fruiting plants and removing plant diaspores from the sugar-rich *Miconia rubiginosa* and the lipid-rich *Xylopia aromatica* in a tropical savanna (Cerrado) from southeastern Brazil. We found 19 bird species feeding on the plants, seven interacting with *Miconia* and 17 with *Xylopia*. Crop size had positive effects on visitors' diversity, QE and frequency of visits made by specialists for *Miconia*, but not for *Xylopia*. Diversity of visitors also positively influenced QE in *Miconia*, but not for *Xylopia*. Large fruit crop size can be an important trait in bird-mediated selection for plants that produce large crops of relatively nutrient-poor fruit (*Miconia*), but not for plants producing highly rewarding diaspores in small crops (*Xylopia*). The attraction of a diverse assemblage of dispersers may help in the maintenance of local diversity of frugivores and indirectly facilitate the regeneration of other plants. In turn, frugivore diversity is linked to higher QE for some plant species and individuals. Our study indicates a positive link between a plant trait, biodiversity and seed dispersal, a critical ecosystem service for plant community diversity and regeneration.

Keywords: bird-mediated selection; biodiversity; ecosystem service; plant regeneration; SDE.

Introduction

The amount of fruit a plant produces is a key trait for attracting dispersers and enhancing plant fitness, playing an important role in natural selection regimes (Palacio & Ordano, 2018; Snow, 1971). The fruit crop size hypothesis states that plants producing larger crops are likely to attract a greater abundance of frugivores, increasing fruit removal and probably positively affecting plant regeneration (Ortiz-Pulido & Rico-Gray, 2000). There are two variants of the fruit crop size hypothesis: (i) the total number of fruits removed and/or (ii) the proportion of fruits removed will be higher in individual plants producing larger fruit crops (Laska & Stiles, 1994). However, no studies have approached this hypothesis using values of quantitative effectiveness of seed dispersal (QE). Approaching the fruit crop size hypothesis through measuring QE could be advantageous, because this method allows to disentangle the effect of crop size over both the number of visits and the number of seeds dispersed per visit.

Calculating quantitative effectiveness (QE) helps to evaluate how quantitatively efficient is the seed dispersal service provided by an animal assemblage. The quantitative effectiveness of seed dispersal is one of the components of seed dispersal effectiveness (SDE) and accounts for the number of seeds dispersed away and is estimated by multiplying the number of visits and number of diaspores consumed per visit (Schupp, 1993; Schupp et al., 2010). If fruit crop is a plant trait under frugivore-mediated selection, as it is often the case (Palacio & Ordano, 2018), producing larger fruit crops could lead to positive quantitative outcomes for QE because it would enhance the number of visits a plant receives and the attraction of high-quality seed dispersers.

By attracting a greater abundance of frugivores, highly productive plants may also attract a greater diversity of visitors. The diversity of species in interaction often improve ecosystem functions and services (e.g. Balvanera et al., 2006). The diversity of visitors can improve quantity of dispersal, enhancing QE (Schupp et al., 2010). Attracting multiple visitors could be beneficial for plants when there is no competition for the resource, i.e., there is enough resource to provide food for all interacting frugivorous. For example, specialist frugivores rely heavily on fruits for food and remove great number of seeds without harming them (Howe & Estabrook, 1977; McKey, 1975). They could get satiated by an overabundant fruit crop, but seeds that otherwise would be wasted can be dispersed by generalist birds (Calviño-Cancela & Martín-Herrero, 2009; Hampe, 2008), that may increase plant fitness even providing low quality of dispersal (see Howe & Estabrook, 1977). However, when the resource is limited and there is competition for it, attracting generalists could reduce overall quantitative effectiveness (Calviño-Cancela & Martín-Herrero, 2009), because seeds that could be dispersed by a specialist (high-quality dispersers) are instead being dispersed by generalists.

Unfortunately, frugivores have been suffering from population declines caused by anthropogenic impacts all over the world (Dirzo et al., 2014), with potential ecological and evolutionary consequences (Galetti et al., 2015). Species richness and abundance are essential for the maintenance of seed dispersal services (García & Martínez, 2012, Bello et al., 2015; Rumeu et al., 2017) and population declines of frugivores could provoke decreases or even loss of this ecological function (McConkey & O’Farrill, 2016). In fact, seed dispersal is one of the most threatened mutualism at global scale (Neuschulz et al., 2016), which reflects in changes in regeneration (Fricke et al., 2017), distribution (Beckman & Rogers, 2013) and genetic diversity of plant populations (Giombini et al., 2017). With time, these alterations could have effects cascading to a broader scale, affecting also community and ecosystem structure in unexpected ways (Culot et al., 2017; Egerer et al., 2018).The current scenario marked by increasing anthropogenic impacts leading to species loss, including key-dispersers, makes it fundamental for us to understand seed dispersal dynamics. Plant traits, such as fruit crop size, may have a role in attracting diverse assemblages of dispersers, helping to maintain biodiversity patterns and the integrity of ecological interactions in some ecosystems. Complementarily, a high diversity of dispersers could increase seed dispersal, a critical ecosystem service for plant community diversity and regeneration. Thus, understanding the role of plant traits, dispersers identity (i.e., specialists and generalists) and diversity over seed dispersal systems could help us comprehend implications of the loss of seed dispersers to plant regeneration and conservation. Here we evaluated how fruit crop size can affect (i) diversity of visitors, (ii) QE received by plants, (iii) frequency of visits by specialists and generalists, and (iv) how visitors' diversity can affect QE.

Methods

Study area

The study was conducted from January to June 2019 in the Cerrado from Estação Experimental de Itirapina (22°12'S, 47°51'W), a tropical savanna fragment of approximately 200 ha, located in São Paulo State, Southeast Brazil. Mean annual rainfall and temperature are ca. 1.459 mm and 21.9ºC, respectively. Climate is characterized by a rainy season from December to March and a dry season from April to September. The fragment is covered by Cerrado, a tropical savanna that grows on nutrient poor and well-drained soils and with most plant species resistant to fire. The area has been suffering from woody encroachment due to the protection from fire for decades and for that reason vegetation is predominantly dense. About 62% of trees and shrubs found in the study area depend on animals for seed dispersal (Mariano et al., 2019). More information about the floristics of the study site can be found in Mariano et al. (2019).

Plant species

Miconia rubiginosa (Bonpl.) DC (Melastomataceae) and *Xylopia aromatica* (Lam.) Mart. (Annonaceae) (hereafter *Miconia* and *Xylopia*, respectively) are amongst the most common tree species in the study area (Mariano et al., 2019). Trees of *Miconia* (2-7 m in height) produce large crops of > 100,000 purple fleshy berries (diaspores, about 0.12 g), arranged in bunches. Fruits are rich in carbohydrates, and contain a mean of 11 seeds, weighing about 1.2 mg (Christianini & Oliveira, 2009). Trees of *Xylopia* (2-6 m in height) produce smaller crops (253 ±179 seeds) and fruits ripe asynchronously. When ripe, fruits, composed by a mean of 15 follicles, expose a pinkish interior containing about 60 arylated seeds, a mean of 5 seeds per follicle. Seeds (diaspore, about 0.06 g) have a bluish tone and present an aril rich in lipids (Christianini & Oliveira, 2010). Fruiting of *Miconia* and *Xylopia* occurs approximately between February and April, and April and June, respectively, and both species are attractive to birds, their primary dispersers (Christianini & Oliveira, 2009, 2010).

Diaspore production

We estimated diaspore production for 10 individuals of each plant species. For each *Miconia* tree we counted the number of bunches with fruits in plant crown and multiplied them by the mean number of diaspores contained in 3 to 4 bunches sampled in the same tree. For each *Xylopia* we counted the number of fruits, multiplied it by the mean number of follicles per fruit (obtained from 30 fruits belonging to six trees) times the mean number of seeds inside the follicles. Mean number of seeds inside the follicles was obtained by counting the number of scars left by the seeds inside a sample of follicles from each tree.

Diversity of dispersers

We performed 320 hours of focal observations of fruiting plants in total (160 hours for each species, 16 hours for each individual plant, N = 10 individuals per plant species). Observations were equally distributed in the mornings and afternoons during the fruiting season. For each visitor we recorded the species, number of diaspores with which it interacted and behavior towards the diaspore (i.e., if removed diaspore away or dropped the diaspore beneath the parental plant canopy). Diaspores that were swallowed by birds that after moved away from the canopy were considered removed. To determine whether bird visitors were specialists or generalists (see Howe & Estabrook, 1977) we used data from focal observations and literature. We calculated (for *Miconia* and *Xylopia* separately) the median values of the contribution of fruits (percentage) to diet (following Wilman et al., 2014), total number of interactions with diaspores and proportion of swallowed diaspores recorded during focal observations. We classified the species presenting values equal or higher than the median on all of these three variables as specialists. The remaining were classified as generalists. We also calculated Shannon-Wiener diversity index of visitors for each plant individual. Shannon-Wiener index was given by $H' = - \sum p_i \ln p_i$, where p_i is the proportion of visits a bird species performed to a tree.

Calculating quantitative effectiveness (QE)

To estimate quantitative effectiveness of seed dispersal (QE) for each plant individual. Some frugivores can interact with diaspores without removing them, dropping the seed under the canopy, providing no dispersal away (cheaters). So, QE received by plant individuals was given by the total number of visits received by each individual during observations multiplied by the mean number of diaspores removed. We also recorded the number of diaspores dropped beneath the parental plant canopy during handling by birds.

To determine if the diversity of visitors influences QE, we obtained the variation in QE received by a plant based on focal observation data and simulations following a gradient of increasing species richness of visitors. To increase the number of records and species covered, we also included data from 261 hours of focal observations made in 2004 and 2005 for the same plant species at the same site (Christianini & Oliveira, 2009, 2010), some *ad libitum* observations and interactions recorded during focal observations in neighbor conspecific plants. With all this data we made a list of visitors in which species were represented repeatedly according to the number of visits for each plant species (i.e. 131 visits for *Miconia* and 235 for *Xylopia*). As the total number of visits to a plant may influence the richness of bird visitors, in the simulations we controlled the number of visits as the total richness of visitors recorded for each plant species (i.e. 17 and 24 species for *Miconia* and *Xylopia*, respectively). First, we calculated for each bird species the mean number of diaspores removed/visit using data obtained from focal observations (Table 1). Then, we started simulations with richness value equal to one to simulate the QE produced for a tree receiving the lowest diversity of visitors. That is, we sorted a bird species from the list of visitors and noted the number of seeds removed in a single visit by this species. We then multiplied this value by the controlled number of visits (17 and 24 visits for *Miconia* and *Xylopia*, respectively), producing an expected amount QE if only this bird species repeatedly visited the plant. We did this same procedure ten times, draughting a new species from the list of visitors at each new trial. Because species had different frequency of visits to the plants, they also differed in their probabilities of being selected in the simulations (i.e., most common visitors were more likely to be selected). Next, we performed new simulations, with two, three, four species (i.e., a gradient of increasing frugivore richness) up to the maximum richness observed per plant species in the field (see above). To calculate QE provided by more than one species we multiplied the controlled number of visits by the mean number of diaspores removed recorded for each species sorted. When richness was at its maximum all bird species contributed with their values of removed diaspores to the calculation of QE (considering one visit of each bird species). For each value of richness (except for the maximum), we had ten values of QE obtained through these simulations, so we took the mean of QE values to investigate the effect of bird diversity on seed dispersal.

Data analysis

We used linear regressions to test the influence of crop size on the diversity of visitors, QE and frequency of visits by different bird species. We tested if crop size (explanatory variable) influences Shannon diversity index, QE, frequency of visits by specialists and generalists, and number of diaspores dropped (response variables). All crop sizes were transformed to log values. We also used linear regression to verify the influence of bird species richness (explanatory variable) on QE values generated in simulations of diversity of visitors (response variable). We opted to set the critical value of p (∞) to 0.10 instead of the usual 0.05 given the sample size of plants (N=10 for each species) and the consequently increase in the chance of Type II error (reduced power) in regressions (Zar, 1999). This departure from ecological tradition was justified because we were limited by the number of replicates we could obtain given the trade-off of increasing sampling effort of observations at each tree or the spread of these observations in more trees, but with a lower effort at each and reducing the chance to record visitors. Since the diversity of visitors for each plant could be influenced by the number of visits recorded (Gotelli & Colwell, 2001) we opted to increase sampling effort per individual tree at the cost of reduced number of trees included in focal observations.

Results

During the focal observations in 2019 we found seven bird species interacting with *Miconia* and 17 with *Xylopia*. Probably because *Miconia* is abundant in the study site and produces large crops, half focal individuals did not receive any visits during observations. However, we were still able to detect that trees of *Miconia* with larger fruit crops attracted more visits while for *Xylopia* there was no such pattern (Table 2). Also, plants with larger crops were more likely to attract a higher diversity of visitors in *Miconia* (Figure 1a), compared to *Xylopia* (Figure 1b). Similarly, trees with large fruit crops were more likely to attain higher QE for *Miconia* (Figure 1c), but not for *Xylopia* (Figure 1d). For *Miconia* and *Xylopia*, fruit crop size did not influence the number of diaspores dropped beneath the plant canopy (Table 2). Specialist frugivorous birds in the local assemblage were more likely to respond sharply to increases in fruit crops in *Miconia* than generalists (Figure 1e). For *Xylopia*, fruit crop size did not influence the likelihood of increasing visits by specialist or generalist birds (Figure 1f). The simulations indicated that attracting a higher diversity of visitors could increase QE for

Miconia (Figure 1g), but not for *Xylopia* (Figure 1h). See Table 2 for a synthesis of regression results.

Discussion

The amount of fruit a plant displays is a key trait involved in bird-mediated selection (fruit-crop size hypothesis, Palacio & Ordano, 2018). However, our data show not all seed removal patterns supports the expectations from the fruit crop size hypothesis (Laska & Stiles, 1994). While for *Miconia* predictions of the hypothesis are supported, for *Xylopia* trees producing large or small crops obtain similar number of visits and diversity of frugivores, QE, as well as attract a similar number of specialists and generalists' visitors. These results are similar to those found for the same plants at the same site in 2004 and 2005 (Christianini & Oliveira, 2009, 2010), indicating consistent temporal patterns of response. Because *Xylopia* trees produce smaller crops compared to *Miconia* and their fruits ripe more asynchronously, it is possible that crop size is not a conspicuous trait for visually driven frugivores interacting with this plant.

Other fruit traits could also advertise rewards to seed dispersers, such as chromatic contrast, also increasing fruit removal (Ordano et al., 2017; Palacio & Ordano, 2018). In this case, *Xylopia* conspicuousness could be more related to the number of ripen fruits, creating a contrast between seeds, which are blue, and inner walls of the follicles, which are pinkish. The presence of neighboring intraspecific (Blendinger et al., 2008) or interspecific (Carlo, 2005) fruiting plants, as well as the characteristics of the surrounding environment (García, 2001) could also dilute the influence on individual crop size and contribute to differential patterns of fruit removal by birds. Thus, other fruit traits and surrounding environment may play a more important role in bird-mediated selection for *Xylopia*.

Miconia trees producing larger crops are more likely to receive a more abundant and diverse assemblage of visitors, and consequently attain higher QE, than *Xylopia*. Because trees of *Miconia* produce much larger crops than *Xylopia*, it is possible that competition between dispersers interacting with *Miconia* is weak because of the abundance of resources it provides. However, the relatively small rate of visits/hour (0.5 visits/hour for *Miconia* and 1.33 visits/hour for *Xylopia*), the absence of records of agonistic interactions between birds feeding on trees and sometimes presence of mixed flocks during focal observations suggest that competition is probably not important. With enough resource for all visitors, *Miconia* trees tend to gain from visits by both specialists and generalists. Meanwhile *Xylopia* trees hardly produce diaspores for all visitors at once, so the firsts to arrive have a better chance of feeding. Possibly for that reason there is a balance between visits by specialist and generalist dispersers interacting with *Xylopia*, also resulting in similar outcomes for QE, irrespective of the crop size. Also, fruit crop size is not tied to the number of diaspores dropped by birds beneath parental plant canopy in 2019. This indicates that the quality of dispersal, at least based on focal observations, does not decrease with increasing crop size. Thus, at least for *Miconia*, producing larger crops may be a fair investment to increase fitness in some years, but not in others (see Ortiz-Pulido & Rico-Gray, 2000).

By attracting a more diverse assemblage of visitors, trees with larger crops in *Miconia* attain higher QE. Including a diverse assemblage of visitors increases the probability that uncommon and large-bodied dispersers, such as the Guan *Penelope superciliaris*, visit fruiting plants. This bird usually swallows a great number of diaspores at once, maximizing quantitative effectiveness of seed dispersal (Campagnoli & Christianini, unpublished data). In contrast, different bird species interacting with *Xylopia* tend to be more redundant and remove a similar small number of diaspores per visit. Birds that often interact with lipid-rich plant species can also only ingest a limited number of diaspores at once, because the gut takes longer to process those kinds of resources (Quintero et al., in press), which could increase species redundancy. Probably for that reason, increasing species richness in *Xylopia* did not have positive effects over QE. However, we did not measure recruitment success in different microhabitats in the field (i.e. the quality component of seed dispersal effectiveness) and did not considered other dispersal agents that may further contribute to seed fate (such as secondary dispersal by ants, Christianini and Oliveira 2009, 2010).

Ants are important secondary dispersers of seeds embedded in feces of frugivorous vertebrates (Pizo & Oliveira, 1999; Christianini & Oliveira, 2009), and feces from different frugivores influence the attraction and removal by different ant species that may influence seed fate in different ways (Pizo et al., 2005). In addition, fruits previously manipulated by primary dispersers are preferred by ants (Bieber et al., 2013). Thus, even if a bird drops a high proportion of plant diaspores during handling (e.g. generalists), those fruits are still attractive to ants and likely to be secondarily dispersed. This rescue of seeds beneath parental plant canopy may lead to increments in recruitment (Christianini & Oliveira, 2009, 2010). In fact, *Miconia* and *Xylopia* are virtually not dispersal limited at the study site, with seeds saturating almost all the available sites (Mariano et al., 2019).The lack of seed limitation is potentially a result from the diverse assemblage of frugivores dispersing those plants from plant canopy (birds) and after reaching the soil (ants).

Frugivorous birds often prefer to consume a wide variety of fruits (Blake & Loiselle, 1992) allowing them to acquire nutritional complementarity. Birds may benefit from patches containing a diverse neighborhood of fruiting trees (Carlo, 2005, Maruyama et al. 2019), preventing satiation from more abundant resources and allowing diet complementarity. By being the most abundant tree species and attracting a high diversity of frugivores, *Miconia* and *Xylopia* may help to maintain the local diversity of frugivores at the site, facilitating seed dispersal of other plants that share the same dispersers (Saracco et al., 2005). Indeed, the birds visiting *Miconia* and *Xylopia* are also frequently recorded feeding on other fruiting trees at the same study site (Campagnoli & Christianini, unpublished data) and elsewhere in Cerrado (Maruyama et al., 2019 and references therein).

Biodiversity has a major role in ecosystem functioning, reflecting in positive outcomes for ecosystem services and consequently for human-wellbeing (Balvanera et al., 2006, Naeem et al., 2009). With the ongoing biodiversity crisis associated with anthropogenic disturbances, frugivores are at great risk (Dirzo et al., 2014), and population declines combined with species extinction could provoke a rapid collapse of seed dispersal services (McConkey & O'Farrill, 2016; Neuschulz et al., 2016; Rumeu et al., 2017). Because seed dispersal and plant community are intrinsically linked, declines in frugivore populations are likely to impair a wide range of ecosystem services provided by plants that depend on animals to complete their life cycles (García & Martínez, 2012, Bello et al., 2015, Culot et al., 2017, Quijas et al., 2010). Thus, there may be a positive link between fruit crop size, diversity of frugivores and seed dispersal, an ecosystem function that is crucial for plant community diversity and regeneration, and consequently for provisioning of ecosystem services.

Concluding remarks

Our study unveils the role of fruit crop size over seed dispersal of two Cerrado plant species. We found that fruit crop size can be an important trait in bird-mediated selection for plant species that produce large fruit crops of relatively cheap, carbohydrate-rich diaspores such as *Miconia rubiginosa*, but crop size may not be important for plant species producing smaller crops of highly rewarding diaspores, such as *Xylopia aromatica*. Fruit crop size may also have a role in attracting diverse assemblages of dispersers, possibly maintaining the local diversity of frugivores and facilitating seed dispersal of other bird-dispersed plant species. In turn diversity of visitors may have a role in promoting greater seed dispersal for individual plants (i.e., increase fitness).

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Table 1. Detailed list of bird species interacting with *Miconia* and *Xylopia* used in simulations, showing total number of visits, mean number of diaspores removed away per visit, percentage of diaspores removed and dropped, and total number of diaspores removed.

Table 2. Linear regressions for all explanatory and response variables and respective values of multiple R-squared (r^2), probability of significance (p) and line equations for *Miconia* and *Xylopia*.

Figure 1. Relationship between crop size and diversity of bird visitors (Shannon index), quantitative effectiveness (QE) and number of visits by specialist (white dots and solid line) and generalist birds (black dots and dashed line), and relationship between visitors' richness and mean simulated QE received by plants for *Miconia* (a,c,e,g) and *Xylopia* (b,d,f,h) based on focal observations of fruiting plants in a Cerrado from southeastern Brazil.

Table 1

Bird species	Total number of visits	Diaspores removed away/visit	% of diaspores		N	Total of diaspores removed
			removed	dropped		
Visiting *M. rubiginosa*						
Tangara cayana	35	1.36	61.3	38.7	106	65
Camptostoma obsoletum	27	1.18	70.3	29.7	37	26
Tangara sayaca	20	2.79	90	10	30	27
Elaenia spp.	10	1.25	100	0	13	13
Piranga flava	10	1.5	100	0	4	4
Turdus leucomelas	8	1.88	48.4	51.6	31	15
Nemosia pileata	6	1.5	50	50	6	3
Tyrannus melancholicus	3	1	75	25	4	3
Cyanocorax chrysops	2	2	28.6	71.4	14	4
Knipolegus cyanirostris	2	2	80	20	5	4
Zonotrichia capensis	2	2.5	100	0	5	5
Penelope superciliaris	1	161	98.8	1.2	163	161
Cyanocorax cristatellus	1	5	100	0	5	5
Pachyramphus castaneus	1	1	100	0	1	1
Serpophaga subcristata	1	1	33.3	66.7	3	1
Myiarchus tyrannulus	1	0	0	100	1	0
Dacnis cayana	1	2	100	0	2	2
Visiting *X. aromatica*						
Elaenia spp.	52	2.08	95.2	4.8	84	80
Camptostoma obsoletum	37	3.43	71.7	28.3	60	43
Tangara cayana	34	2.83	97.6	2.4	41	40
Dacnis cayana	26	2.91	85	15	40	34
Turdus leucomelas	24	4.26	96.1	3.9	77	74
Nemosia pileata	10	2.97	95.7	4.3	23	22
Myiozetetes similis	8	2.92	100	0	21	21
Pitangus sulphuratus	7	2.8	81.8	18.2	11	9
Myiarchus tyrannulus	6	3.14	100	0	11	11
Piranga flava	6	3.07	71.9	28.1	32	23
Zonotrichia capensis	4	1	33.3	66.7	3	1
Tangara palmarum	3	2	100	0	2	2
Conirostrum speciosum	3	2.3	0	100	7	0
Phaeomyias murina	2	2	100	0	4	4
Tyrannus melancholicus	2	2.82	100	0	5	5
Cnemotriccus fuscatus	2	2.5	60	40	5	3
Cyanocorax cristatellus	1	14	92.9	7.1	14	13
Cyanocorax chrysops	1	3	33.3	66.7	9	3
Pachyramphus validus	1	3	100	0	3	3
Serpophaga subcristata	1	1	100	0	1	1
Megarynchus pitangua	1	1	100	0	1	1

Tangara sayaca	1	1	50	50	2	1
Coereba flaveola	1	0	0	100	1	0
Brotogeris chiriri	1	2	50	50	4	2

Table 2

Variables		***Miconia***			***Xylopia***		
Explanatory	**Response**	**r^2**	**p**		**r^2**	**p**	
Crop size	Total of visits	0.40	0.04	y = 3.69x - 15.49	<0.001	0.99	ns
Crop size	QE	0.35	0.07	y = 10.39x - 45.1	<0.001	0.94	ns
Crop size	Shannon index	0.31	0.08	y = 0.33x - 1.42	0.03	0.58	ns
Crop size	Visits by specialists	0.30	0.09	y = 2.25x - 9.89	0.01	0.70	ns
Crop size	Visits by generalists	0.10	0.36	ns	0.01	0.71	ns
Crop size	Diaspores dropped	0.09	0.38	ns	0.20	0.19	ns
Richness	QE	0.71	<0.001	y = 9.85x + 6.38	0.03	0.36	ns

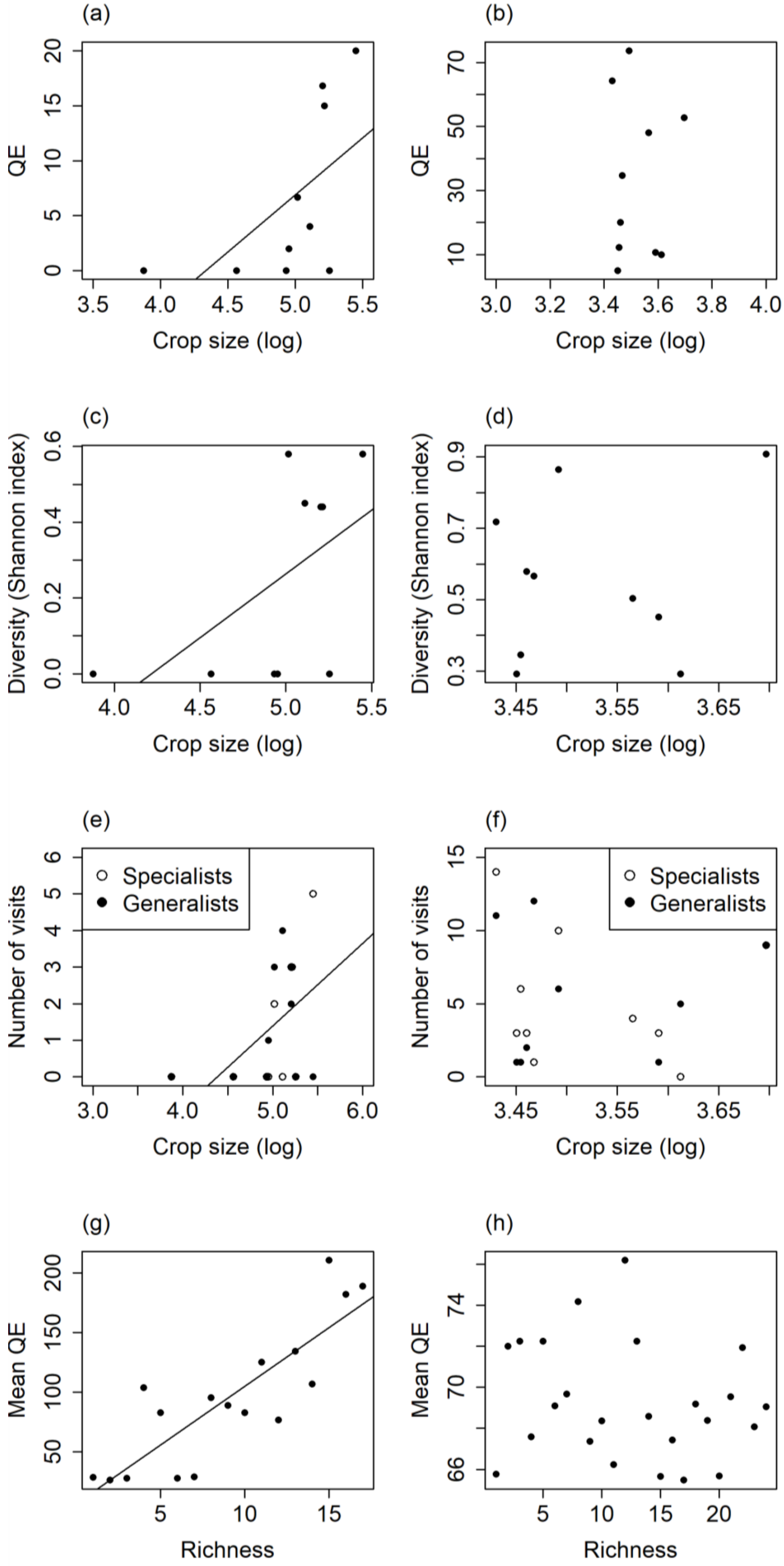
(a)
QE
0
5
10
15
20
3.5
4.0
4.5
5.0
5.5
Crop size (log)
(b)
QE
10
30
50
70
3.0
3.2
3.4
3.6
3.8
4.0
Crop size (log)
(c)
Diversity (Shannon index)
0.0
0.2
0.4
0.6
4.0
4.5
5.0
5.5
Crop size (log)
(d)
Diversity (Shannon index)
0.3
0.5
0.7
0.9
3.45
3.55
3.65
Crop size (log)
(e)
Number of visits
0
1
2
3
4
5
6
○ Specialists
● Generalists
3.0
4.0
5.0
6.0
Crop size (log)
(f)
Number of visits
0
5
10
15
○ Specialists
● Generalists
3.45
3.55
3.65
Crop size (log)
(g)
Mean QE
50
100
150
200
5
10
15
Richness
(h)
Mean QE
66
70
74
5
10
15
20
Richness

Figure 1

Chapter 2

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Long-term temporal variation in quantitative effectiveness of seed dispersal in a Neotropical savanna

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Abstract: Seed dispersal is often a complex process involving two or more subsequent phases performed by different dispersal agents. It is common that the assemblage of dispersers varies in space contributing to variation in the quantity component of seed dispersal effectiveness (QE). Although QE may also vary over time, ecological studies usually ignore long-term temporal variation. We compared QE provided by disperser assemblages of two native plants in savanna (Cerrado) in southeastern Brazil, *Miconia rubiginosa* and *Xylopia aromatica*, temporally separated by 15 years. We performed focal observations of birds (phase I) and ants (phase II) interacting with diaspores in plant canopy and ground, respectively, and calculated the contribution to QE from each agent. We found a total of 26 bird species and 20 ant genera interacting with *Miconia* and *Xylopia* and those were classified as common, uncommon, specialists and generalists. A core of bird and ant taxa (almost 83% common or specialists) changed relatively little over time and was responsible for about 47% and 90% of contributions to QE in phase I and II, respectively. Bird and ant taxa had similar turnover over the years (72.5% and 68% respectively), but the contribution of ants to QE was more consistent over time than birds. In contrast, fluctuations in bird contributions caused variations in levels of specialization and generalization in phase I of seed dispersal. Overall, number of diaspores removed from plants in phases I and II varied temporally, mainly due to differences in visits by seed dispersers in time. Both consistencies and variations in seed dispersers contributions could cause a temporal variation in the susceptibility of dispersal systems to species loss, helping to explain why some plant life stages are more susceptible to anthropogenic disturbances than others.

Key words: Cerrado; functional redundancy; generalization; mutualism; plant recruitment; seed removal.

Introduction

Seed dispersal performed by frugivorous animals plays a crucial role in survival and establishment of new plant individuals, potentially influencing plant population and community dynamics (Christian 2001, Rogers et al. 2017). However, predicting the factors influencing seed dispersal and its consequences are not easy tasks. For instance, many animals may interact with fleshy fruits and produce variable outputs for the seeds (death, damage, survival, dispersal or not to safe sites adequate for recruitment, etc, Calviño-Cancela and Martín-Herrero 2009, González-Castro et al. 2015). Moreover, seed dispersal can be a complex process involving two or more subsequent phases performed by different dispersal agents (i.e., diplochory, Vander Wall and Longland 2004), such as birds feeding on fruits in plant canopy and depositing seeds in droppings on the ground that are further removed by rodents or ants. In diplochory, the primary seed disperser transports a seed away from the parent plant canopy (phase I of dispersal), while the secondary disperser provides a subsequent movement, with the seed already on the ground (phase II). While phase I of dispersal can move seeds farther away, phase II often rescue seeds from risks (e.g. from fire or seed predators) and may deposit them in more adequate sites for germination and recruitment (Briggs et al. 2009, Christianini and Oliveira 2010). All these steps, as well as the contribution of different animals that take part in them, are likely to be subject to temporal variations within and among fruiting seasons, but the empirical evidence is scant (Herrera 1998, González-Varo et al. 2018, Pizo and Camargo 2018) limiting our ability to detect common patterns.

Calculating seed dispersal effectiveness (SDE) is one way of evaluating how efficient is seed dispersal provided by an animal assemblage and what is the impacts over plant regeneration (Schupp 1993, Schupp et al. 2010). SDE involves quantity and quality components (Schupp et al. 2010). The quantity component (i.e. quantitative effectiveness or QE) is associated with the number of seeds dispersed and involves, for example, the number of visits and seeds dispersed per visit of a frugivore. The quality component measures the probability that a dispersed seed will recruit and generate an adult and it is estimated by seed treatment by a frugivore (e.g. if removes or drops the seed), the site of deposition and post-dispersal likelihood of survival and recruitment. Standardized variables to measure QE are useful to compare the role of different interacting species in a site or between years (Calviño-Cancela and Martín-Herrero 2009)

Many animals disperse seeds of the same plant species in the tropical region and that is why there is an assumption that these interactions are often diffuse and generalized. However, recent studies detailing animal contributions to plant regeneration have been providing different evidences (Blendinger 2017 and references therein). Although it is common to find certain redundancy in species contribution to seed removal, some species can dominate the influence on plant regeneration by providing better quality of dispersal (Christian 2001, Manzaneda and Rey 2009, Ordano et al. 2017, Camargo et al. 2019a). However, we are not aware of how consistent outcomes of plant-frugivore interactions are in the long-term. This is an important gap, since spatial and temporally recurrent interactions are more likely to shape traits of interacting species (Thompson 2005). If different species of the same animal group (for example, birds) provide similar quantitative effectiveness for a plant, this redundancy would be important for maintaining seed dispersal services over time. For example, if a decrease in abundance of one frugivore in a year is compensated by an increasing number of visits by other functional equivalent species (but see Fricke et al. 2017)). Thus, redundancy could turn these systems less susceptible to species extinction, and temporal fluctuations than more specialized dispersal systems that rely on a few species (Zamora 2000). The absence of long-term studies makes it difficult to identify temporal patterns and predict how seed dispersal systems may change over time, the consequences and what are the evolutionary processes that shape them.

Quantitative effectiveness of seed dispersal (QE) can vary according to population or intrinsic characteristics of interacting animals. For example, the number of visits of birds to a fruiting plant may vary with local species abundance and migratory patterns (Herrera 1998), while the number of dispersed seeds per visit depends on morphological features of the disperser (such as body size) and the availability of alternative resources in space and time (Jordano 2000). Dispersers that don't harm the seeds, remove them from the vicinity of the parent tree and visit the tree on a regular basis are considered more reliable for seed dispersal (Howe and Estabrook 1977). For example, specialized frugivorous birds are usually more dependent on fruits for food (i.e. present higher percentages of fruits in their diets) and void or regurgitate seeds in viable conditions, while generalist birds mix lower amounts of fruit with arthropods in their diets and sometimes can harm seeds to a varying extent. For species with similar diets, more abundant species would be more likely to visit a plant and affect plant fitness on a regular basis (e.g. common species) compared to uncommon species, which tend to interact with fruits occasionally due to their lower abundance. Thus, different species can contribute to variation in QE (Calviño-Cancela and Martín-Herrero 2009, González-Castro et al. 2015). Also, QE received can vary according to intrinsic plant characteristics such as fruit quantity and nutritional quality, which can also vary among individual plants and years (Van Schaik et al. 1993, Herrera 1998, García and Ortiz-Pulido 2004, Blendinger et al. 2016, Pizo and Camargo 2018). For instance, conspecifics plants producing large fruit crops as well as more nutritive fruits can attract more frugivores and disperse more seeds (Ortiz-Pulido and Rico-Gray 2000, Blendinger et al. 2016).

The assemblage of plant visitors can vary in space and time according to population abundance fluctuations due to migratory patterns, food preferences and abundance of other food resources, among others (Loiselle and Blake 1994, Blendinger 2017). Fruit production can vary spatially and temporally in response to changing environmental conditions (Herrera 1998, Ortiz-Pulido and Rico-Gray 2000). Since QE depends on the assemblage of frugivores and fruit production, it should probably be variable in a spatiotemporal context (Schupp et al. 2010) Although spatial context has been approached by a reasonable number of studies (Nathan and Muller-landau 2000, McCarty et al. 2002, Wright et al. 2005, Schupp et al. 2010, Camargo et al. 2019), there are few studies evaluating temporal variation over seed dispersal in the long-term (Pizo and Camargo 2018). Besides, no studies approach long-term temporal variation over seed dispersal services provided by primary and secondary dispersers. It is likely that the longer the timeframe, the higher is the turnover of species participating in mutualistic interactions (Díaz-Castelazo et al. 2010), so temporal shifts in dispersers assemblages could have effects over QE (González-Varo et al. 2018).

Here we investigated the role of temporal variation over seed dispersal of two native plants in Cerrado, a Neotropical savanna. We compared the QE provided by disperser assemblages and the number of diaspores removed from a sample of plants, both temporally separated by 15 years. We assessed temporal variation over (i) contribution of primary and secondary dispersers to QE, (ii) contribution of common, specialist, uncommon and opportunist dispersers to QE and (iii) total number of diaspores removed from plants in phase I and II of dispersal.

Methods

Study area

We conducted the study from February 2004 to June 2005 (hereafter period of 2004/2005) and January to June 2019 (hereafter period of 2019) in Estação Experimental de Itirapina (22°12’S, 47°51’W), a ca. 200-ha protected Cerrado fragment located in Southeast Brazil. Mean rainfall and temperature are 1.360 mm and 21.8°C (data for 1994 to 2004) and 1424 mm and 23.8 °C (2014 to 2018). Climate is marked seasonal, with a wet season concentrated from December until March and a dry season from April until September. The fragment is covered by a species-rich tropical savanna (Cerrado) growing in poor and well drained soils and with several plant species resistant to fire and that benefit from primary seed dispersal by birds and secondary dispersal by ants (Christianini and Oliveira 2009, 2010). The study site is suffering from woody encroachment due to 40 years of absence of fire disturbance. The physiognomy is predominantly dense with a continuous cover of shrubs and trees, the most abundant species being *Xylopia aromatica* (Lam.) Mart., *Miconia albicans* (Sw.) Triana, and *M. rubiginosa* (Bonpl.) DC., which represent almost 75% of the woody individuals > 3 cm diameter at the base (Mariano et al. 2019). The surroundings are mainly covered by timber plantations with exotic trees such as *Pinus* spp. and *Eucaliptus* spp. and pasturelands that experienced few land-use changes during the study period (Figure S1).

Plant species

We selected two trees among the most common at the study site *Miconia rubiginosa* (Bonpl.) (Melastomataceae) DC and *Xylopia aromatica* (Lam.) Mart. (Annonaceae) (hereafter *Miconia* and *Xylopia*). In each fruiting season, each *Miconia* (2-7 m in height) produce >100,000 purple fleshy berries (diaspores, about 0.12 g) rich in carbohydrates, with a mean of 11 seeds that weighs about 1.2 mg (Christianini and

Oliveira 2009). Trees of *Xylopia* (2-6 m in height) produce a mean of 253 ±179 seeds (Christianini and Oliveira 2010). When ripe, fruits (divided into follicles) expose a reddish inside containing about 60 bluish seeds (diaspores, about 0.06 g), each one having an aril that covers one quarter of the seed. The aril is rich in lipids (Christianini and Oliveira 2010). Fruiting of both species occurs from February to June and they are dispersed primarily by birds and secondarily by ants (Christianini and Oliveira 2009, 2010).

Diaspore production and removal

We estimated diaspore production from seven to ten individuals of each plant species in the beginning of each fruiting season. For each plant we calculated the area of the canopy projected in the ground. Beneath each plant canopy we installed 3 to 5 fruit traps supported by 4 stakes at about 20 cm above ground. Each stake received a layer of Tanglefoot® to prevent ant access. Diaspore production for both species were estimated by visual counts of fruits in plant canopy confirmed by a sample of reproductive structures in each tree (see Christianini and Oliveira 2009, 2010 for details of methods). We removed and sorted fruits and seeds inside the traps every 7-15 days. For *Miconia* and *Xylopia* the number of diaspores wasted beneath the canopy was obtained by total number of diaspores found inside the traps, divided by the fraction of canopy area sampled with the traps. The number of diaspores removed from the canopy by primary dispersers was obtained subtracting the number of wasted diaspores by the total plant production estimated by visual counts (Jordano 1995).

To access the contribution of ants and rodents to the fate of diaspores that reached the ground we performed removal experiments with the aid of selective exclosures. Removal stations were set beneath the canopy of 30 to 60 trees of each species in each year. Each tree was at least 20 m away from each other and considered as a replicate. Removal stations consisted in two paired treatments, one covered by an exclusion cage (15 x 15 x 10 cm, mesh 1.5 cm), excluding vertebrates and allowing only invertebrate access, and the other an open control. Treatments beneath each plant received ten diaspores of *Miconia* or five diaspores of *Xylopia* placed above the ground (for further details see Christianini and Oliveira 2009, 2010). After 24hs we recorded how many diaspores were removed or destroyed *in situ*.

Dispersers' contributions

To sample the contribution of phase I dispersers to QE we performed focal observations in 20 to 44 fruiting plants in each year totaling 581hs of sampling effort for both species. For each bird visitor we recorded the species, number of interactions and behavior towards the diaspores, i.e. if removed it or dropped it beneath plant canopy. Diaspores consumed by birds that latter flew away to another plant were considered dispersed. To investigate the contribution of phase II dispersers we sampled interactions of ants with diaspores fallen to the ground. We placed ripe diaspores of *Xylopia* and *Miconia* over white filter paper (4 x 4 cm), disposed in five stations (10 m from each other) along 8 transects (100 m from each other). Filter paper was used to facilitate visualization on the leaf litter and had no detectable effect on ant behavior. Diaspores were checked each 15 minutes during 2hs. We recorded the ants and their behavior towards the diaspores (i.e. if removed or cleaned the diaspore at the spot). In case of removal, we followed the ant until it reached the nest or until we lost it in the leaf litter, and then measured the removal distance. Due to uncertainties regarding taxonomic classification of some ants, such as *Pheidole*, we analysed the contribution of ants at genus level. Observations were made during the day and night, so we were able to sample ants active during both periods. We also registered interactions *ad libitum*.

We classified dispersers (birds and ants) as common, uncommon, specialist and generalist dispersers. To obtain estimates of bird abundance, we performed 6 point counts in 2004/2005 and 9 point counts in 2019, lasting 10 minutes each. We calculated the median of abundance.period^{-1} using data of all species which interacted with diaspores. Species were arranged by abundance and those with values equal or higher than the median were considered common while the remaining species were classified as uncommon. To classify bird species as specialist or generalist frugivores (see Howe and Estabrook 1977) we calculated separately, for visitors of *Miconia* and *Xylopia*, the median values of percentage of fruits in the diet (based on Wilman et al. 2014), the total number of interactions with diaspores and proportion of swallowed diaspores. Species presenting values of these three variables equal or higher than the median were considered specialist frugivores. The remaining were classified as generalists. To classify ant genera as common or uncommon we used data from a local ant survey (Salles et al. 2018). Samples were based on 64 pitfall traps operated for 48 hours, 32 during the rainy season and the other half during the dry season. Abundance was estimated based on the percentage of pitfalls that presented a certain ant genus. We ordered ant genera interacting with diaspores according to their estimated abundance. Genera with abundances equal or higher than the median were considered common and the remaining were considered uncommon. *Pachycondyla*, *Odontomachus*, *Atta* and *Ectatomma*, previously recorded as important removers of fleshy diaspores (Passos and Oliveira 2004, Christianini and Oliveira 2010, Lima et al. 2013), were considered specialists, and the remaining genera were considered generalists.

Quantitative effectiveness (QE) values

To evaluate the contribution of each species to quantitative effectiveness of seed dispersal (QE) we considered the number of visits to fruiting plants or fallen diaspores and the number and proportion of diaspores removed. Therefore, our measure of seed dispersal captures the immediate consequences of interactions to seed fate. Some frugivores can interact with diaspores without removing them, only consuming the pulp in the spot, providing no dispersal away (cheaters). Thus, QE provided by each primary disperser species was calculated by the number of visits divided by the number of sampled hours in each period, multiplied by the mean number of diaspores removed away. QE provided by secondary dispersers was calculated by the number of interactions of each genus with diaspores, divided by the total amount of interactions, multiplied by likelihood of diaspore removal (i.e. the proportion of *Miconia* fruits and *Xylopia* seeds that were indeed removed). Total number of diaspores removed from plants was calculated by the proportion of diaspores removed from the canopy (canopy removal, phase I) and from the ground (secondary removal, phase II).

Data Analysis

We used linear regression to check if there was consistency in the frequency of visitors between periods (2019 versus 2004/2005; Part of this data were presented in Christianini and Oliveira 2009, 2010). We calculated the percentage contribution that each species or genera (the last in the case of ants) had over total values of QE and compared these values between periods using linear regression. In order to compare the contribution of different assemblages between periods we plotted dispersal agents in quantitative component landscape graphs in order to compare the contribution of different assemblages between periods, using number of visits/h and mean number of diaspores removed per visit (for birds) and proportion of visits and proportion of diaspores removed (for ants) in each axis (Schupp et al. 2017). Each dot (data) was a bird species or ant genera and its respective values of visit rate (X axis) and number or proportion of seeds removed (Y axis) combined. Curved lines (isoclines) represented all combinations of axis that yield the same QE. We used linear regressions to verify how consistent were the contributions of each category of disperser (common, uncommon, specialist and generalist) between periods. At last we looked at total number of diaspores removed from the plants between periods in the canopy and the ground and applied Student *t*-tests for comparisons. We also looked at removal distances provided by birds and ants and compared both years using bar plots and Student *t*-tests.

Results

We found from 10 to 13 and 8 to 20 bird species visiting *Miconia* and *Xylopia*, respectively, in 2004/2005 and 2019. For ants, we found from 7 to 9 and 15 to 6 ant genera, respectively interacting with diaspores of *Miconia* and *Xylopia* in 2004/2005 and 2019. For *Miconia*, frequency of visitors was consistent over time (Figure 1a, birds: $r^2 = 0.54$, $p = 0.0006$; Figure 1b, ants: $r^2 = 0.72$, $p = 0.0002$). For *Xylopia*, frequency of visitors was less consistent for birds (Figure 1a, $r^2 = 0.22$, $p = 0.02$) than for ants (Figure 1b, $r^2 = 0.79$, $p < 0.0001$), indicating a higher variation in visitors assemblage over time for phase I than phase II of dispersal.

Phase I (primary) dispersers contributions to QE were not correlated between periods (Figure 1c, *Miconia*: $r^2 = 0.001$, $p = 0.89$, *Xylopia*: $r^2 = 0.099$, $p = 0.14$), probably because many species interacted with diaspores in only one time period. Some species dominated QE contributions (e.g. had the highest QE values, detaching from others). In 2019, for example, *Penelope superciliaris* dominated QE compared to other species interacting with *Miconia* (Figure 2a). Furthermore, there was a temporal increase in the contribution of some species to QE, such as *Thraupis sayaca*, *Camptostoma obsoletum* e *Tangara cayana*, and a decrease in the number of species providing lower values of QE in 2019, compared to 2004/2005 (Figure 2b). *Elaenia* spp. and *C. obsoletum* had increased contributions to QE for *Xylopia* in 2019 and some new species emerged in comparison to 2004/2005, such as *T. cayana* and *Dacnis cayana* (Figure 2c).

Different from birds, phase II dispersers' contributions to QE were more consistent between time periods (Figure 1d, *Miconia*: $r^2 = 0.98$, $p < 0.001$, *Xylopia*: $r^2 = 0.64$, $p = 0.054$). However, ant genera with the highest contributions to QE for *Miconia* (*Atta*, *Pheidole* and *Ectatomma*) decreased their contribution to seed dispersal in 2019 compared to 2004/2005 (Figure 2e). Anyway, *Atta* was yet the most dominant genus in both periods and their relative positions in the landscape remains very similar, indicating their relative contributions were alike between periods. For *Xylopia*, there was an increase in QE provided by *Pheidole* ants (Figure 2d).

Common and specialist dispersers tended to contribute more consistently to QE when compared to uncommon and generalists, which tended to interact with diaspores in only one of the time periods (Figure 3). Ant specialists were more temporally consistent in their contribution to QE for *Miconia* than *Xylopia* (Figure 3g), probably because most of *Xylopia* seed dispersal was attributed to *Pheidole*, a genus of generalist and common ants that contributed consistently (Figure 3h). *Pachycondyla* and *Odontomachus*, relatively uncommon genera, contributed consistently for QE of *Xylopia* (Figure 3f). For birds, some common species interacting with diaspores had consistent contributions between periods, but there were also some of them contributing to QE in only one period, such as *D. cayana*. *T. cayana*, *T. leucomelas* and *Cyanocorax* *cristatellus* (Figure 3a). Most specialist birds contributed considerably to qe (more than 10%) but not so much consistently in time for *Xylopia*, while for *Miconia* they were more consistent (Figure 3c). One exception was the uncommon and specialist *P. superciliaris*, that was responsible for about 50% of QE contribution received by *Miconia* in 2019 (Figure 3c). Other uncommon species tended to contribute less to QE and in only one period (Figure 3b). Contribution of generalists QE was mostly inconsistent in time, except for species such as *T. cayana*, *Nemosia pileata* and *Piranga flava* (Figure 3d).

Birds (phase I) removed a mean of 25% of the crop of *Miconia* trees in 2004/2005 and 48% in 2019, while for *Xylopia*, birds removed a mean of 31% of diaspores in 2004/2005 and 52% in 2019. Ants (phase II) removed a mean of 26.6% and 76.4% of fruits of *Miconia* in 2004/2005 and 2019, respectively, and 84.6% and 83.2%, of seeds of *Xylopia* in 2004/2005 and 2019, respectively. The proportion of diaspores removed in phase I of dispersal was higher in 2019 for both plant species (*Miconia*: $t = -3.22$, $p = 0.004$, *Xylopia*: $t = -2.57$, $p = 0.02$, Figures 4a, 4c). Phase II removal was higher in 2019 only for *Miconia*, while for *Xylopia* it was similar to 2004/2005 (*Miconia*: $t = 9.28$, $p < 0.0001$, *Xylopia*: $t = -0.35$, $p = 0.72$, Figures 4b, 4d). Removal distances provided by dispersal agents did not vary between time periods for *Xylopia*, but ants provided lower distances of removal for *Miconia* fruits in 2019 compared to 2004/2005 ($t = 5.72$, $p < 0.0001$).

Discussion

Temporal consistency in the frequency of visits by birds and ants indicates that even after a long period of time (15 years) there is some predictability about the most frequent interacting species. A large portion of the seed dispersal service is provided by a few species that visit the plants more frequently (see also Vázquez et al. 2005, Schupp et al. 2010). This pattern persists over time, and more frequent species tend to exhibit low temporal turnover. Less frequent species usually provide lower values of QE and tend to suffer more from species turnover, being replaced by functionally equivalent species (see Zamora 2000). This turnover is probably associated with quantity inefficiency of species (i.e., uncommon species, Schupp et al. 2010) or marginal dependency of fruits on their diets (i.e., generalists, Blendinger 2017). However, some bird species can contribute with a considerable portion of QE in only one period (see *P. superciliaris* for *Miconia* in Figure 3b, *C. cristatellus* for *Xylopia* and *T. leucomelas* for *Miconia* in Figure 3d), contributing to a higher temporal variability in phase I compared to phase II of dispersal. This could be related to the long-term establishment of feeding territories by some individuals (Pizo and Camargo 2018), as well as fluctuations in the abundance of other resources (Loiselle and Blake 1994). Long-term variability in phase I is probably unrelated to fluctuations in species abundances, because abundances were similar between periods (pers. obs.).

Common and specialist dispersers (among birds and ants) tend to contribute more consistently to seed dispersal services in time, compared to uncommon and generalists. This reinforces the idea that there is a consistent core of species responsible for keeping seed dispersal over time. Yet some generalists, mostly also common species, could contribute consistently to QE, such as the bird *T. cayana* (for *Miconia*) and the ant *Pheidole* (for *Xylopia*). The maintenance of *Miconia* and *Xylopia* as the most common plant species in the fragment across the study periods (pers. obs.), associated to the low alterations in the surrounding landscape over time (see Fig. S1), could contribute to the dependency of some frugivores on these plants (Loiselle and Blake 1994). A consistent contribution of dispersers to QE may have evolutionary consequences as it could reinforce the coevolution of a set of traits that allow plants to offer rewards attractive to efficient dispersers and animals to better exploit those resources (Thompson 2005).

Phase II of dispersal seems to be temporally more stable compared to phase I, probably as a result of *Atta* (responsible for about 73% of QE contribution for *Miconia*) and *Pheidole* (responsible for about 65% of QE contribution for *Xylopia*) strongly dominating seed dispersal along the time. While populations of frugivorous birds may fluctuate broadly over time (Herrera 1998, Pizo and Camargo 2018), ant colonies may persist for several years (Morrison 1998), indicating they could be less likely to suffer from fluctuations in population abundance. Ant nests could facilitate recruitment due to provision of safe sites for germination (at least to some seeds) and seedling survival (Levey and Byrne 1993, Christianini and Oliveira 2009, 2010, Farji-Brener and Werenkraut 2017). Yet, although ants provided seed dispersal services more consistently than birds, removal distances provided by ants are considerably lower (Christianini and Oliveira 2009, 2010). In a scenario where ants remain as the sole seed dispersers, removal distances would decrease, possibly affecting plant regeneration due to increasing mortality rates related to high density dependence (Spiegel and Nathan 2010).

Loss of *Atta* could cause considerable decreases in QE received by the *Miconia* plants. However, this scenario is unlikely because this genus is ecologically dominant and, in some cases resistant to anthropogenic impacts (Wilson 2003, Leal et al. 2014). Nevertheless, removal distances provided by ants for *Miconia* decreased considerably in 2019, compared to 2004/2005. *Atta* sp. may become more selective about the resources they carry to their nests as foraging distances increase (Costa et al. 2018). It is possible that *Miconia* fruits were amongst the most valuable resources available for *Atta* colonies in a certain year, or that an increase in *Atta* nest density through those years have decreased removal distances (Gómez and Espadaler 1998). Because benefits provided by seed removal increase with removal distances (Ness et al. 2004), the contribution of ants to the quality component of SDE could vary more in time, compared to the quantity component.

Birds interacting with *Xylopia* fluctuated more over time, compared to those interacting with *Miconia* (Figure 1a). After controlling for tree size, *Miconia* and *Xylopia* had higher crop sizes in 2019 than in 2004/2005 (Campagnoli and Christianini unpubl data). It is possible that they attracted a more diverse and abundant assemblage of primary dispersers in 2019 than in 2004/2005 as bird preferences may be driven by resource abundance (Loiselle and Blake 1994, Ortiz-Pulido and Rico-Gray 2000). Differences between frequency of visits to these plant species could also be related to the chemical compounds in fleshy tissues exploited by birds and ants: while *Xylopia* are lipid rich, *Miconia* are carbohydrate rich. Usually lipid rich diaspores are also the most energetically rewarding ones (Quintero et al. 2020), attracting a wide range of specialists and generalists (Figure 3d, 3h). Furthermore, as the seed of *Xylopia* is small it is unlikely a size-matching constraint to partners in interaction. These may help to explain the lower temporal consistency in bird visitors to *Xylopia*, but the higher consistency for ants, as *Pheidole* have nests in virtually every square meter of Cerrado (see also Salles et al. 2018) and their foragers are faster at discovering food items on the ground than most other ants.

The susceptibility of dispersal systems to species loss can probably vary temporally, in special if there is high dominance of species and low functional equivalence in a given year. This scenario is more likely to happen for birds than ants, considering that QE contributions from bird species fluctuated more over time. For instance, *P. superciliaris* dominated most of the contribution to QE for *Miconia* in

2019. Since this species is more typical from forests, its appearance in 2019 could be due to woody encroachment in the area. Large animals are usually the most affected by anthropogenic disturbances (Dirzo et al. 2014) and the loss of this species would decrease QE considerably for *Miconia* in that year. However, *Miconia*'s dispersal system was much more generalized in 2004/2005, with several species performing functionally similar contributions to QE (Figure 2b). Therefore, the relative impact of the loss of the most important disperser in a given year could be much greater for *Miconia* in 2019 rather than 2004/2005. Bird species providing most of seed dispersal for *Xylopia* in 2019 had very similar contributions to QE. Nevertheless, in 2004/2005 this same system was not generalized, with a few species interacting with *Xylopia* diaspores, and *T. leucomelas* and *C. cristatellus* concentrating the contribution to QE. Another example is phase II dispersal for *Xylopia* in 2019 compared to 2004/2005. In 2019, *Pheidole* ants increased their contribution to QE. This caused *Pheidole* to dominate seed dispersal services for *Xylopia* in 2019, while in 2004/2005, genera interacting with *Xylopia* were more functionally redundant. Thus, systems can vary from generalized to specialized (see Waser et al. 1996) following temporal fluctuations in interactions with animal assemblages, which can make the susceptibility of interaction systems to species loss to fluctuate over time.

We showed that seed dispersal dynamics vary in the long-term. Approaching QE has revealed an interesting pattern: in some years a few species are responsible for most seed dispersal (see also Blendinger 2017), but not in others. Our study unveils that most common and specialist species provide consistent seed dispersal services, suggesting that there may be a core of species that keeps QE over time. Plant-animal interactions maintained over long periods of time could have evolutionary consequences for both sides, for instance, increasing specialization and trait-matching (Thompson 2005, Barker and Bronstein 2016). However, we also found some fluctuations in dispersers contributions over time, causing variations in levels of specialization and generalization of seed dispersal systems. Both consistencies and fluctuations could affect the temporal susceptibility of those dispersal systems to impacts, such as frugivore populations decrease and species loss that could be compensated by common species in some years, but not in others. These temporal fluctuations may help to explain why plant life stages heavily depending on animals, such as pollination and seed dispersal, are more susceptible to anthropogenic disturbances than other stages (Neuschulz et al. 2016).

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Figure 1. Linear regressions showing frequency of visits by birds (a) and ants (b) interacting with *Miconia* and *Xylopia* in 2004/2005 and 2019, and birds (c) and ants (d) contributions to QE of *Miconia* and *Xylopia* in 2004/2005 and 2019. Labels represent species and genera presenting the highest frequency of visits to fruiting plants (a,b) and the highest contributions to QE (c,d). Solid and dashed black lines represent relationships between frequency of visitors of *Miconia* and *Xylopia* in 2004/2005 and 2019. Red lines represent the expected relationship if the frequency of visits were totally consistent between periods. Bird species: *Penelope superciliaris* = Pen sup, Tan cay = *Tangara cayana*, Ela spp. = *Elaenia* spp., Cam obs = *Camptostoma obsoletum*, Tur leu = *Turdus leucomelas*, Thr say = *Thraupis sayaca*, Dac cay = *Dacnis cayana*, Pir fla = *Piranga flava*, Nem pil = *Nemosia pileata*, Con spe = *Conirostrum speciosum*. Ants genera: Atta = *Atta*, Phe = *Pheidole*, Pac = *Pachycondyla*, Ect = *Ectatomma*, Odo = *Odontomachus*, Was = *Wasmannia*.

Figure 2. Quantitative component landscapes showing variation in QE provided by birds interacting with *Miconia* (a, b) and *Xylopia* (c); and ants interacting with *Miconia* (d) and *Xylopia* (e) in 2004/2005 and 2019. Figure 2a shows the dominance of *Penelope superciliaris* over QE provided for *Miconia*, while figure 2b shows detailed contributions of the remnant bird species. Bird species: see legend from Figure 1 for species abbreviations. Ant genera: Cam = *Camponotus*, see legend from Figure 1 for other genera abbreviations. Arrows indicate species and genera presenting alterations in QE between periods. Red points refer to 2004/2005 and blue points refer to 2019.

Figure 3. Linear regressions showing percentage of contribution to QE provided by common (a,e), uncommon (b,f), specialist (c,g) and opportunist (d,h) bird species and ant genera interacting with *Miconia* and *Xylopia* in 2004/2005 and 2019. Solid and dashed black lines represent relationships between dispersers contributions to QE for

Miconia and *Xylopia* in 2004/2005 and 2019. Red lines represent the expected relationship if contributions were consistent between periods. Bird species: Myi tyr = *Myiarchus tyrannulus*, Myi sim = *Myiozetetes similis*, Cya cri = *Cyanocorax cristatellus*, see legend from Figure 1 for other species abbreviations. Ant genera: Meg = *Megalomyrmex*, Myc = *Mycocepurus*, Cyp = *Cyphomyrmex*, see legend from Figure 1 for other genera abbreviations.

Figure 4. Proportion of diaspores removed in the canopy (a,c) and in the ground (b,d) in 2004/2005 and 2019 for *Miconia* **(**a,b) and *Xylopia* (c,d).

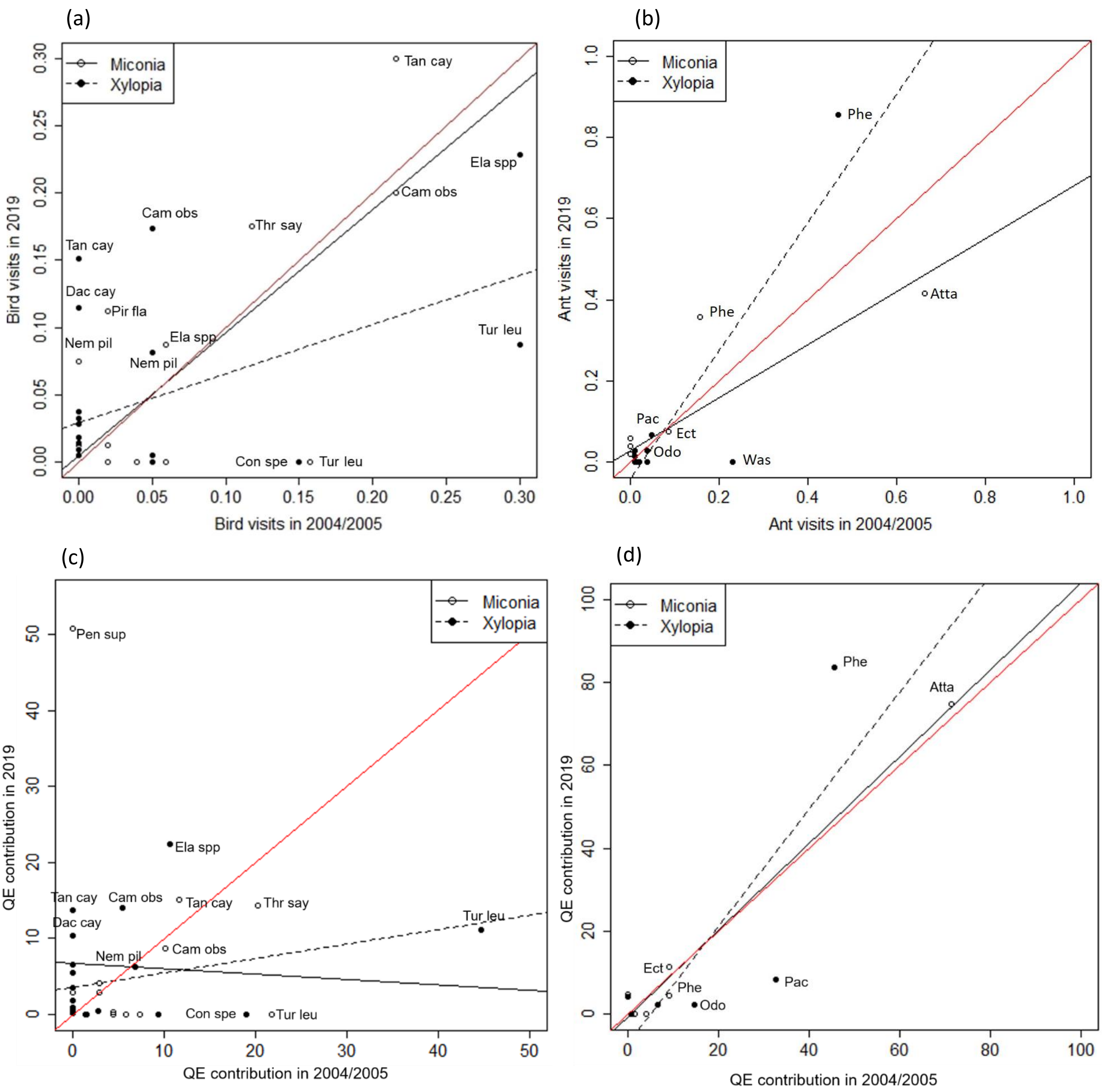
(a)
Miconia
Xylopia
Bird visits in 2019
Bird visits in 2004/2005
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Tan cay
Ela spp
Cam obs
Thr say
Cam obs
Tan cay
Dac cay
Pir fla
Ela spp
Nem pil
Nem pil
Tur leu
Con spe
Tur leu
(b)
Miconia
Xylopia
Ant visits in 2019
Ant visits in 2004/2005
0.0
0.2
0.4
0.6
0.8
1.0
Phe
Phe
Atta
Pac
Ect
Odo
Was
(c)
Miconia
Xylopia
QE contribution in 2019
QE contribution in 2004/2005
0
10
20
30
40
50
Pen sup
Ela spp
Tan cay
Cam obs
Tan cay
Thr say
Dac cay
Tur leu
Nem pil
Cam obs
Con spe
Tur leu
(d)
Miconia
Xylopia
QE contribution in 2019
QE contribution in 2004/2005
0
20
40
60
80
100
Phe
Atta
Ect
Phe
Pac
Odo

Figure 1

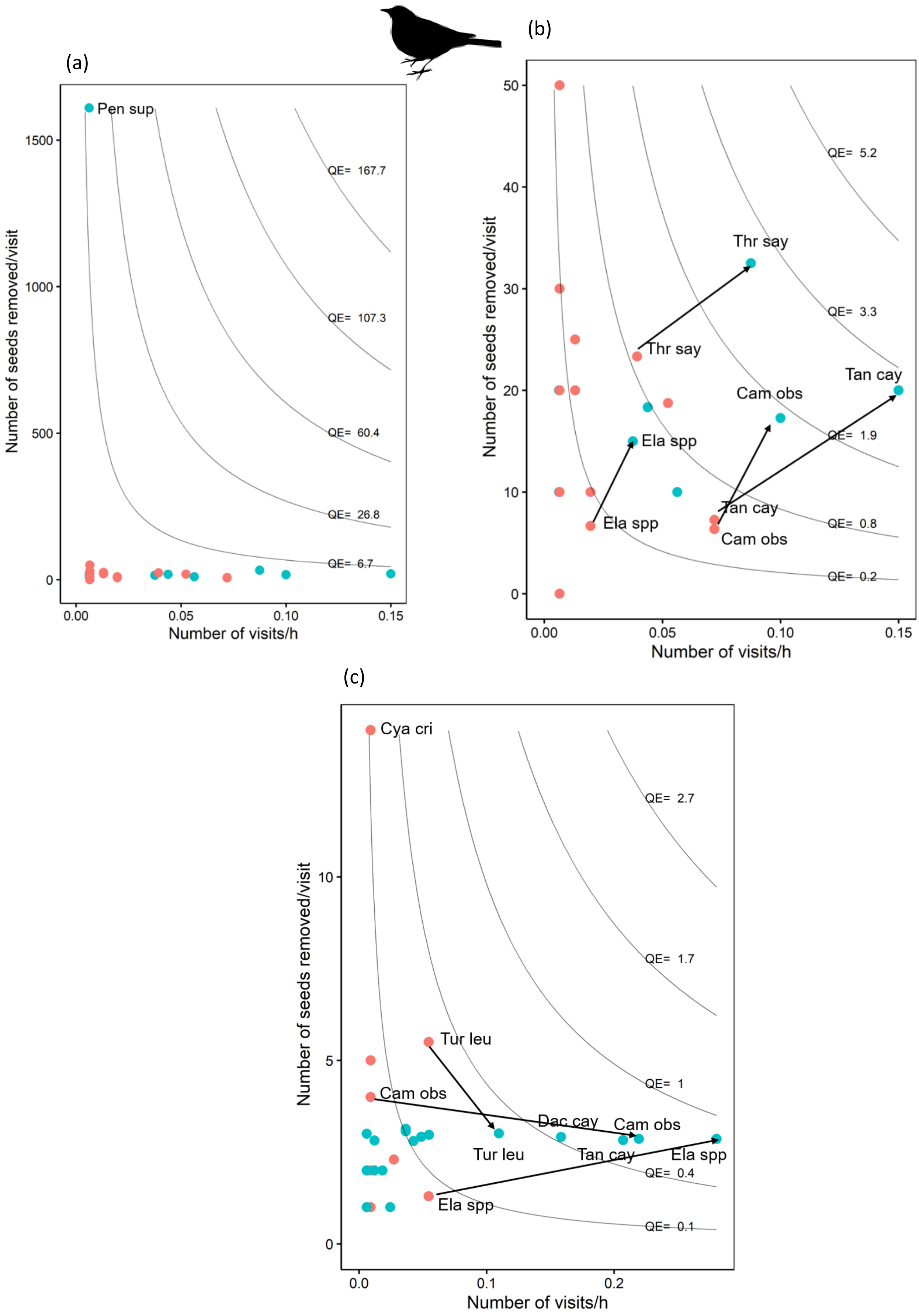
(a)
Pen sup
QE= 167.7
QE= 107.3
QE= 60.4
QE= 26.8
QE= 6.7
1500
1000
500
0
0.00
0.05
0.10
0.15
Number of seeds removed/visit
Number of visits/h
(b)
50
40
30
20
10
0
QE= 5.2
QE= 3.3
QE= 1.9
QE= 0.8
QE= 0.2
Thr say
Thr say
Tan cay
Cam obs
Ela spp
Ela spp
Tan cay
Cam obs
0.00
0.05
0.10
0.15
Number of seeds removed/visit
Number of visits/h
(c)
Cya cri
QE= 2.7
QE= 1.7
QE= 1
QE= 0.4
QE= 0.1
Tur leu
Cam obs
Dac cay
Cam obs
Tur leu
Tan cay
Ela spp
Ela spp
10
5
0
0.0
0.1
0.2
Number of seeds removed/visit
Number of visits/h

Figure 2

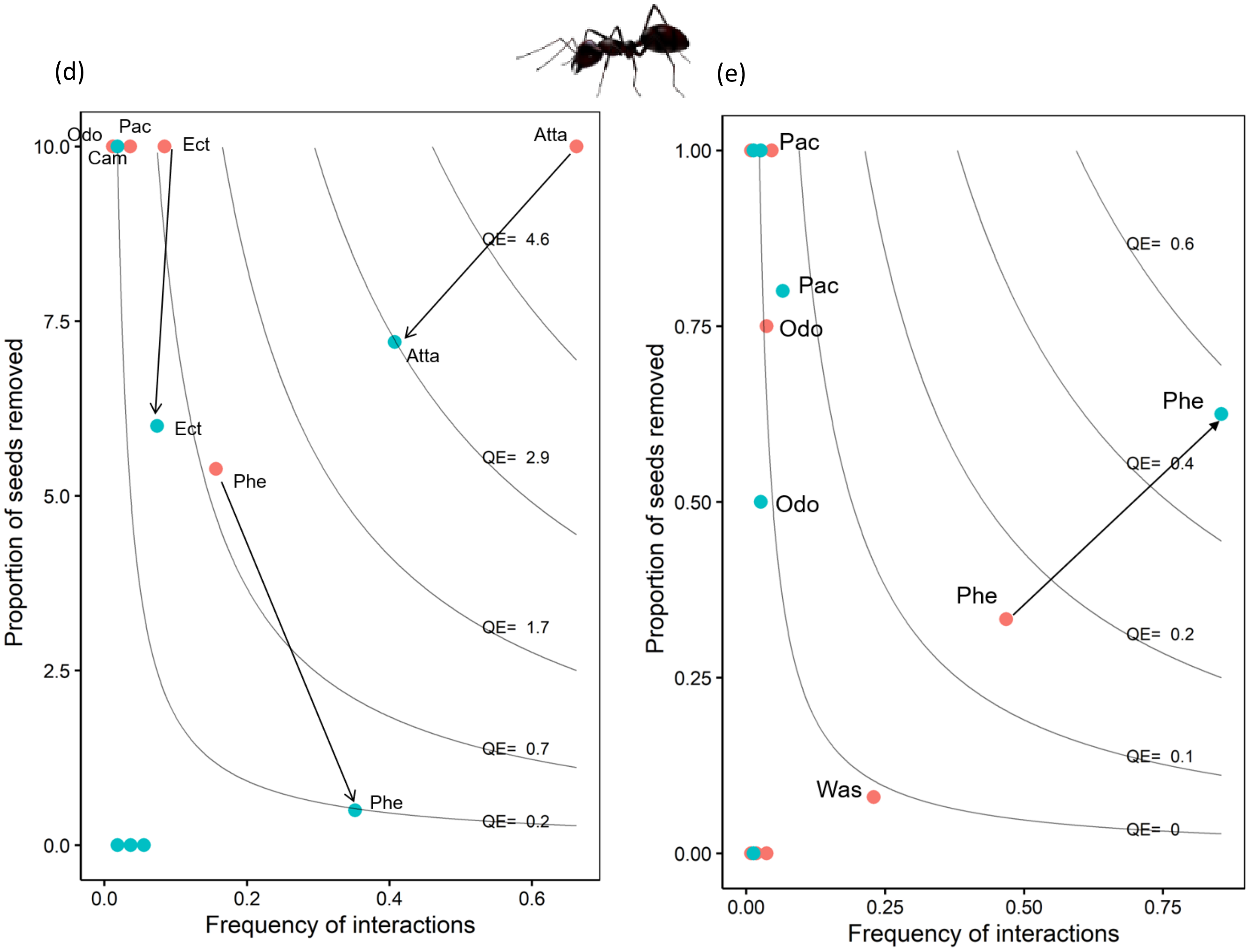

Figure 2 continued

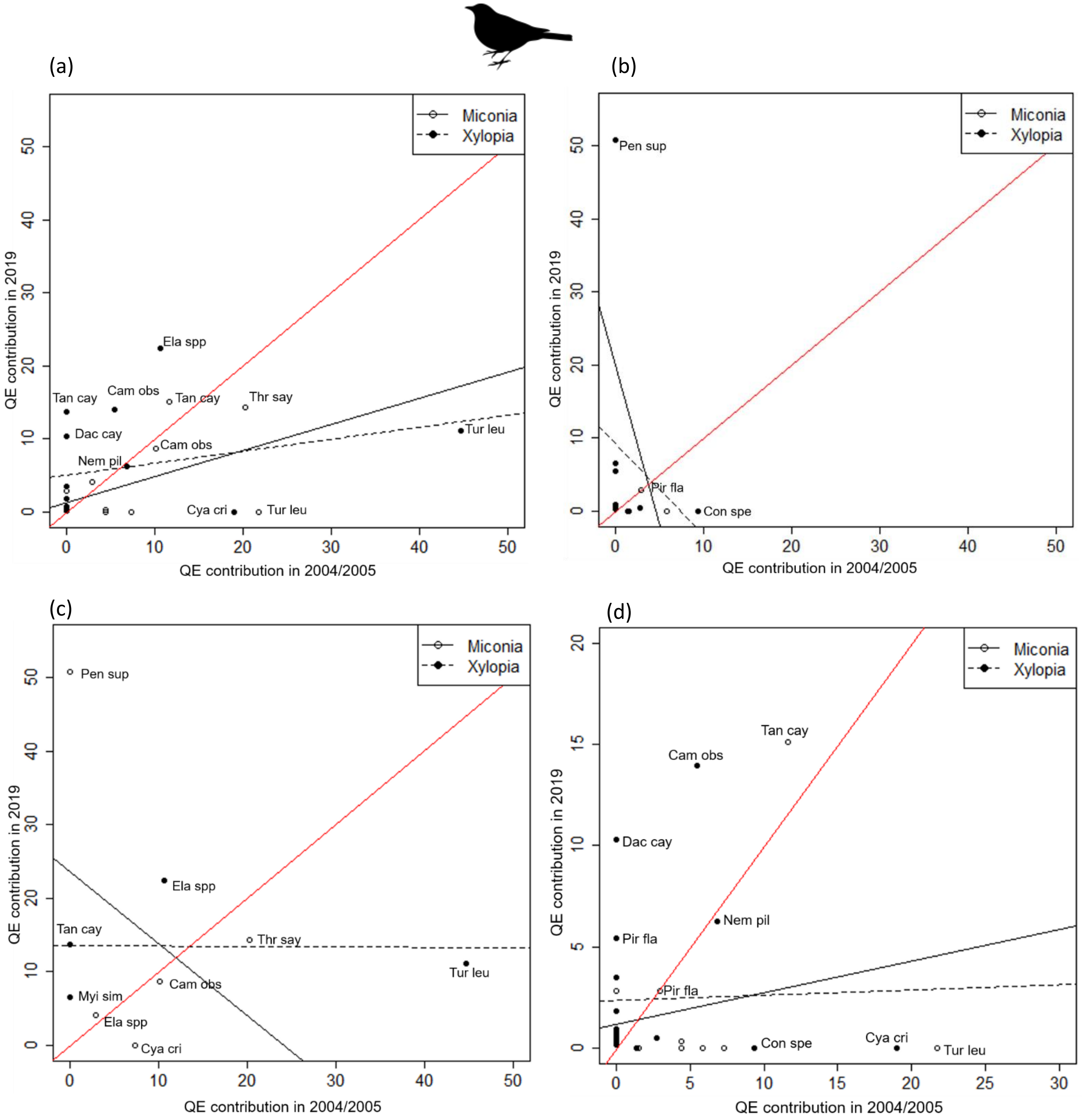
(a)
Miconia
Xylopia
QE contribution in 2019
QE contribution in 2004/2005
0
10
20
30
40
50
Ela spp
Tan cay
Cam obs
Tan cay
Thr say
Dac cay
Tur leu
Cam obs
Nem pil
Cya cri
Tur leu
(b)
Miconia
Xylopia
QE contribution in 2019
QE contribution in 2004/2005
0
10
20
30
40
50
Pen sup
Pir fla
Con spe
(c)
Miconia
Xylopia
QE contribution in 2019
QE contribution in 2004/2005
0
10
20
30
40
50
Pen sup
Ela spp
Tan cay
Thr say
Tur leu
Cam obs
Myi sim
Ela spp
Cya cri
(d)
Miconia
Xylopia
QE contribution in 2019
QE contribution in 2004/2005
0
5
10
15
20
25
30
Tan cay
Cam obs
Dac cay
Nem pil
Pir fla
Pir fla
Con spe
Cya cri
Tur leu

Figure 3

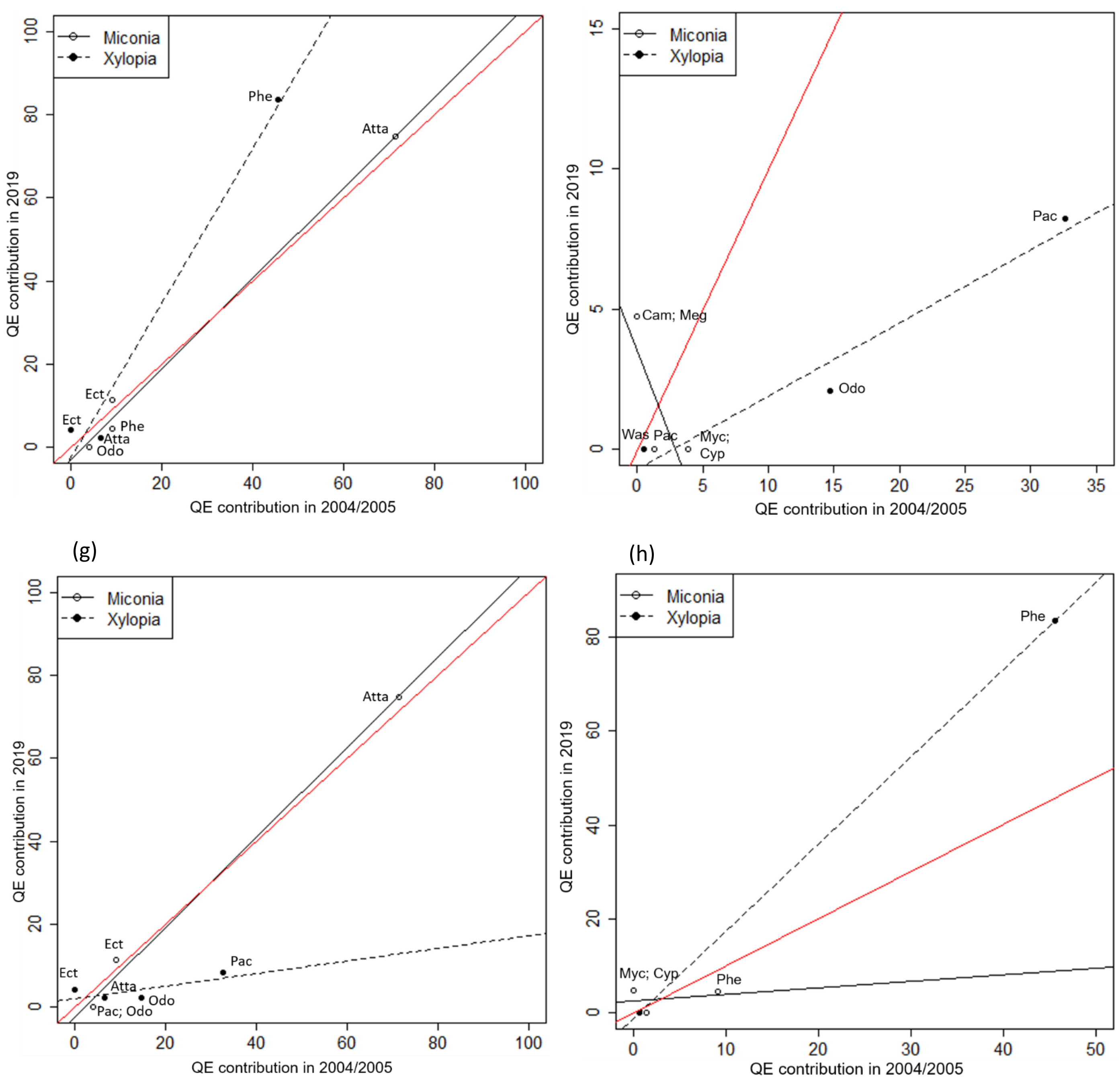

Figure 3 continued

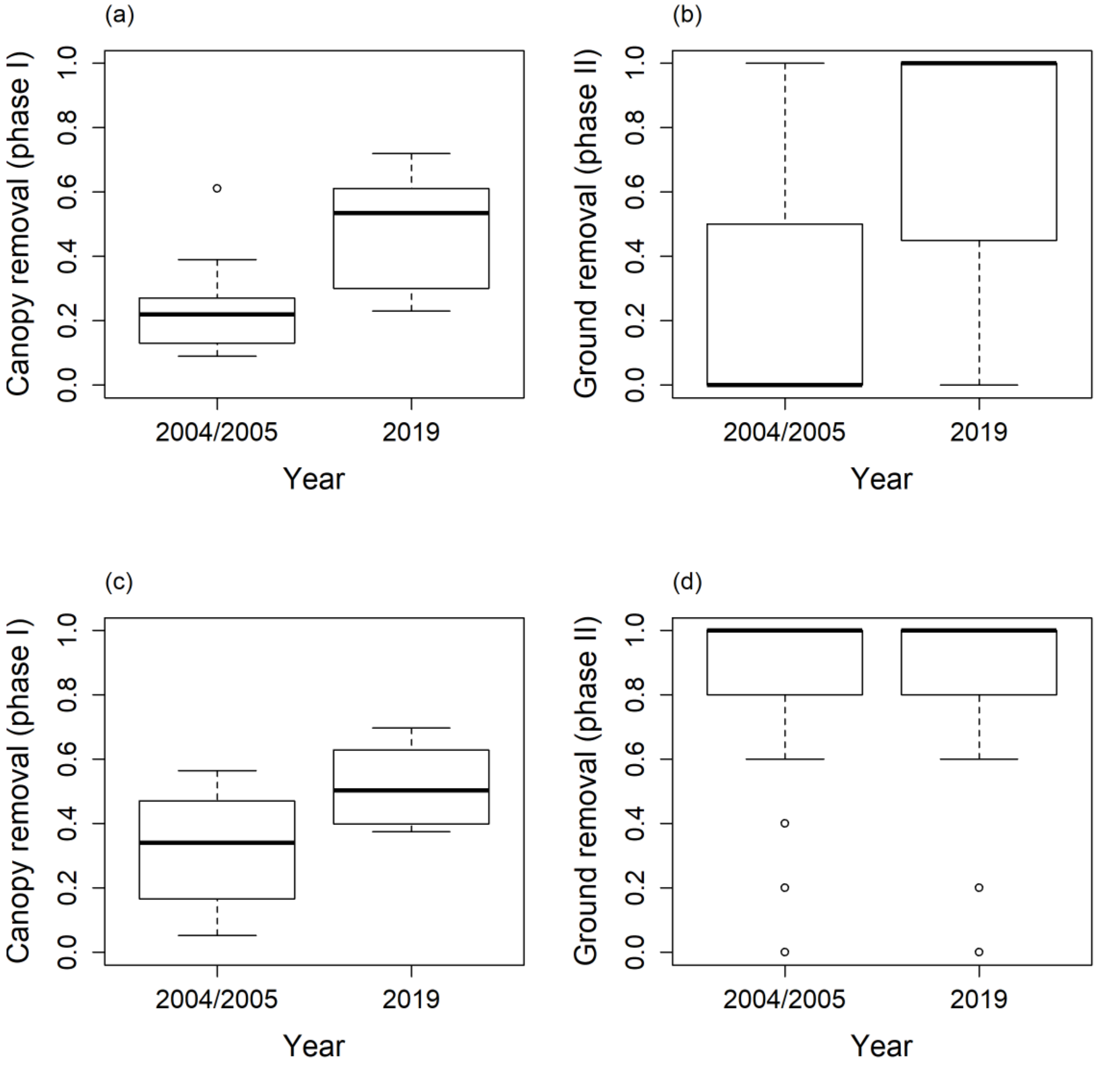
(a)
Canopy removal (phase I)
0.0
0.2
0.4
0.6
0.8
1.0
2004/2005
2019
Year
(b)
Ground removal (phase II)
0.0
0.2
0.4
0.6
0.8
1.0
2004/2005
2019
Year
(c)
Canopy removal (phase I)
0.0
0.2
0.4
0.6
0.8
1.0
2004/2005
2019
Year
(d)
Ground removal (phase II)
0.0
0.2
0.4
0.6
0.8
1.0
2004/2005
2019
Year

Figure 4

Supporting information

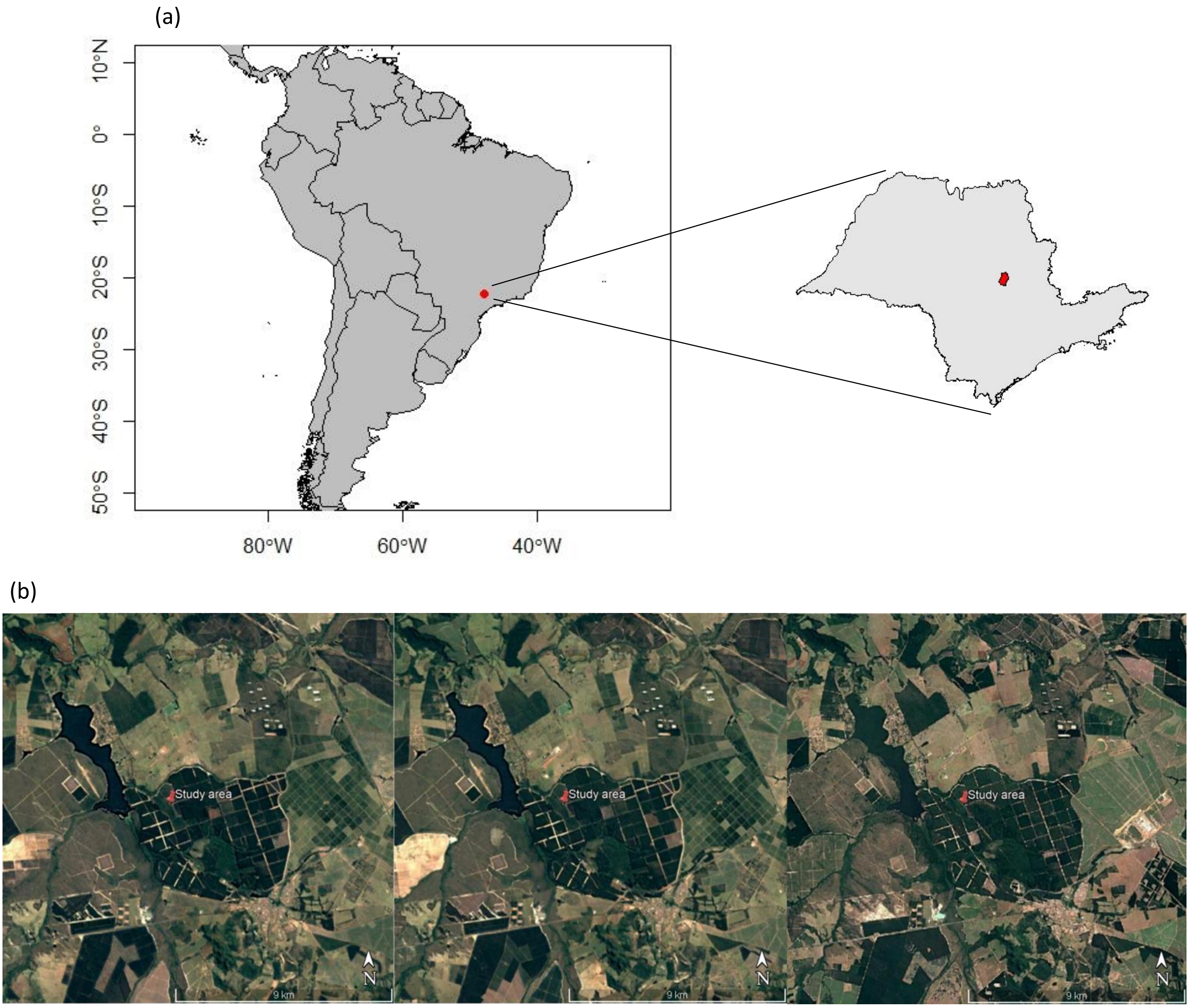

Figure S1. Location of Estação Experimental de Itirapina in Southeast Brazil, São Paulo State (a), and surroundings of the study area in 2004, 2005 and 2019, respectively (b).

Concluding remarks

For some plant species fruit crop size may have a role in attracting diverse assemblages of dispersers, enhancing overall quantitative effectiveness (i.e., increase fitness, Ortiz-Pulido and Rico-Gray 2000, Palacio and Ordano 2018). However, species producing lipid-rich diaspores (e.g. *Xylopia*) limit the number of diaspores frugivores can ingest (Quintero et al. in press) and consequently, dispersers interacting with lipid-rich diaspores are more likely to be redundant, providing similar QE (i.e., functional redundancy), irrespective of crop size. It is possible that seed dispersal services are maintained over time by a core of species, mostly common and specialists (but also a few generalists), which could result in coevolutionary patterns for both plants and animals interacting (Thompson 2005). However, more inconsistent dispersers undergo fluctuations in their contributions to QE over time, causing seed dispersal systems to range from specialized to generalized in time (Waser et al. 1996). This possibly reflects in temporal variation of vulnerability of dispersal systems to anthropogenic impacts, such as population declines and species extinction. To improve our understanding about how QE could be affected by fruit crop size, diversity of frugivores and long-term temporal variation, future studies should focus on accessing the quality component of qe through direct approaches, by measuring seed fate and recruitment success of dispersed seeds (Jordano et al. 2007, Carlo and Tewksbury 2014).

Seed dispersal is a key ecosystem function for plant community diversity and regeneration (Christian 2001), influencing the provision of ecosystem services by plants, such as provisioning of plant products (i.e., food, fodder, timber, firewood), erosion control, invasion resistance, regulations of pathogens and carbon stocking (Quijas et al. 2010, Bello et al. 2015, Culot et al. 2017). Recently Egerer et al. (2018) elucidated the importance of frugivores in maintaining a socially and economically valuable plant in Mariana Islands, *Capsicum frutescens*, and suggested that resource managers may use such mutualisms to get support for conservation of frugivores and forests. In fact, threats to frugivorous animals leading to population declines and species extinction are specially threatening to seed dispersal services they provide (McConkey and O'Farrill 2016). Accessing the factors influencing seed dispersal, as well as how temporal scales affect seed dispersal systems, reveal how fragile those systems could be and how problematic such threats are to plant regeneration and conservation. Thus, we reinforce the importance of biodiversity conservation, as well as the establishment and maintenance of protected areas, to reduce anthropogenic impacts to key mutualisms, such as seed dispersal, ensuring plant regeneration and maintenance of ecosystem functions and services.

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