



Evaluating the significance of native vegetation surrounding orange orchards for the pollinator community

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ABSTRACT

Recognizing the importance of native vegetation close to agricultural fields in sustaining biodiversity and ecosystem services, this study aimed to evaluate the influence of native forests on the floral visitor's community and provision of pollination services. We assessed floral visitor communities and conducted pollination-tests within orange orchards, in both Atlantic Forest and Cerrado biomes, in SP, Brazil. We selected four farms, with two experimental areas, with orange orchards adjacent to native vegetation. Floral visitors survey and pollination-tests were performed in five transects distant from the forest at: 0 m, 100 m, 200 m, 300 m, and 400 m. Floral visitors survey was performed by active collection. Pollination-tests were executed by three treatments: open pollination, pollinator exclusion and supplementary cross pollination. The experimental areas were mapped according to their coverage and land use and the Generalized Additive Models was employed to verify any correlation between the landscape metrics and the floral visitors. We recorded 3084 individuals from 30 taxonomic groups visiting orange flowers, with *Apis mellifera* L. being the most abundant species. While floral visitors' richness showed no biome-specific differences, the abundance was higher in Cerrado. Species richness declined significantly with distance from forest edges, and abundance increased with greater landscape-scale forest cover. Despite demonstrating the critical role of forest remnants in maintaining biodiversity, we observed suboptimal fruit set likely attributable to anomalous climatic conditions rather than pollination deficits. These findings highlight the dual importance of local (proximity) and landscape (coverage) forest conservation for sustaining orchard pollinators, and the necessity of multi-year studies to disentangle climatic effects from true pollination service dynamics.

1. Introduction

Brazil is one of the top five food producers in the world, considered the largest net exporting country in 2021 (FAO - Food and Agriculture Organization of the United Nations, 2023). Orange cultivation, for instance, is among the ten principal crops in the country, being the most cultivated fruit (IBGE - Instituto Brasileiro de Geografia e Estatística, 2022a). In 2019, Brazil led the global ranking, surpassing important

countries such as China, India, and the European Union (CROPLIFE - CropLife Brasil, 2020; FAO - Food and Agriculture Organization of the United Nations, 2021). Currently, it remains the main country producing oranges, mainly for juice exportation (IBGE - Instituto Brasileiro de Geografia e Estatística, 2022a; CROPLIFE - CropLife Brasil, 2023; Shahbandeh, 2023; USDA - United States Department of Agriculture, 2025), with Europe leading the main destination, followed by the United States, which imported 298,781 tons and 170,190 tons of orange juice,

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respectively, in the first half of 2023 (CITRUSBR - Associação Nacional de exportadores de sucos cítricos, 2024).

The genus *Citrus* comprises species of Asian origin, some of which were introduced to Brazil due to favorable climatic conditions, ideal for producing fruits on a commercial scale (Silva and Landau, 2020). The flowers of orange varieties are perfect (e.i., hermaphrodite), highly aromatic, and with white corolla (Lorenzi et al., 2006; Abrol, 2015; Malaspina et al., 2022). In general, *Citrus* species are self-compatible and self-pollinated and can show from moderate to high dependence on pollinators for fruit production (BPBES/REBIPP - Plataforma Brasileira de Biodiversidade e Serviços Ecosistêmicos/ Rede Brasileira de Interações Planta-Polonizador, 2019; Layek et al., 2023). However, in the *Citrus* species with moderate dependency of pollinators, the visit of these insects, especially bees, increases the success of crop pollination, increasing productivity by up to 50 % (Chacoff and Aizen, 2007; Malerbo-Souza et al., 2004; Monasterolo et al., 2022; 2024, Ribeiro et al., 2017).

Optimal pollinator conservation in agricultural systems requires food resources, available nesting sites and accessible water sources (Patrício-Roberto and Campos, 2014). Cultivated crops and strategic planting of co-blooming and pollinator-attractive plants can offer food resources of quality enhancing the pollinator abundance and increasing the visitation rates to crop flowers, therefore contributing to the fruit production during the flowering period of the crop. Also, extends pollinator persistence beyond the crop flowering period (Layek et al., 2021; Gilpin et al., 2022). Nesting sites and water sources can be provided by natural vegetation patches with minimal anthropogenic disturbance (Patrício-Roberto and Campos, 2014; Ranalli et al., 2025).

In this context, the presence of pollinators is more pronounced in regions with higher natural vegetation coverage, once these habitats provide nesting sites and permanent resources for this guild (Patrício-Roberto and Campos, 2014; Ranalli et al., 2025). Therefore, conserving the forests around cultivated areas and planting neighboring co-blooming species contributes to the pollination service (Murta et al., 2008; Patrício-Roberto and Campos, 2014; Huais et al., 2020; Layek et al., 2021; Gilpin et al., 2022).

In Brazil, the main citrus-producing region is in the citrus belt, extending through the States of São Paulo and Minas Gerais, where two Brazilian biomes predominate – Cerrado (Brazilian Savanna) and Atlantic Forest. Cerrado is the second largest biome in South America, present in all Brazilian regions, occupying about 23.3 % of the national territory (IBGE – Instituto Brasileiro de Geografia e Estatística, 2019; IBGE - Instituto Brasileiro de Geografia e Estatística, 2022b). Its continuous area covers several Brazilian States, including Minas Gerais and São Paulo. Considering the entire extension of this biome, it contains springs of the three largest hydrographic basins in South America (Amazonas/Tocantins, São Francisco, and Prata), which results in a high aquifer potential and favors its biodiversity, considered the richest Savanna in a global level (MMA - Ministério do Meio Ambiente, 2023a). Despite this, due to agricultural expansion, it is considered the most threatened Brazilian biome, with few conservation units and programs compared to other biomes (Santelli, 2021).

The Atlantic Forest is the third largest biome in South America and the largest one in Brazil. Its conservation status is even more worrying since there is only 12 % of the original vegetation remaining, of which 80 % is represented by small fragments (Ribeiro et al., 2009; IBGE – Instituto Brasileiro de Geografia e Estatística, 2019; IBGE - Instituto Brasileiro de Geografia e Estatística, 2022b). It is considered one of the most important biomes in the world due to its high biodiversity and endemism (MMA – Ministério do Meio Ambiente, 2023b). Like Cerrado, it is another highly threatened biome, which has been modified / anthropized since the 1500 s, culminating in great loss of biodiversity (Pinto et al., 2009; Ribeiro et al., 2009; IBGE – Instituto Brasileiro de Geografia e Estatística, 2019). At least for this biome, there are some restoration and conservation initiatives, such as the ‘Pact for the Restoration of the Atlantic Forest’ (Rodrigues et al., 2009), still in force

to recover 15 mi ha of its vegetation (<https://pactomataatlantica.org.br/o-movimento/>).

Given the domain and the importance of both Cerrado and Atlantic Forest biomes in the citrus belt, understanding the influence of the natural vegetation on orange crop productivity is crucial. The presence of these natural vegetations close to cultivation areas favors the maintenance of bee diversity in the orchards, contributing to their productivity and the sustainability of several crop production, including orange cultivation (Patrício-Roberto and Campos, 2014; Halinski et al., 2018; Huais et al., 2020; MacInnis et al., 2020; Power et al., 2021; Li et al., 2022; Alexandre et al., 2023). Thus, this study aimed to evaluate the influence of native forests adjacent to orange orchards on the floral visitors' community and the provision of pollination services. The objectives were to: (i) identify the floral visitor insects in orange orchards, (ii) explore how they are affected by the extent of spillover from remnant forest patches and (iii) by the cover of natural forest in the landscape and (iv) measure the contribution of pollinators to fruit production across a distance gradient from the native vegetation.

2. Material and methods

2.1. Sites and studied species

To select the farm, several criteria were considered: (i) the presence of native vegetation immediately adjacent to *C. sinensis* (L.) Osbeck – pear variety; (ii) do not have managed / commercial colonies of Africanized honeybees in the farm; (iii) the orchard must extend at least 400 m from the forest edge, and at this distance, it must be no less than 400 m away from another forest fragment; (iv) the farm must contain two areas that meet the above criteria and are separated by a minimum distance of 800 m and, (v) the owner must provide consent to participate in the project. So, with the help of Fundecitrus team (Fund for Citrus Protection - an association maintained by citrus producers and juice manufacturers from the State of São Paulo to foster the sustainable development of the citrus industry), four commercial orange farms met all the requirements and were selected to this study - two of them located in the Atlantic Forest biome and the other two in Cerrado (Fig. 1).

C. sinensis (L.) Osbeck – pear variety belongs to the Family of Rutaceae. Like other citrus plants, they are originally from Asia and were introduced to Brazil by the Portuguese in the 16th century. Due to the favorable environmental conditions in Brazil, these plants adapted more successfully than in their original habitats (Lorenzi et al., 2006; Cunha-Sobrinho et al., 2013; Mattos et al., 2014). These plants are perennial and can remain productive for approximately 20 years. Their phenology is highly influenced by local weather conditions (Martins et al., 2014). To initiate floral induction, known as pre-flowering, the plants must undergo a period of low temperatures and water stress. During this phase, the plants remain dormant until the onset of the rainy season, at which point they transition into their reproductive phase (Moretto, 2019).

The flowers of *Citrus sinensis* (L.) Osbeck, specifically the pear variety, are aromatic, hermaphroditic, and characterized by five white petals (Lorenzi et al., 2006). The flower's duration, from bud opening to abortion or the onset of fruiting, lasts approximately 25 h (Malerbo-Souza and Halak, 2013). This variety is not strictly dependent on bee pollination; however, the presence of pollinators enhances both fruit production and quality (Ribeiro et al., 2017), with *Apis mellifera* L. been considered the primary pollinator (Malaspina et al., 2022).

2.2. Experimental design

Two experimental areas were installed in each selected farm, totaling 8 study plots (four located in Atlantic Forest biome and four in Cerrado). Then, five transects were installed at five different distances parallel to the native vegetation - 0, 100, 200, 300, and 400 m (Fig. 2) in each experimental area (Halinski et al., 2018; Huais et al., 2020;

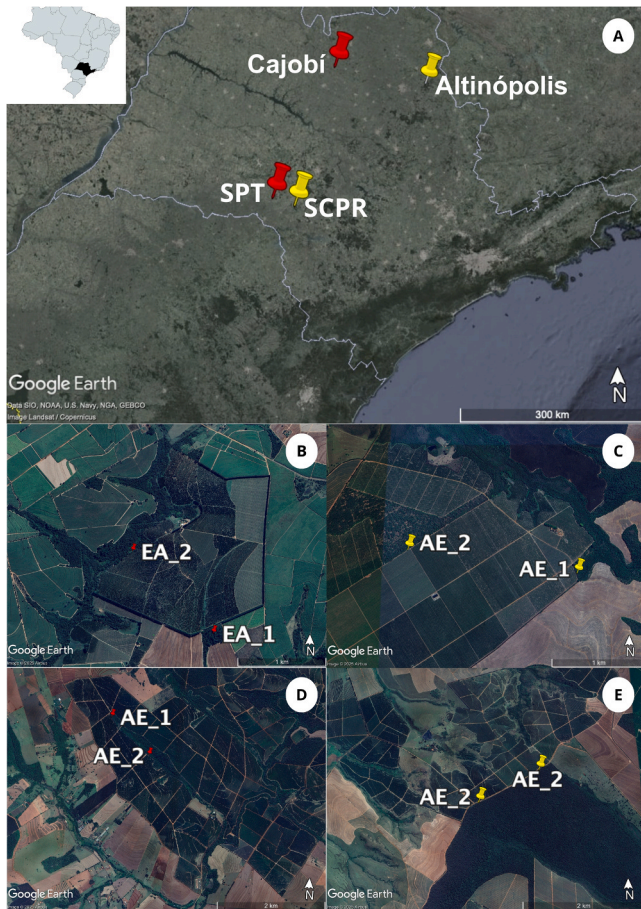


Fig. 1. A- Location of the study sites. The farms in the Atlantic Forest biome are in the cities of Cajobi and São Pedro do Turvo (SPT) - red pins. The farms in the Cerrado biome are in the cities of Altinópolis and Santa Cruz do Rio Pardo (SCRPR) - yellow pins. The biomes were identified through the Mapbiomas Brasil (mapbiomas.org). B, C, D, E - satellite view of the experimental areas in each study farm: Cajobi (B), Altinópolis (C), SPT (D) e SCRPR (E).

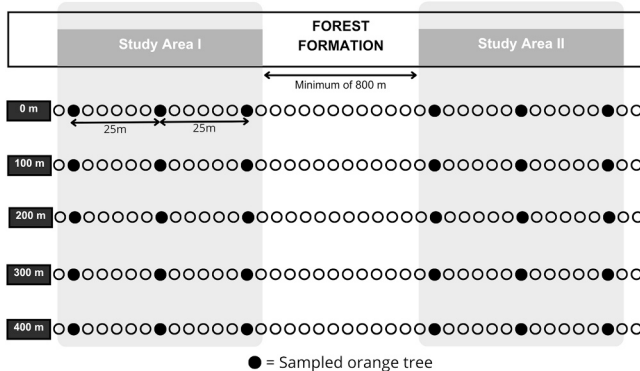


Fig. 2. Experimental design inside one orange farm, with two 'study plots', adapted from Patrício et al. (2012).

MacInnis et al., 2020; Power et al., 2021; Li et al., 2022).

We performed the data collection in three orange trees, apart 25 m from each other in each transect, as demonstrated in Fig. 2.

2.3. Floral visitors' assessment

The floral visitors' assessment was performed during the main blossom, in July of 2023. Collections were carried out during two

periods of the day: twice in the morning, between 7 am and 11 am, and twice in the afternoon, between 1 pm and 5 pm, on two consecutive days at each orange farm, once the flower viability is 25 h. The surveys were executed in the 50 m transects, through the "marked spot" method, adapted from Krug and Alves-dos-Santos (2008) and Patrício et al. (2012), assessing 3 orange trees in each transect – one at each end of the transect and one in the middle of it (Fig. 2). The collector remained at each tree in the transect for five minutes. Insects that landed on the flowers were captured with the assistance of an entomological net, within 25 % of the canopy orange tree.

Each experimental area was sampled by two collectors simultaneously. On the first day of sampling, one collector began the collections on the transect closest to the forest (0 m), while the other started collecting on the farthest transect (400 m). Thus, collections progressed from peripheral distances towards the center of the sampling design, finishing the collection at the 200 m transect. On the second day of collection, both collectors started the assessments within the sampling design (200 m) and moved towards peripheral distances, ensuring that all distances were sampled at the same time intervals.

Captured insects were euthanized in ethyl acetate and stored in 50 mL plastic bottles with 70 % alcohol. In the laboratory, insects were mounted on entomological pins and identified to the lowest possible taxonomic level, aided by identification keys or taxonomic experts. Floral visitor insects captured in this research were deposited in the entomological collection at the Environmental Services and Bee Research Laboratory (ASA's), located at the Federal University of São Carlos - UFSCar, Araras campus, State of São Paulo, Brazil.

The surveys were performed always on sunny days, with temperatures above 13 °C (Silveira et al., 2002).

2.4. Landscape analysis

Land use and cover maps were generated by utilizing satellite images with a spatial resolution of 1 m, accessible in the QGIS version 3.26.2 software with the Google Satellite plugin database. Landscape feature boundaries were digitally delineated manually at a 1:5000 scale, and the polygons were classified into nine categories: citrus, sugarcane, road, bare soil, river and lake, forest plantation, forest formation, pasture, and other perennial crops.

These maps encompassed eight sampled areas (400 m transects), comprising two transects per farm (four farms), with a minimum distance of 800 m between each. We employed a landscape size of 750 m radius following a scale of effect selection determined by the lowest value of the Akaike information criterion (AICc), considering sizes of 500 m, 750 m, and 1000 m. As outlined by Jackson and Farig (2014), the scale of effect refers to the spatial extent to which a landscape variable exerts its most pronounced impact on a specific response variable.

Landscape metrics were computed utilizing RStudio software version 2023.06.0 and the landscape metrics package (Hesselbarth et al., 2019). The PLAND metric was employed to determine the percentage of the landscape covered by each focal class.

2.5. Pollination tests

The pollination tests were executed during main flowering peak, in July of 2023, following the protocol used by Patrício et al. (2012) to evaluate both the importance of native vegetation on the pollinator's contribution to orange fruit set and the indication of a possible pollination deficit (opportunity to improve yield and quality through better pollination). Three different pollination tests were performed (open pollination, pollinator exclusion and supplementary cross-pollination), always marking the terminal bud of an inflorescence to guarantee an optimal standardized protocol, as the inflorescence of an orange tree has floral buds at different stages and locations, and these characteristics imply different fruiting rates (Gonzatto et al., 2018). For each treatment, 30 inflorescences were randomly selected and one floral bud in the

pre-anthesis stage of each inflorescence, was marked with a colored string in each of five distances 50 m transects (0 m, 100 m, 200 m, 300 m, and 400 m away from the forest), totalizing 30 floral buds per distance.

In the open pollination test, the flower buds were marked with a blue string and remained freely accessible to insect visits. For the pollinator exclusion test, the buds were marked with white string, and the inflorescences were bagged inside a PP plastic net for 25 h after bud opening - the period of stigma viability (Malerbo-Souza and Halak, 2013). After this, the buds were exposed to ensure they were subjected to the same environmental conditions as in the other treatments. In the supplementary cross-pollination test, the buds were marked with red string, and in the following day, just after the anthesis, manual pollen addition was performed using pollen collected from three different plants, with the aid of a brush.

To evaluate the fruit set, the formed fruits were counted when they reached the size of a tennis-table ball (the same stage used by farmers to estimate the harvest). In that stage, the abortion of fruits due to lack of pollination is rare.

2.6. Data Analysis

We employed Generalized Additive Models (GAMs) with a single fixed-effect factor and the farm (four distinct farms) as random factors. The fixed-effect factors included the percentage of citrus, sugarcane, forest formation, and pasture, as these four land-use classes are present across all landscapes. We separately applied Generalized Additive Models (GAMs) to each response variable and explanatory variable. The response variables considered were floral visitors' species richness and floral visitors' abundance. We also incorporated a null model into the roster of competing models, signifying the absence of a landscape effect (Martensen et al., 2012).

We selected the models using the AICc corrected for small sample sizes (Burnham and Anderson, 2002). The selected models were those exhibiting the lowest AICc values. Models with AICc values differing from the best model but having a $\Delta AICc$ less than 2.0 were deemed equally plausible. When multiple models were considered equally viable, we assigned weights (w_i) to the models using the weight of evidence (weight; 0.5) as the threshold.

To investigate the impact of the distance from the forest formation patch on the community of floral visitors, we performed the Pairwise Wilcoxon test. And for comparing fauna between the Cerrado and the Atlantic Forest, we performed the Kruskal-Wallis non-parametric test, given the non-normal distribution indicated by the Shapiro-Wilk normality test for the data. The results of the pollination tests did not allow us to apply any statistical analysis, due to the very low fruit set, so we performed a descriptive analysis, calculating the fruiting rate, considering the total number of buds found during the final reading of the experiments, joining the data from all sampled areas for each distance.

All analyses were carried out in R Studio software version 2023.06.0

using the *mgcv* (Wood, 2011), *bbmle* (Bolker, 2022), *stats* (R Core Team, 2023), and *dplyr* (Wickham et al., 2023) packages, except for the descriptive analysis (used to evaluate the pollination tests), which were carried out in Microsoft® Excel for Mac version 16.95 (25030928).

3. Results

We recorded a total of 3084 insects visiting the orange flowers, distributed in 30 different taxonomic groups. *Apis mellifera* was the most abundant ($n = 2551$), followed by *Trigona* spp. ($n = 346$), both from the Apidae family (Table 1; floral visitor species with the most expressive abundance). Representing the bees, we also captured specimens from the families Andrenidae ($n = 2$), Colletidae ($n = 1$), and Halictidae ($n = 5$). We registered floral visitors other than bees, such as wasps (Vespidae - Hymenoptera; $n = 5$), flies (Syrphidae, Tachinidae, and Otitidae - Diptera; $n = 18$), butterflies (Lepidoptera; $n = 13$) and two individuals of dragonfly (Odonata). The table with all data is available in the Supplementary Material (Table S1).

There were no significant differences in the richness of insects visiting the flowers comparing the Cerrado and the Atlantic Forest biomes. However, the richness of these insects was higher at distances closer to the forest (Fig. 3; $p = 0.0043$), independent of the biome, being significantly lower beyond 300 m from native vegetation (Table 2).

Floral visitor abundance exhibited no significant relationship with distance from forest edges (Figure S1, Supplementary Material; $p > 0.05$). Whereas, it was positively correlated with the forest cover extent ($R^2 = 0.54$), with sites in the Cerrado biome exhibiting both higher forest coverage and significantly greater floral visitor abundance compared to those in the Atlantic Forest ($p = 0.09$). Fig. 4 presents the land use mapping of each one of the eight sampled areas. Landscape-level analysis identified forest formation as the only predictor strongly associated with pollinator abundance (Table 3; $w_i = 0.9984$), and this relationship was consistent in orange orchards, where forest cover positively influenced floral visitor abundance (Fig. 5).

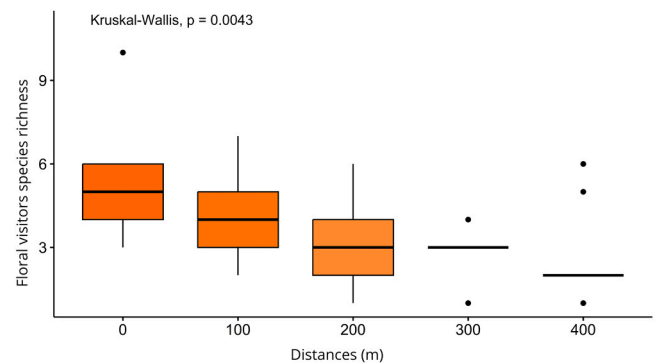


Fig. 3. Floral visitors' species richness in the five different sampled distances from the native vegetation.

Table 1

Floral visitor species with higher abundance registered in the study area at different distances from the forest. AF = Atlantic Forest biome; C = Cerrado biome. The data with all taxonomic groups registered in the floral visitor's assessment can be consulted in the Table S1; Supplementary Material.

Taxon	0 m		100 m		200 m		300 m		400 m	
	AF	C	AF	C	AF	C	AF	C	AF	C
<i>Apis mellifera</i> Linnaeus (Fam. Apidae)	116	404	111	448	76	395	77	309	112	503
<i>Trigona spinipes</i> Fabricius (Fam. Apidae)	11	111	2	12	0	7	0	2	0	5
<i>Trigona hyalinata</i> Lepeletier (Fam. Apidae)	2	74	0	0	0	1	0	0	3	0
<i>Trigona</i> sp. (Fam. Apidae)	22	54	2	26	2	1	1	1	5	0
<i>Paratrigona subnuda</i> Moure (Fam. Apidae)	1	27	0	12	0	6	0	0	0	2
<i>Xylocopa</i> sp. (Fam. Apidae)	6	4	2	20	4	3	1	2	2	0
Other Bees	8	19	5	9	2	4	1	5	4	2
Other insects	6	8	2	6	4	3	4	2	1	2
Total abundance	144	458	125	478	88	409	87	322	121	511

Table 2

Pairwise Wilcoxon test for significant differences between distances (0 m, 100 m, 200 m, 300 m, and 400 m) for pollinator species richness, Wilcoxon test. Table presenting *p* values (significant *p* values are in bold).

	0 m	100 m	200 m	300 m
100 m	0.360	-	-	-
200 m	0.052	0.194	-	-
300 m	0.014	0.075	0.926	-
400 m	0.046	0.075	0.612	0.200

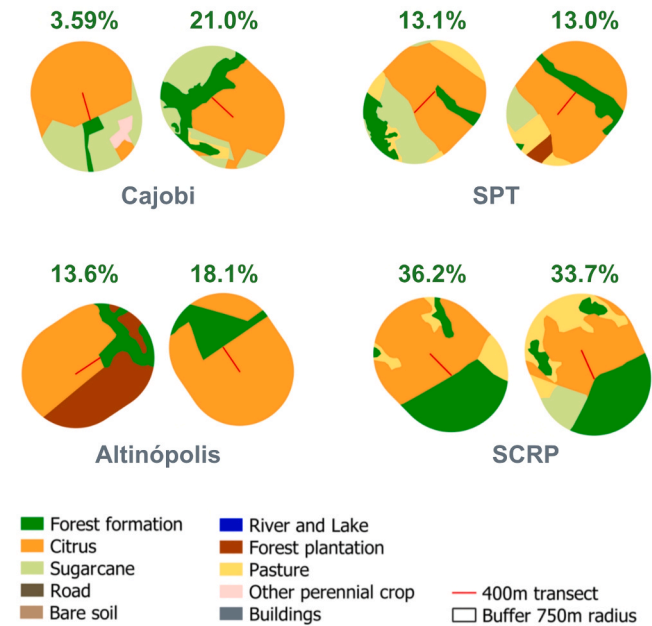


Fig. 4. Orange farms land use mapping. The farms 'Cajobi', and 'SPT' are in the Atlantic Forest biome, while the farms 'Altinópolis' and 'SCR2', are inserted within the Cerrado domain. The value above each buffer represents the percentage of natural vegetation cover.

Table 3

Result of the model selection for the floral visitors' community. We fitted Generalized Additive Models and showed their respective AICc, the relative difference ($\Delta AICc$), and the model of weight of evidence (w_i). Models with $w_i > 0.5$ were considered as equally plausible. Percentage of each focal class (citrus, sugarcane, forest formation, and pasture), and Null (uncertainty of the relationship between response variables and explanatory variables). Only the plausible model is presented here - complete results are presented in [table S2 - Supplementary Material](#).

Model	AICc	$\Delta AICc$	w_i
Pollinator abundance ~ forest formation	127.3	0.0	0.9984

The pollination tests resulted in a very low fruit set - during the week of experiment setup, the region experienced an extreme and atypical heatwave due to the El Niño phenomenon, with temperatures exceeding 40°C for several consecutive days. Furthermore, after the implementation of the tests, some farms managed the orchards by drastically pruning the cultivation lines, leading to the loss of some experiments. This management resulted in an unstandardized sample size, rendering any statistical analysis of the collected data unfeasible. So, we performed a descriptive analysis, calculating the fruiting rate, considering the total number of buds found during the final reading of the experiments, joining the data from all sampled areas for each distance ([Table 4](#)).

In general, the pollinator exclusion test showed the lower fruiting percentage, as expected, once *C. sinensis* (L.) Osbeck does not depend on

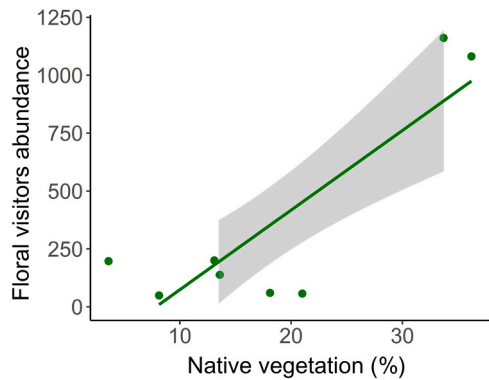


Fig. 5. Correlation between the native vegetation coverage and the floral visitor abundance.

Table 4

Percentage of fruit set in the pollination tests, considering all sampled areas collectively, along a distance gradient from the forests.

	0 m	100 m	200 m	300 m	400 m
Pollinator exclusion test	5.29	1.83	2.19	0.20	4.52
Open pollination test	10.21	10.22	11.06	5.29	9.21
Supplementary cross-pollination test	25.53	15.28	15.18	15.21	17.65

pollinators for fruit formation, but the presence of pollinators increases fruit production ([Ribeiro et al., 2017](#)), as observed in the open pollination test. The supplementary cross-pollination test presented the highest percentage for fruits formed. These results indicate that a pollination deficit may have occurred during the testing period. However, due to climatic conditions and orchard management practices immediately following experimental setup, we cannot attribute this potential deficit to any specific cause.

4. Discussion

We recorded four different insect orders visiting the orange flowers: Hymenoptera, represented mainly by the bees; Diptera; Lepidoptera, and Odonata.

We did not find differences in the floral visitors richness between Atlantic Forest and Cerrado biomes. Furthermore, all taxa observed in this study have a wide geographic distribution ([Silveira et al., 2002](#)), even recorded in both biomes. *Melissoptila* sp., *Nannotrigona* sp., *Ceratina* sp., *Hylaeus* sp., recorded only in farms within the Atlantic Forest biome in our study, were also observed occurring in the Cerrado ([Pedro and De Camargo, 1991](#); [Anacleto and Marchini, 2005](#); [Andena et al., 2005](#); [Rocha-Filho et al., 2022](#)). We observed one specimen of *Xylocopa frontalis* Cockerell only in the Cerrado biome, but other species of *Xylocopa* were observed in both biomes. Like other groups, bees of this genus have a broader geographic distribution, including the Atlantic Forest and Cerrado biomes ([Silveira et al., 2002](#); [Gonçalves and Brandão, 2008](#)).

Considering the visits in orange flowers, these results corroborate with other studies, that also observed non-bee insects visiting flowers of *C. sinensis* (L.) Osbeck, such as Lepidoptera ([Malerbo-Souza and Halak, 2013](#)) and Diptera ([Malerbo-Souza and Halak, 2013](#); [Vanlalhmangaiha et al., 2023](#)). Dipteran insects were observed visiting other varieties of *Citrus* sp. as well ([Cholis et al., 2020](#); [Layek et al., 2020](#); [da Santos et al., 2021](#)). We did not observe Coleoptera insects visiting orange flowers, but this group was mentioned in other papers visiting flowers of some citrus varieties ([Malerbo-Souza and Halak, 2013](#); [da Santos et al., 2021](#)).

Apis mellifera L. was the most abundant floral visitor, representing almost 83 % of the visits. It is known that citrus flowers emit highly attractive scents to honeybees (Hymenoptera, Apidae) ([Afik et al., 2006](#))

which can fly long distances searching for floral resources (Visscher and Seeley, 1982). This species has been reported as an effective pollinator of *C. sinensis* (L.) Osbeck (Malerbo-Souza et al., 2003; 2004; Nascimento et al., 2011; Toledo et al., 2013; Ribeiro et al., 2017; Witter et al., 2018). While collecting nectar and pollen, the behavior of *A. mellifera* L. in orange flowers facilitates continuous contact between the stamens and stigma, thereby promoting pollination (da Santos et al., 2021). However, successful pollination typically requires each flower to be visited by *A. mellifera* L. at least ten times (Malerbo-Souza et al., 2003).

In Brazil, the Africanized honeybee (*Apis mellifera* L.) is the result of hybridization between the African honeybee (*Apis mellifera scutellata*) and European subspecies (*Apis mellifera ligustica* and *Apis mellifera iberiensis*), which were introduced in the 1950s. These bees had a great adaptation in different habitats and established themselves in the wild, forming feral colonies presented in agricultural areas and fragments of native vegetation (De Jong, 1996).

Several studies have reported the high abundance of the Africanized *A. mellifera* compared with other bee species (Ricketts, 2004; Thomson, 2004; Patrício et al., 2012; Pundek and Gonçalves, 2025). Although the presence of *A. mellifera* being considered a threat to native bees, specially to the solitary species (Patrício-Roberto and Campos, 2014; Thomson, 2004), the real impacts have not been conclusively (Martins et al., 2013) until recently.

Garibaldi et al. (2021) conducted a three-year study at national level to investigate the effects of Africanized honeybees presence on native bee species. The authors installed over 10,000 pan traps across the country in seven different crops. The results indicate that, overall, *A. mellifera* L. is the most dominant bee species across the national territory. However, the negative impacts associated with this dominance were not significantly different from those observed for other native bee species, such as *Trigona* sp., which exhibited the highest abundance in certain regions. These findings suggest that the negative effects on bee communities are not specifically attributable to *A. mellifera* but rather to the dominance of any single species within agricultural landscapes (Garibaldi et al., 2021).

The second most abundant insect observed in our study was the stingless bee *Trigona spinipes* Fabricius (Hymenoptera, Apidae). However, in citrus flowers, this species can be considered a resource robber, once the bee contact with the stigma is deemed rare during floral resource exploitation (da Santos et al., 2021). Its behavior on the flowers generates contradictions in its relationship with the reproduction of the angiosperms they visit, since the individuals puncture flower buds to collect flower resources, especially nectar (Malerbo-Souza et al., 2004).

Because of this behavior, this stingless bee is considered a pest of some crops, including citrus, bananas, passion fruit, and acerola. Due to the injuries in the floral structure caused by the puncturing, this species impairs the production of fruits suitable for commercialization (Braga-Sobrinho et al., 1998; Witter et al., 2018). Despite this evidence, Malerbo-Souza et al. (2004) confirmed that visits by *Trigona* sp. to orange flowers do not interfere with fruit set rates, contradicting claims that this species is considered a pest of oranges. This information is quite important because *Trigona* sp. is a native bee from Brazil with a geographical distribution throughout the national territory and is of great importance for the pollination of other crops and native plants in degraded areas (Giannini and Jaffé, 2015).

From the 23 groups of bees observed in this study, ten can exhibit solitary habits (do not live in colonies), such as *O. flavescens*, *C. aenea*, *Centris* sp., *Melissoptila* sp., *Xylocopa frontalis*, *Xylocopa* sp., *Ceratina* sp., *Hylaeus* sp., *Dialictus* sp. and some individuals from Halictidae family. *Xylocopa* sp., *Ceratina* sp., *Centris* sp., *Hylaeus* sp., and Halictidae were also reported by Klein et al. (2020) as floral visitors of citrus flowers.

The solitary bees are important in the pollination of several cultivated plants, such as acerola, tomato, eggplant, canola, and others (Patrício et al., 2012; Gaglianone et al., 2018; Bertoli et al., 2019; Klein et al., 2020; Marsaro et al., 2023). Moreover, it is known that the functional traits of pollinators can be important to the crop yield, as

demonstrated by Woodcock et al. (2019) to oilseed rapes. The presence of wild insect pollinators in cropped areas, even if they are not considered the main pollinator, are also important in the maintenance of the pollinators persistence in the cultivation and surrounding, guaranteeing the pollination services and food security in long terms (Senapathi et al., 2021).

The highest species richness of floral visitors was found at the closest distance to the forest (0 m), gradually decreasing as the distance from the forest increased. Our findings corroborate with several studies in which the diversity of species is higher in the distances closest to the forests (Ricketts, 2004; Patrício-Roberto and Campos, 2014; Halinski et al., 2018; Huais et al., 2020; MacInnis et al., 2020; Power et al., 2021; Li et al., 2022; Alejandre, 2023).

The importance of natural and semi-natural habitats to the diversity and abundance of pollinators and other insect groups in agricultural areas is strongly consolidated (Patrício-Roberto and Campos, 2014; Garratt et al., 2017; 2023; Togni et al., 2021). These habitats usually provide all the necessary resources for pollinator survival, such as reproduction and nesting sites and water and food resources. Beyond this, natural habitats are rarely managed, which means that these sites may be permanent, guaranteeing the presence of pollinators and other guilds in the region during all the seasons (Williams and Kremen, 2007; Patrício-Roberto and Campos, 2014; Reverté et al., 2019).

Natural landscapes within or adjacent to agricultural areas are biodiversity refuges, creating habitats for pollinators. They harbor greater resource richness, such as plants and flowers, facilitating the establishment and maintenance of pollinator communities. Consequently, it facilitates the flow of these agents between natural and cultivated areas (Ricketts et al., 2008; Blitzer et al., 2012; Kennedy et al., 2013; Jachula et al., 2018). The benefits associated with the proximity of natural and cultivated habitats have been described by Landaverde-González et al. (2017) as ensuring greater richness and abundance of pollinators in agricultural lands. Additionally, it provides recurring visits to cultivated plant species, as observed in our results, regarding the richness of pollinators.

Considering that many observed groups in our study present solitary habits or have small body sizes, the presence of native vegetation near the orchards becomes even more important. Typically, solitary bees or those with small body sizes tend to forage at distances close to their nesting site, thus avoiding unnecessary energy expenditure (Schmid-Hempel and Wolf, 1988; Peterson et al., 2006; Greenleaf et al., 2007; Zurbuchen et al., 2010). Indeed, we observed very few solitary bees or those with small body sizes visiting orange flowers beyond 300 m from the forest. Similar patterns were observed by Ricketts (2004) in coffee plantations, where bee species richness was higher in coffee plantations near forest patches, while the increased abundance within cultivated areas was primarily attributed to the presence of *A. mellifera* L.

Our landscape analysis findings further elucidated the significance of native vegetation coverage in facilitating the presence of floral visitors, some considered as orange pollinators within orchards. The abundance of floral visitors, mainly *A. mellifera* L. was significantly correlated with the percentage of forest cover, in which the orchards situated in areas with higher percentages of native vegetation cover exhibited greater bee abundance. Similar outcomes were observed in blueberry cultivation, where farms surrounded by large expanses of native forest demonstrated increased pollinator abundance within orchards (Vega et al., 2023).

Lázaro and Alomar (2019), in a broader context, evaluated the importance of habitat heterogeneity in almond crops, considering different land uses and occupations. Among all the parameters analyzed, forest patch size was positively correlated with pollinator richness and flower visitation rate in the crop. In this context, the size of forest patches within agricultural landscapes has been established as a crucial factor in sustaining pollinator populations across various crop species worldwide.

In different regions of Wisconsin, United States, the positive

correlation between forest patch size and the richness and abundance of pollinators was demonstrated in apple orchards, being important to consider the preservation of these natural habitats in land management strategies in agroecosystems (Watson et al., 2011).

Hansen et al. (2020) identified a positive correlation between the proportion of forest cover within a landscape spanning 1.5–10 km and the diversity of guava pollinators in Southern Thailand. In this scenario, however, the fruit set did not improve with the presence of forests in that landscape context. Despite this, guava is recognized as a predominantly self-pollinating species, thus less reliant on external pollinators for fruit production (Hansen et al., 2020).

In Argentina, the size of forest fragments has been shown to impact the provision of pollination services for soybean crops, with effects extending proportionally to the sizes of forest patches. This implies that smaller fragments exerted a greater influence at shorter distances, while pollination services were more effective in orchards located farther from larger forest patches (Huais et al., 2020). Similarly, in Brazil, the percentage of forest cover has been identified as significant for both the richness and species composition of soybean pollinators (Ferreira et al., 2022) and for the provision of pollination services in eggplant cultivation (Patrício, 2013).

Many studies have reported the importance of natural habitats for the diversity and abundance of pollinators in agricultural areas, both in terms of crop distance and forest size. Despite this, further research is still needed to understand how these natural landscapes could be restored and preserved and how it should be distributed within the agricultural context to achieve the best optimization of pollination services in target crops (Martin et al., 2019).

Another parameter we tested in this study to understand the influence of the native vegetation in the pollination service was the orange fruit set. However, the pollination tests resulted in a very low fruitification rate. We attribute this result to two factors: (i) possibly due to the atypical heat wave caused by El Niño in 2023 (NOAA – National Oceanic and Atmospheric Administration, 2024), resulting in high temperatures in the period of the test execution and, (ii) and due to the management of some farms, where the producer carried out drastic pruning in the orchards, leading to the loss of several buds used in the experimental treatments and consequently losing the standardization of the tests.

Even so, the descriptive analysis (Table 4) indicates a possible pollination deficit, once the climate certainly interfered with the pollination service, since the bees drastically reduce foraging activities in higher temperatures (Silveira et al., 2002). Another hypothesis to explain the high fruit abortion rate considers the ideal weather conditions for orange cultivation, which comprehend temperatures from 22 °C to 34 °C. Higher temperatures can jeopardize the fruit set and development (Balfagón et al., 2022). Citrus flowering is typically a critical period for fruit production (Shafqat et al., 2021). Following flower pollination, increased temperature and water stress can inhibit ovule fertilization (Wheeler et al., 2000; Nagaz et al., 2020; Shafqat et al., 2021), reducing fruiting, increasing fruit drop, and decreasing tree yield (García-Tejero et al., 2010). In addition to the high abortion rate, the estimated fruit weight for this year's harvest was the lowest in the last 10 years (FUNDECITRUS, 2023), corroborating our findings.

Despite the difference in fruit set between the open pollination and the supplementation tests suggesting a possible pollination deficit, we cannot attribute this result to the absence of pollinators in the region, as the survey data indicated a high abundance, particularly of *A. mellifera* L. - an effective pollinator of *C. sinensis* (L.) Osbeck (Malerbo-Souza et al., 2003; 2004; Nascimento et al., 2011; Toledo et al., 2013; Ribeiro et al., 2017; Witter et al., 2018).

Considering the atypical weather scenario we faced during the pollination tests, it is necessary to repeat the experiments in better weather conditions, so we can exclude this variable, attributing the results to the landscape conditions.

5. Conclusion

Our results provide important evidence of the native forest's contribution to the maintenance of the richness and abundance of floral visitors in orange crops, thereby supporting enhanced pollination service provision in orange orchards. The richness of floral visitors was higher until the distance of 100 m from the forests, while higher coverage percentage of forests was positively correlated with the abundance of floral visitors.

The suboptimal fruit set observed likely reflects the anomalous climatic conditions during our study period (atypical heat wave caused by El Niño). While these results preclude definitive conclusions about pollination deficits, they highlight the necessity for multi-year studies controlling for interannual climatic variability to accurately assess pollination service reliability.

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CRediT authorship contribution statement

Osmar Malaspina: Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Gabriel Bacoccina Motta:** Writing – review & editing, Resources, Investigation, Data curation. **Graziele Luna Silva:** Writing – original draft, Resources, Investigation, Data curation. **Marília Fernandes Giroto:** Writing – original draft, Investigation, Data curation. **Roberta Cornélio Ferreira Nocelli:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Jaqueline Aparecida da Silva:** Writing – original draft, Resources, Investigation, Data curation. **Victor Ribeiro Sales:** Writing – original draft, Investigation, Data curation. **Paula Carolina Montagnana:** Resources, Methodology, Investigation, Formal analysis. **Patrício-Roberto Gleiciani Bürger:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Roberta Nocelli reports financial support was provided by Innocent Drinks and the Sustainable Juice Platform. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109830.

Data availability

Data will be made available on request.

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