Universidade de Brasília
Instituto de Ciências Biológicas
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THE ROLE OF MULTIPLE SPATIAL SCALES IN SUSTAINING NATIVE BEES AND POLLINATION SERVICES IN ORGANIC AGROECOSYSTEMS

Rafaela Mendes Assunção

Orientador: Prof. Dr. Pedro Henrique Brum Togni
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O PAPEL DE MÚLTIPLAS ESCALAS ESPACIAIS NA MANUTENÇÃO DE ABELHAS NATIVAS E NO SERVIÇO DE POLINIZAÇÃO EM AGROECOSSISTEMAS ORGÂNICOS

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Tese de doutorado apresentada ao **Programa de Pós-Graduação em Ecologia** da Universidade de Brasília (UnB) como requisito para obtenção do título de Doutora em Ecologia.

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RAFAELA MENDES ASSUNÇÃO

Tese de doutorado apresentada em 18 de dezembro de 2025, junto ao Programa de Pós-graduação em Ecologia do Instituto de Ciências Biológicas da Universidade de Brasília, sob orientação do Prof. Dr. Pedro Henrique Brum Togni e coorientação da Dra. Carmen Silvia Soares Pires, com o apoio financeiro da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), como parte dos requisitos para obtenção do título de Doutora em Ecologia.

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RESUMO

A polinização por abelhas é um serviço ecossistêmico ameaçado pela expansão agrícola. Mitigar essa ameaça exige a compreensão de como a estrutura da paisagem molda as comunidades de abelhas, suas interações ecológicas e sua estrutura genética. Portanto, o objetivo desta tese foi investigar, por meio de uma abordagem multi-escala, os fatores que afetam a diversidade de abelhas e seus efeitos na polinização em cultivos de tomate orgânico no Cerrado. Avaliamos como fatores em nível de paisagem e de fazenda influenciam as comunidades de abelhas silvestres e suas redes de interação (Capítulo I). Revisamos sobre a aplicação de marcadores moleculares na pesquisa com abelhas para avaliar a adequação dessa ferramenta para estudos de padrões genéticos espaciais das populações de abelhas (Capítulo II). Investigar como a estrutura da paisagem influencia o fluxo gênico e a abundância em uma espécie de abelha nativa modelo, a *Exomalopsis analis* (Capítulo III). Por fim, avaliamos a introdução de colmeias manejadas de *Melipona quadrifasciata* como uma estratégia de polinização complementar e para compreender as ressalvas da polinização assistida em agroecossistemas (Capítulo IV). Nossos resultados mostraram que o serviço de polinização emerge de uma complexa interação de escalas espaciais. No nível de paisagem, a cobertura de vegetação natural influenciou a abundância de abelhas, enquanto no nível da fazenda, a riqueza de plantas não-cultivadas moldou as interações planta-abelha e, consequentemente, a qualidade dos frutos (Capítulo I). Com base na utilidade de marcadores genéticos para estudos com abelhas (Capítulo II), descobrimos que a abundância de *E. analis* foi sensível a composição da paisagem numa escala pequena, mas a espécie manteve o fluxo gênico genético pela paisagem, utilizando remanescentes naturais como 'stepping stones' (Capítulo III). A introdução de colmeias manejadas de *M. quadrifasciata* não melhorou a qualidade dos frutos, e as abelhas silvestres permaneceram como os principais agentes da polinização (Capítulo IV). Esta tese mostra que a simples introdução de polinizadores manejados não pode compensar a perda de comunidades funcionais de abelhas silvestres. Enquanto a paisagem circundante determina o pool regional de espécies e dita o potencial para a conectividade genética, as condições em na escala da fazenda, especificamente a diversidade floral, modulam quais espécies estão presentes, como elas interagem e se as abelhas manejadas introduzidas conseguem se estabelecer com sucesso. Portanto, o manejo eficaz da polinização em agroecossistemas tropicais deve priorizar a conservação de habitats naturais em escala de paisagem e, simultaneamente, promover a biodiversidade na fazenda para sustentar comunidades locais robustas de abelhas e as interações complexas que impulsionam o serviço de polinização.

ABSTRACT

Pollination by wild bees is a key ecosystem service threatened by agricultural expansion, and effectively mitigating this threat requires understanding how landscape structure shapes bee communities, their ecological interactions, and their genetic structure. Therefore, this thesis aimed to investigate the drivers that sustain wild bees in and its effects on pollination in organic tomato agroecosystems in the Cerrado, using a multi-scale approach. First, we assessed how landscape and farm-level factors influence wild bee communities and their interaction networks (Chapter I). After that, we systematically reviewed the application of molecular markers in bee research to establish a methodological framework on the suitability of using this approach to understand spatial patterns of bee populations (Chapter II). This framework was then applied to investigate how landscape structure shapes gene flow and abundance in a native model species, *Exomalopsis analis* (Chapter III). Finally, we evaluated the introduction of managed *Melipona quadrifasciata* hives as a complementary pollination strategy and to understand the caveats of assisted pollination vs. natural pollination by wild bees (Chapter IV). Our results revealed that pollination services emerge from a complex interplay of spatial scales. At the landscape-level, natural vegetation cover predicted bee abundance, while on-farm non-crop plant richness shapes plant-pollinator network interactions and, ultimately, tomato fruit quality (Chapter I). Furthermore, based on the usefulness of genetic markers for bee studies (Chapter II), we found that *E. analis* abundance was highly sensitive to fine-scale habitat, while maintained broad-scale genetic connectivity across the landscape, using natural remnants as 'stepping stones' for gene flow (Chapter III). Finally, the introduction of managed *M. quadrifasciata* hives did not enhance fruit quality, with wild bees remaining the primary drivers of pollination (Chapter IV). This thesis demonstrates that simply introducing managed pollinators cannot compensate for the loss of functional wild bee communities. While the surrounding landscape determines the regional species pool and dictates the potential for genetic connectivity, farm-level conditions, specifically floral diversity, modulate which species are present, how they interact, and whether introduced managed bees can thrive. Therefore, effective pollination management in tropical agroecosystems must prioritize the conservation of landscape-scale natural habitats while simultaneously enhancing on-farm biodiversity to support robust local bee communities and their complex interaction that drives pollination service.

GENERAL INTRODUCTION

The critical role of biotic pollination in global food production and ecosystem sustainability is widely recognized (Potts et al. 2016; Dicks et al. 2021). About 75% of global crop species are highly dependent on animal pollination, and more than 40% of their production depends on pollinators (Siopa et al. 2024). The economic value of pollination is estimated at \$152 billion globally, representing 10% of the world’s agricultural output for human food (Porto et al. 2020; Khalifa et al. 2021). In countries such as Brazil, which is recognized as a major agricultural powerhouse, pollinator-dependent crops contribute billions of dollars annually (Basualdo et al. 2022; Oliveira et al. 2024). Therefore, without animal pollination global crop production would decrease by 5–8%, and the quality of many foods would decline, leading to economic and nutritional losses (Gazzea et al. 2023). Animal pollination directly improves both the quantity and quality of crops by improving key marketable traits, including fruit size, appearance, nutritional content, and seed set, which are fundamental to market crop value and food security (Eilers et al. 2011; Khalifa et al. 2021; Porto et al. 2021). However, a global decline in pollinator populations has been documented, threatening the stability of agricultural production and food security (Potts et al. 2010; Garibaldi et al. 2022).

The global decline of pollinators is primarily driven by land-use change, especially the expansion of intensive agricultural and pesticide use (Winfree et al. 2009). This process involves natural habitat loss and fragmentation, decreasing connectivity and intensifying harmful edge effects for pollinators (Fahrig et al. 2011; Zeller et al. 2020; Griffin and Haddad 2021). Consequently, the extinction rate is estimated to be approximately 16% to vertebrate and 40% to invertebrate pollinators (IPBES 2016). The latter group includes wild bees, which are considered the most important pollinators for most crops (Khalifa et al. 2021; Requier et al. 2022). The conversion of natural habitats into simplified agricultural landscapes results in the systematic depletion of essential resources for bees, such as nesting sites and diverse floral sources (Ferreira et al. 2015; Rosanigo et al. 2020; De Sousa et al. 2022). On the other hand, larger and more interconnected natural remnants enhance the long-term viability of bee populations by facilitating recolonization and spillover among natural and crop habitats (Boscolo et al. 2017; Fragoso et al. 2023; da Silva Carneiro et al. 2024). Thus, it is possible to improve the movement of bees between managed agroecosystems and adjacent natural or semi-natural habitats (spillover effect), given that wild bees nest and reproduce in natural habitats and can move into adjacent crop fields to forage.

The movement of individuals across human-modified landscapes may translates directly into gene flow, a fundamental process that shapes the genetic diversity of bee populations (Suni et al. 2014; Jaffé et al. 2016b, 2019). Functionally permeable agroecosystems are those that facilitate this dispersal, often by providing corridors that connect different habitats (Gutiérrez-Chacón et al. 2020; Machado et al. 2020; Togni et al. 2021). For example, native stingless bees can maintain gene flow by dispersing through degraded habitats, as long as movement between natural remnants is still possible and the matrix is permeable for different species (Jaffé et al. 2016a; de Matos Barbosa et al. 2022). This gene flow increases the effective population size and reduces the loss of genetic diversity over generations, buffering populations against genetic drift and inbreeding (Allendorf 2017). To understand these dynamics, landscape genomics offers a powerful approach. By analyzing genetic data from molecular markers, such as microsatellites, researchers can assess dispersal patterns and the degree of population connectivity across large scales, providing critical data to monitor the conservation status of bee populations and design effective management strategies (Lozier et al. 2013; Jaffé et al. 2016a; de Matos Barbosa et al. 2022).

Another key aspect of the success of gene flow and bee spillover involves the quality of the agricultural matrix itself, which determines its attractiveness and permeability to bee species from nearby natural habitats (Morandin and Kremen 2013; Wu et al. 2021; Killewald and Gibbs 2025). For instance, the use of synthetic pesticides on conventional agroecosystems imposes severe physiological risks on bees (e.g. direct mortality and sub-lethal impairments of flight), creating a barrier and reducing landscape permeability for many bee species (Pires et al. 2014; Steinhübel et al. 2022). Conversely, agroecological approaches, including organic and agroforestry systems, can mitigate this physiological risk (Holzschuh et al. 2008; Gutiérrez-Briceño et al. 2023; Lorandi et al. 2023; Silva Neto et al. 2023). Moreover, while monocultures often represent a homogenous font of floral resources, the incorporation of agri-environment schemes, such as wildflower strips or hedgerows, creates a more diverse and temporally stable resource availability for pollinators (Fijen et al. 2024). Particularly, the management of non-crop plants (traditionally referred to as weeds) within farms represents a powerful and low-cost strategy to enhance spillover of bee communities (Nicholls et al. 2013; Laha et al. 2020; Ferreira et al. 2023).

However, while the benefits of managing non-crop plants for pollinators are becoming increasingly recognized, a knowledge gap remains regarding the specific interactions that structure these communities. Moving beyond simple species lists requires characterizing the plant-bee interactions networks within farms to predict community resilience by identifying key species that support bee diversity, interaction preferences, and the overall degree of specialization (Escobedo-Kenefic et al. 2020; Landaverde-González et al. 2021; Assunção et al. 2022; Rafferty and Cosma 2024). Additionally, a network-based approach provides a powerful framework for assessing the susceptibility of bee communities to land-use changes in the landscape level (Ferreira et al. 2013; Escobedo-Kenefic et al. 2022; Proesmans et al. 2024). For instance, landscape simplification can disrupt plant-pollinator networks by compositional (e.g., the loss of specialist and rare species) (Winfree et al. 2014; Astegiano et al. 2024), numerical (shifts in the relative abundances of species) (Moreira et al. 2015; Ferreira et al. 2020), and behavioral changes (altered foraging patterns and diet generalization) (Dalsgaard et al. 2020; Gómez-Martínez et al. 2022). In turn, there is a growing recognition that the emergent properties of interaction networks can serve as predictive indicators of ecosystem function, including pollination service (Ferreira et al. 2013; Arceo-Gómez et al. 2020). For example, generalization in networks increases functional redundancy, which buffers against species fluctuations and makes pollination services more resilient (Kaiser-Bunbury et al. 2017; Domínguez-Garcia et al. 2024; Maurer et al. 2024). Nevertheless, studies that simultaneously quantify how landscape features shapes network structure, and how network structure, in turn, impacts pollination service and crop yield, remains scarce, especially in tropical regions (Sritongchuay et al. 2022).

This scarcity of integrated knowledge leaves farmers in a vulnerable position, forcing them to face the immediate challenge of ensuring pollination in landscapes where the pool of wild bees may be too depleted (Gazzea et al. 2023; Millard et al. 2023). In these cases, strategies focused solely on enhancing floral resources often prove insufficient, needing more direct interventions such as the management of social bee hives for applied pollination. In such scenarios, enhancing floral resources alone may not be enough to maintain pollinator populations in agroecosystems. Thus, the management of social bee hives for assisted pollination have emerged as a complementary approach widely implemented worldwide (Velthuis and Van Doorn 2006; Abbasi et al. 2021; Bonneau et al. 2021; Grant et al. 2021; Roubik 2023). However, the widespread management of the exotic *Apis mellifera* has raised significant concerns, especially in the Neotropical region. These concerns include potential negative ecological impacts on native bees, plant-bee interactions, transmission of pathogens, and pollination dynamics (Fleites-Ayil et al. 2023; Tavares-Brancher et al. 2024; da Silva Cardoso et al. 2025; Assunção et al. 2025). Consequently, the management of native bee species is emerging as an alternative, most notably the stingless bees of the tribe Meliponini (Apidae) (Slaa et al. 2006; Roubik 2023). As an exmple, the agricultural significance of the genus *Melipona* in Brazil is surpassed only by that of *A. mellifera*, as these native bees are key pollinators for many of the nation's most economically valuable crops (Giannini et al. 2020).

The value of managing native stingless bees becomes particularly evident in crops where *A. mellifera* is not an efficient pollinator, like in the tomato (*Solanum lycopersicum*) plants (dos Santos et al. 2009). Research in Brazil demonstrated that supplementing tomato fields with managed hives of *Melipona quadrifasciata* yielded remarkable results: a 33% increase in seed set and up to a 16% gain in fruit mass (Silva-Neto et al. 2019). This is highly significant given that Brazil ranks the world's top ten tomato producers (3.9 million tons in 2019), with its cultivation employing approximately 10% of the national rural labor (Treichel et al. 2016; FAO 2021). This underscores tomato as an important crop for Brazilian farmers, whose productivity is clearly enhanced by assisted pollination from native bees (Del Sarto et al. 2005; Bartelli et al. 2014). Despite these benefits, the full ecological implications of introducing managed hives into new environments are still not completely understood and deserves a critical evaluation, especially when beehives are translocated across biomes (Mallinger et al 2017; dos Santos et al. 2022). Moreover, robust management protocols to ensure the long-term health and viability of these hives and practical knowledge on their management for crop pollination are still underdeveloped in the tropics (Requier et al. 2019; Giannini et al. 2020; Zaman and Dorin 2023). To address these gaps and move towards a more efficient and sustainable assisted pollination, it is fundamental to understand the foraging ecology of these managed bees within complex agroecosystems, their efficacy on different target crops and its impacts on wild communities (da Silva Correia et al. 2020; Vaidya et al. 2023).

The challenge of addressing these ecological and management questions becomes particularly pertinent in global biodiversity hotspots where the ongoing expansion of conventional large-scale agriculture and pastures poses a severe and immediate threat. An example is the Brazilian Cerrado, the world's most biodiverse savanna (Myers et al. 2000; Klink & Machado 2005; Sano et al. 2019), which has already lost over 45% of its native vegetation to agricultural conversion (MapBiomas 2021). The Cerrado harbors an estimated 820 bee species, which accounts for 12% of the Neotropical bee diversity and includes a high number of endemic species (Raw 2007). In the Cerrado's smallholder organic farms, for instance, bee richness is positively correlated with the integrity of surrounding natural areas (Assunção et al. 2022). Additionally, non-crop plants can increase landscape permeability for bees and mediate key interactions in the local plant-pollinator network, reinforcing the interconnection between local farm management and broader landscape structure (Assunção et al. 2022). These findings indicate that it is possible to develop strategies that enhance pollination services in Cerrado agroecosystems without compromising the biome's natural habitats. Moreover, it clearly shows a path to understanding the natural vegetation as a key element of agroecosystems and the basis for a long term resilient and biodiversity-friendly tropical agriculture. The key to this approach lies in considering ecological factors and biological interactions that operate across different spatial scales.

Therefore, this thesis aims to assess how environmental resources at multiple spatial scales influence bee communities and their provision of pollination services within organic agricultural systems of the Cerrado biome. Specifically, we aim to: (1) Quantify the relative contributions of local farm management practices and broader landscape structure to the maintenance of both wild and managed bee populations; (2) Evaluate the consequences of these multi-scale interactions for pollination effectiveness in organic tomato crops; (3) Assess the scope and effectiveness of microsatellite markers as a primary tool for bee population genetics research; (4) Apply this genetic approach to investigate how landscape composition influence gene flow and shape the genetic structure of a native bee that is an effective pollinator of tomato crops; and (5) based on these findings, propose a sustainable management strategy that integrates the conservation of natural pollination with the applied use of managed native stingless bee hives.

This thesis is structured into four chapters, each centered on bee communities and their interactions within organic tomato farms in the Federal District of Brazil. Chapter I assesses the role of habitat structure and heterogeneity across multiple scales (landscape, farm, and local level) on the maintenance of wild bee communities. This chapter further investigates the consequences of these multi-scale factors for plant-bee interactions and the efficiency of pollination services to the crop. Chapter II provides the methodological foundation for our genetic analysis by systematically reviewing the use of microsatellite markers in bee research. This review was conducted to inform and validate the approach used in the subsequent chapter. Chapter III applies this genetic approach to investigate how landscape composition influences gene flow and shapes the genetic structure of a native model species. Chapter IV evaluates whether the introduction of managed *M. quadrifasciata* hives provides pollination services that are complementary to those of wild bees in open-field tomato cultivation. This chapter also aims to identify which crop and non-crop plants serve as supplementary resources for the maintenance of this managed species.

We established a set of integrated hypotheses to guide this thesis. First (Chapter I), we hypothesized that landscapes with greater cover of natural vegetation of Cerrado would support a richer regional pool of bee species. This positive landscape effect would be enhanced by farm-level management, where greater floral diversity creates more permeable agroecosystems, fostering more complex and robust plant-pollinator interaction networks, and ultimately leading to improved pollination services. Second, we hypothesized that for our model species, SRR markers as useful tools for genetic analyses of these species population (Chapter II), so that natural vegetation remnants at landscape-level would facilitate gene flow and enabling bee population dispersal, thereby sustaining the species' presence across the entire landscape mosaic (Chapter III). Finally (Chapter IV), we hypothesized that the introduction of managed native bee hives would serve as a viable complementary strategy, enhancing pollination particularly in landscapes where wild bee communities are depleted.

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CHAPTER 1: Natural vegetation types in the landscape and local flower resources shape pollinator network interactions and crop yield in organic agroecosystems

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Abstract

Pollination by wild bees is a key ecosystem service, which is fundamental to the sustainability of food systems. To predict and improve pollination service, it is necessary to understand how different environmental conditions and landscape aspects affect wild bee communities and their interactions with plants. This study investigated how habitat structure at multiple spatial scales (landscape, farm and local) affects wild bee communities, their interactions with plants, and pollination service. We conducted the study in tomato fields located in the Brazilian Cerrado. Our results demonstrate that pollination services emerge from a complex interplay between these scales. Forest cover in the landscape was the main predictor of bee abundance, while non-crop plant richness within farms was crucial for structuring interaction networks, increasing modularity and specialization. Open-pollinated tomato fruits showed greater weight, size, and seed number compared to self-pollinated ones. Notably, fruit quality was explained by network interaction metrics, such as nestedness and modularity, rather than bee abundance or richness alone. Network nestedness explained 60% of the variation in fruit weight and 91% in fruit size, while modularity and specialization were the main predictors of seed number. We conclude that agroecological management strategies must adopt a multi-scale approach. The conservation of natural habitats in the landscape, combined with the management of non-crop plants on farms, is essential to sustain complex ecological interactions and ensure efficient pollination services in tropical agroecosystems.

1. Introduction

The transition toward sustainable agricultural systems requires minimizing environmental impact while maintaining food production and farmer livelihoods (Eyhorn et al., 2019). Organic farming plays a central role in this transition by supporting biodiversity, improving soil quality, reducing pollution, and increasing farm incomes (Reganold and Wachter, 2016; Wan et al., 2025). Smallholder farmers are more inclined to adopt organic management practices and nature-based solutions in crop management (Lavandero et al., 2025), and they also maintain greater crop and non-crop diversity than large-scale farmers worldwide (Ricciardi et al., 2021). These characteristics make smallholder farmers both highly dependent on and especially benefitted by ecosystem services, while also establishing them as important agents in the transition to a more sustainable production system (Sandhu et al., 2008; Astegiano et al., 2024; Sritongchuay et al., 2022, 2026).

Pollination is one of the most critical ecosystem services for agricultural production, directly influencing crop yields and fruit quality (Gallai et al., 2009; Porto et al., 2020). In Brazil, for example, almost 80% of crops depend to some extent on pollinators, and about one-third of food crops rely heavily or essentially on animal pollination (BPBES and REBIPP 2019, Oliveira et al., 2024). Pollinator absence can reduce yields by 40-100% with accentuated economic impacts (Giannini et al., 2015a). While multiple taxa contribute to pollination, wild bees remain the primary pollinators in agroecosystems (Requier et al., 2023), making their conservation and proper management essential for sustaining agricultural productivity (Osterman et al., 2021). The maintenance of wild bee populations is strongly linked to the landscape-level availability of natural vegetation, which provides a mosaic of nesting and foraging niches that shapes the regional wild bee species pool (Ferreira et al., 2015; de Sousa et al., 2022). Conversely, intense reduction and fragmentation of natural vegetation disrupt connectivity and intensify edge effects, which can impair bee movement and reduce pollination services (Nemésio and Silveira, 2010; Zeller et al., 2020).

Agricultural intensification is widely recognized as a key driver of natural vegetation reduction and fragmentation, creating a dynamic interaction between farm-level management design and the broader landscape context (Concepción et al., 2012; Nicholson et al., 2017). However, certain agricultural practices can mitigate the negative impacts of natural vegetation fragmentation on bee communities. For example, maintaining proximity to forest remnants and managing non-crop plants (weeds), enhancing farm permeability by providing complementary and continuous resources for bees (Nicholls and Altieri, 2013; Klaus et al., 2021; Campbell et al., 2022). Nevertheless, the effectiveness of these farm-level practices is influenced by the surrounding landscape structure, the spatial scale considered, and the biological traits of bee species (e.g., body size, foraging and nesting preferences) (Ricketts et al., 2004; Saturni et al., 2016; Coutinho et al., 2021; Campbell et al., 2022; Kammerer et al., 2024).

The complex interplay between landscape structure, farm management, and bee species traits ultimately shapes the dynamics of local resource use by bees and the outcomes on pollination (Moreira et al., 2015; Assunção et al., 2022; Gómez-Martínez et al., 2022). To understand this, the analysis of plant-pollinator interaction networks provides a robust framework for quantifying how the structure of a community translates into ecosystem functions (Bascompte and Jordano, 2007; Moreira et al., 2015). Properties of interaction networks are shaped by ecological and functional factors, including morphological matching (Maruyama et al., 2016; Maglianesi et al., 2024), degree of specialization (Dorado et al., 2011; Carvalho et al., 2014), and the availability and quality of both natural habitats and floral resources (Landaverde-González et al., 2021). These factors determine network persistence and stability, and thereby impact the provision of pollination service at the local level (Sritongchuay et al., 2022; Astegiano et al., 2024).

While analytical tools such as interaction networks have advanced our understanding of how farm management and landscape context affect pollinator communities (Memmott et al., 2004; Felipe-Lucia et al., 2020; Monteiro et al., 2025), critical knowledge gaps remain. First, the relative importance of spatial scales, ranging from local and farm-level management to landscape-level habitat structure, in shaping wild bee assemblages and pollination services remains unresolved (Boscolo et al., 2017; Kammerer et al., 2024). Second, most empirical evidence comes from temperate systems. Tropical agroecosystems, where biodiversity is higher and smallholder farming is widespread, remain comparatively understudied (Viana et al., 2012; Escobedo-Kenefic et al., 2020; Assunção et al., 2022; Monteiro et al., 2025). Finally, while network approaches are valuable for highlighting the functional roles of species, there is a lack of empirical evidence directly linking interaction network properties to measurable pollination outcomes (Sritongchuay et al., 2022; Astegiano et al., 2024). Addressing these gaps is essential for designing biodiversity-based strategies that effectively enhance conservation and pollination services, especially in the tropics.

Here we aimed to understand how wild bee assemblages and the structure of plant-bee interaction networks are explained by habitat features of a single spatial scale and by combined features of multiple scales, with a focus on smallholder farmers with organic systems. Our central hypothesis is built on a multi-scale framework. At landscape-level, the composition of different natural vegetation cover determines the potential pool of colonizing bees, while at farm-level, heterogeneous habitats can promote farm permeability for bees moving from natural habitats (spillover effect). At local-level, this interplay of scale-dependent effects will drive bee diversity and, consequently, the structure and complexity of plant-bee interaction networks, as these interactions at more restricted spatial scales can be highly dependent on dynamics of local resource use. Overall, we hypothesize that more heterogeneous farms surrounded by natural vegetation remnants will support more diverse bee assemblages with complex and structured plant-bee interaction networks, leading to greater pollination efficiency.

2. Materials and Methods

2.1. Study farms

We carried out the study in tomato fields across 12 organic vegetable farms in the Federal District, Brazil (15°46′48″S, 47°55′45″W) between July–October 2022 and May–October 2023 (Fig. 1). The farms (6.81 ± 1.13 hectares, mean ± SE) were 6 km apart, and cultivated tomatoes and other vegetables in open fields. Our selection of 12 study sites was a deliberate methodological decision shaped by the needs for high-quality sampling and the logistical realities of field research. First, the number of available and suitable farms was constrained by producer willingness to participate and the need for sites with comparable tomato cultivation and flowering phenology. Second, our high-frequency sampling, combined with the need to standardize effort during peak activity hours at each site, required substantial time investment per farm. Additionally, given the constraints on available human resources and institutional logistics, focusing our efforts on these 12 sites was essential to maintain the scientific rigor and consistency of our data collection, which would have been compromised with a larger, more superficially sampled set of locations.

The farms were located in the Cerrado biome, the Brazilian tropical savanna in distinct landscape contexts (Fig. 1). They are embedded within an agricultural matrix interspersed with remnants of natural vegetation, including mandatory riparian forest conservation areas (Sano et al., 2019). The Cerrado exhibits mean annual temperatures of 22-27 °C, with a rainy summer (October-April) accounting for approximately 90% of the total annual rainfall (1100-1600 mm), followed by a dry winter (May-September) (Nascimento and Novais 2020). Farmers have been certified as organic for at least three years. For pest control, they used mass releases of *Trichogramma pretiosum*, along with applications of *Bacillus thuringiensis* (Bt), neem-based products and other bioproducts, following Brazilian organic regulations (Togni et al., 2019).

2.2. Experimental design

The Italian tomato cultivar (Solanum lycopersicum var. Matinella), commonly cultivated by Brazilian farmers, particularly in organic systems (CONAB 2019) were used in the experiments. The study period covered two complete flowering cycles to capture variation in pollination and fruit development across phenological stages. Farmers cultivated tomatoes in an area of approximately 400 m², with 400-700 plants. Soil preparation followed local organic farming practices, incorporating simple thermophosphate and poultry litter. Plants were vertically staked and arranged in double rows with 60 cm spacing between plants and plant rows. Sprinklers were used to maintain adequate soil moisture and irrigation. Pollination services provided by wild bees were assessed in tomato crops on six of the 12 study farms. To ensure uniform cultivation conditions in farms with pollination treatments, we supplied farmers with 400 seedlings and fertilizers, standardizing plant density and nutritional inputs. Seedlings were transplanted to the field 30 days after germination.

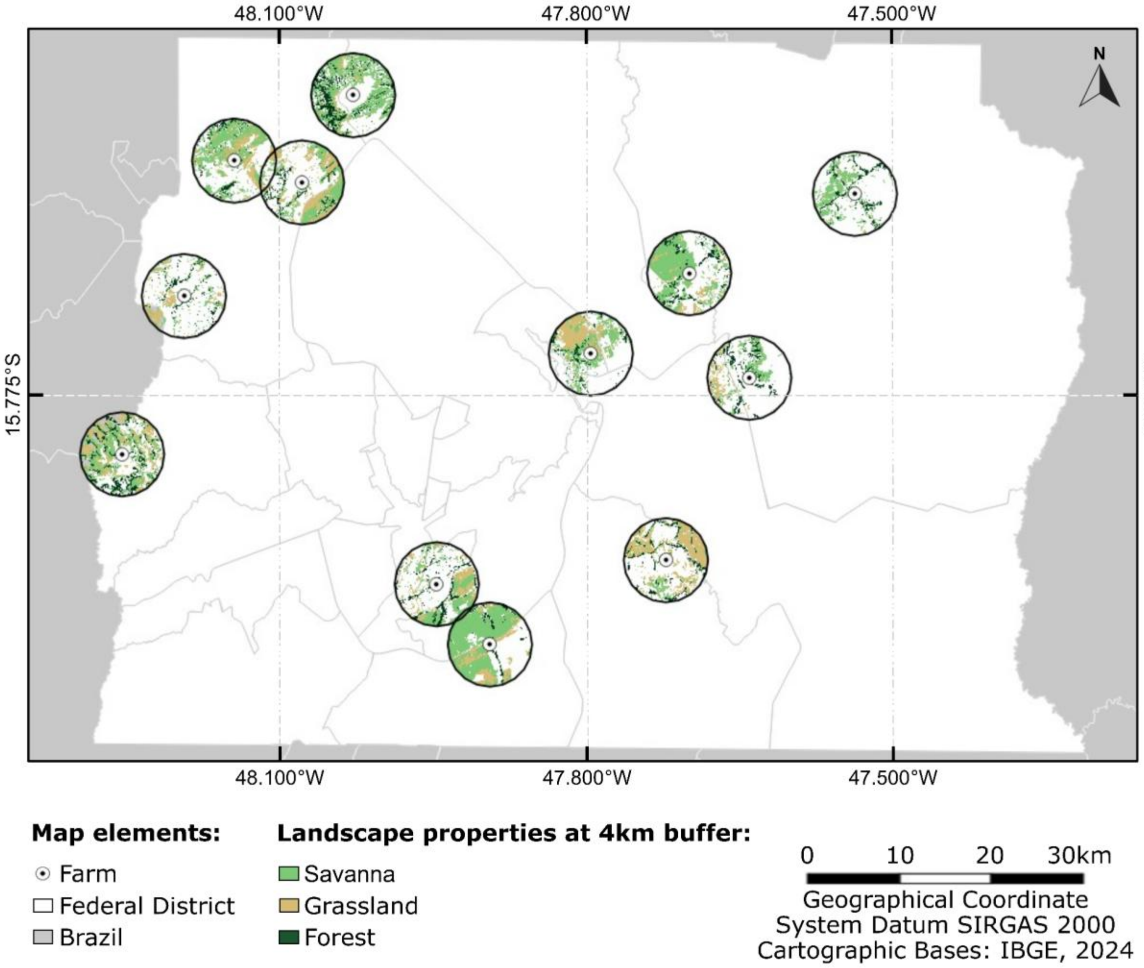

Fig. 1 Location and landscape composition surrounding study farms. The map shows the geographic distribution of the 12 tomato organic crops in the Brazilian Federal District (15°46′48″S, 47°55′45″W). Each circle represents a 4 km buffer around a farm, showing the distribution of Cerrado natural vegetation types: savanna (light green), grassland (dark yellow), and forest (dark green). Data refers to a map of MapBiomas Collection 2 land cover classification for 2023 (Souza et al. 2020), which classifies Sentinel-2 satellite images with 10 m spatial resolution. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

2.3. Bee sampling

To characterize wild bee assemblages in the farms, we sampled bees throughout the tomato flowering season (July–October) in 2022 and 2023 across the 12 farms. Direct samplings were carried out once a week during four weeks of the flowering window, which typically occurs around 5-6 weeks after germination, ensuring representation of all floral stages and associated bee visitors. We assessed bee visitation to both tomato and non-crop plants adjacent to cultivated areas. Non-crop plants were typically used as live or dead soil cover and formed permanent vegetated borders surrounding all cultivated areas in organic farms, contributing to soil protection and nutrient cycling during both cropping seasons and fallow periods. Non-crop plants were selected due to their documented role in maintaining wild bee populations and their interactions in agricultural systems (Assunção et al., 2022), they are well known by farmers, and they lack any specific management outside the crop.

The samplers walked through the area (within 10 m radius of the tomato crop), actively collecting bees using plastic pots, from 9:00 am to 12:00 pm (peak of bee activity). Only bees that landed directly on the flowers were collected. The daily sampling effort was quantified in minutes of collection and multiplied by the number of collectors. Sampling effort was standardized at 20 h per farm (10 h each in tomato and non-crop plants), totaling 240 h across all 12 study farms. The bees were identified at the lowest possible taxonomic level (Silveira et al., 2002). Bee specimens were deposited in the Entomological Collection of the University of Brasília (DZUB) and the Entomological Collection of Embrapa Genetic Resources and Biotechnology, both located in the Federal District.

2.4. Resource availability at different spatial scales

To assess the landscape-level effects, we evaluated landscape composition of the Cerrado vegetation across multiple spatial scales (0.5-10 km buffers) surrounding each of the 12 study farms. We characterized the landscape composition using the land cover and land use map of the MapBiomas Project Collection 2 land cover classification for 2023 (Souza et al., 2020). This map classifies Sentinel-2 satellite images in a matrixial map (raster format) with a spatial resolution of 10 m (pixels represent areas of 10 x 10 m) using only validated data. We calculated the proportional coverage area of three natural vegetation types in the Cerrado (forest, savanna, and grassland) within buffers, a simple and robust metric of landscape composition. This metric quantifies the relative area of each vegetation type within a given spatial scale (buffers), directly reflecting habitat availability. Forest is dominated by continuous tree canopies (>80% cover), which typically occur near river courses; savanna feature 20–70% tree and shrub cover, interspersed with a broad graminoid stratum; grassland consists of a graminoid stratum and include less than 5% tree and shrub cover (Ribeiro and Walter, 2008). This approach allowed us to identify the optimal spatial scales and critical amounts of each natural Cerrado vegetation cover that influence wild bee diversity patterns, as well as plant-bee interactions and pollination service efficiency. This approach allowed us to identify the optimal spatial scales and critical amounts of each natural Cerrado vegetation cover that influence wild bee diversity patterns, as well as plant-bee interactions and pollination service efficiency in agroecosystems.

We defined farm-level as the area encompassing the individual farms, considering the average area of the farms, this represents buffers < 0.2 km radius. To quantify the farm-level effects, two key metrics were analyzed: non-crop plant richness and crop distance to the nearest natural fragment. All non-crop plant species visited by bees during samples were identified to species level with the assistance of a taxonomist (Moreira and Bragança, 2011). Non-crop plant identification provided a relative estimate of plant richness availability across farms, as well as detection of specialized plant-bee interactions and keystone floral resources based on visitation frequency. During the study, non-crop plants within and around the crops were kept unmanaged. Additionally, we calculated the distance from each tomato crop to the nearest natural vegetation fragment (greater than 1 ha) using the QGIS software (QGIS Development Team, 2022). This data was incorporated because flower visitors are known to be affected by distance to natural habitat (Carvalheiro et al., 2010, 2011) and fragments below 1 ha lack the capacity to sustain stable bee populations in agroecosystems (Brosi et al., 2008; Kennedy et al., 2013).

At the local level, we focused our sampling efforts on crop plots and their immediate field margins. It is at this scale that key ecological processes, such as the dynamics of foraging choices by bees, occur and directly shape pollination services. To quantify and analyze these local level interaction dynamics, we constructed a bipartite interaction network for each farm, including wild bees, tomato plants, and non-crop plant species. Interactions were organized in a matrix with plant species as rows and bee species as columns, where cell values represented the number of individual bee visits. Therefore, the network metrics were calculated using quantitative (instead of presence–absence) data.

At the network-level (a single value, per metric, obtained for the entire network), we estimated nestedness using the NODOF metric (Almeida-Neto and Ulrich, 2011), which captures the extent to which interactions of specialist species are subsets of those of generalists. Modularity was determined using the QuanBiMo algorithm (Dormann and Strauss, 2014), which identifies distinct clusters of species that interact more frequently with each other than with species outside their group. We also calculated specialization index (H2), a standardized measure of overall network-level specialization that accounts for interaction frequencies and species abundances (Blüthgen et al., 2006).

At the group-level (a value per metric obtained for both the bee and plant groups), we assessed network specialization using niche overlap among bee species (niche overlap high level - NOHL) and among plant species (niche overlap low level - NOLL). NOHL measures the similarity in floral resource use between bee species by calculating the pairwise overlap in their interaction partners (plants). Values near 0 indicate no common use of niches, 1 indicates perfect niche overlap (Dormann et al., 2009; Dormann, 2020). We evaluated network robustness at two levels: robustness at the low level (RLL), indicating the resistance of plant species to bee extinctions, and robustness at the high level (RHL), indicating the resistance of bee species to plant extinctions (Burgos et al., 2007; Dormann, 2020).

At the species-level (= node level; a value per metric obtained for each species), we quantified the structural importance of bee and non-crop plant species within the interaction network. First, we calculated weighted betweenness centrality (WB) and weighted closeness centrality (WC). WB measures the relevance of a node (species) as a connector between different portions of the network, whereas WC measures the proximity of a node to all other nodes in the network (Mello et al., 2015). Species with high WB are key connectors that support network integrity and maintain network cohesion. In contrast, those with high WC can affect many others through indirect interactions in response to changes in abundance or extinction. Second, we characterized the species' functional roles by their module-interaction distributions using two metrics: within-module connectivity (z-score) and among-module connectivity (c-score) (Guimera and Amaral, 2005). Z-score measures how strongly a species is linked to others in its own module. C-score quantifies how evenly a species' interactions are distributed across all modules. We classified species into four functional groups by applying z-c values: peripherals (low z, low c), poorly connected both within and between modules; connectors (low z, high c), bridge modules but lack within-module dominance; module hubs (high z, low c), highly connected within their module but not beyond; and network hubs (high z, high c), integrate the entire community (Guimera and Amaral, 2005; Olesen et al., 2007).

2.5. Bee pollination in tomato crops

Across all 12 farms, we conducted weekly surveys of the wild bee and plant communities to characterize landscape and fam levels effects on bee communities and to construct local-level plant-pollinator interaction networks. However, pollination services provided by wild bees were assessed in tomato crops on a subset of 6 of these 12 farms. This approach allowed us to link broad landscape and community patterns (all 12 farms) to specific functional outcomes (the 6-farm subset). This 6-farm subset was necessary due to the logistical and financial constraints of the experimental setup. The protocol required a high degree of standardization, which we achieved by providing tomato seedlings of a uniform variety to all 6 farms, an expense that was feasible only for this number of sites. Furthermore, the time-intensive nature of pollination experiments limited the number of sites we could manage while maintaining high data quality.

Two pollination treatments were applied simultaneously to each selected plant: (1) self-pollination (SP), in which inflorescences were bagged before anthesis using voile fabric that excluded bees but allowed wind passage; and (2) open pollination (OP), where flowers remained accessible to bee visits. A random sample of 600 plants (100 per farm) was selected for treatment application, for a total of 1,200 plants analyzed across both treatments (600 SP + 600 OP). Inflorescences were unbagged once fruit development began. In the OP treatment, flowers showing anther necrosis (a characteristic mark of mandible insertion by buzz-pollinating bees) were classified as bee-pollinated (Pires et al., 2024). The tomato variety used (Italian tomato cultivar) has indeterminate growth, producing new buds and clusters along the main stem, with more vigorous fruits typically forming on lower clusters. To control for the influence of fruit position and developmental stage, the position of flower clusters and buds was recorded, and treated buds were marked with white water-based liquid paper to allow posterior recognition. From each experimental plant, we harvested one to two mature fruits per pollination treatment. After harvest, fruit quality was evaluated by measuring fruit weight (g), diameter (cm), and seed number per fruit.

2.6. Statistical analysis

To assess whether the observed network metrics differed significantly from what would be expected by chance (i.e., no ecological effects acting), we used the ‘nullmodel’ function, a wrapper for generating null models for quantitative and binary networks (Dormann et al., 2009). We applied 10,000 randomizations, which preserve the network size and marginal totals (row and column sums), maintaining species' interaction frequencies while randomizing the distribution of interactions (Vázquez et al., 2007).

We evaluated the effects of resource availability at the landscape and farm levels on tomato bee diversity patterns and plant-bee interaction network structure using linear and nonlinear models. Bee diversity analyses included only individuals of bee species considered effective pollinators of tomato plants (Deprá et al., 2014; Santos et al., 2014; Silva-Neto et al., 2016; Vinícius-Silva et al., 2017; de Moura-Moraes et al., 2021; Toni et al., 2021). To quantify diversity patterns, we quantified for each farm: (1) species richness; (2) abundance; (3) diversity (Shannon diversity index, H'); (4) P. lineata abundance, and (5) Exomalopsis spp. abundance (main pollinators of tomato crops in the region; Assunção et al., 2022). We used bee diversity patterns and network-level metrics (NODOF, QuanBiMo and H2) per farm as response variables and (1) the percentage of each vegetation type (savannah, grassland and forest) at buffers of 0.5 to 10 km (landscape-level); (2) and non-crop plant richness and distance to the nearest natural vegetation area (farm level) (Crawley 2012), as explanatory variables. We focused on network-level metrics because it captures emergent structural properties of the plant-bee interactions at the community level (Dormann et al., 2020). Separate models were fitted for each response and explanatory variable.

To identify the optimal spatial scale (buffer) for each response variable, we constructed a set of independent models. Each model represented a distinct spatial scale, corresponding to a buffer radius from 0.5 km to 10 km. We then compared these models by calculating the corrected Akaike information criterion weights (AICcWt) for each one (Akaike, 1998; Anderson and Burnham, 2004; Mazerolle, 2020). The AICcWt is interpreted as the probability that a given model is the best among the others. The model with the highest weight corresponds to the one with the strongest evidence for that particular combination of predictor variables.

To assess the differences in fruit quality parameters (weight, size and seed number per fruit) between pollination treatments (SP and OP), we fitted Generalized Linear Mixed Effect Models (GLMM). We also analyzed the effects of tomato bee diversity patterns and plant-bee interaction network structure on pollination service through tomato fruit quality. To isolate the effects of wild bee visitation on pollination effectiveness, we fitted a GLMM with only fruit quality parameters of OP treatments as response variables and bee diversity patterns and network-level metrics as explanatory variables (Crawley, 2012). We included “plants” (replicates), “farms” and “clusters” as random factors in the analyses. “Plants” and “clusters” were included to control for developmental variation in tomato fruits. Farms were included as random effects to account for environmental and management differences across study areas. Separate models were fitted for each response and explanatory variable.

To understand the relative importance of different drivers of tomato pollination, we also employed an AICcWt approach. First, to determine whether bee community structure was primarily shaped by landscape-level or farm-level factors, we constructed a set of candidate models for bee diversity patterns and plant-bee network-level metrics (response variables). This set included: (1) a model with only landscape-level predictors (separate models for each natural vegetation type), and (2) a model with only farm-level predictors (separate models for non-crop plant richness and distance to natural vegetation area). We then compared these models using their associated AICcWt to assess the best-supported model for bee community structure. Second, to identify the strongest predictors of tomato pollination service, we formulated another set of candidate models for fruit quality parameters (response variables). In this case, using either bee diversity metrics or network-level metrics as predictors. By again comparing their AICcWt values, we could determine which set of predictors provided the best explanatory power for fruit quality. For all analyses, model selection was conducted independently for each response variable.

All analyses were performed with the software R (R Core Team, 2018). Geospatial analyses were conducted with the packages ‘landscapemetrics’ and ‘terra’ (Hesselbarth et al., 2019; Hijmans et al., 2022). Analyses of the network of interactions were performed using the package ‘bipartite’ (Dormann et al., 2009). We fitted several GLM and GLMM with a Poisson, negative binomial, beta, or Gaussian distribution using the ‘lmerTest’, ‘lme4’, and ‘glmmTMB’ packages (Venables and Ripley, 2002; Cribari-Neto and Zeileis, 2010; Crawley, 2012; Bates et al., 2015). Nonlinear relationships were explored using polynomial models, as visual inspection of the data suggested potential nonlinearity. Model assumptions were verified by inspecting residual distribution and variance homogeneity (Crawley, 2012). For model comparison and selection, we used the Akaike Information Criterion with a correction for small sample sizes (AICc) (Akaike, 1998). The significance of the model was assessed by an analysis of deviance (ANODEV) with a F-test or $\chi 2$ test (Crawley, 2012). We calculated AICcWt values using the package ‘MuMIn’ (Barton et al., 2012).

3. Results

3.1. Bee community

Across the 12 farms included in this study, we recorded a total of 4,231 bees, which were distributed among 76 species, 36 genera and 5 families (Table S1). Among them, 32 species were classified as tomato pollinator bees, based on their behavior observed on the flower and the available literature. The most abundant species on tomato flowers were *Paratrigona lineata* (n = 1,507 individuals), *Exomalopsis analis* (n = 221), *Pseudaugochlora* spp. (n = 106), and *Exomalopsis auropilosa* (n = 49). Most bees were collected on non-crop plants (51.57%). We identified 35 species of non-crop plants in 25 genera and 12 families (Table S2). The most abundant bee species in non-crop plants were *Apis mellifera* (n = 1,037), *P. lineata* (n = 230), *Trigona spinipes* (n = 198) and *Scaptotrigona* sp.1 (n = 107).

3.2. Landscape-level – influence of vegetation type cover on bee diversity

Landscape-level effects on tomato bee assemblages varied across response variables and natural vegetation types of Cerrado (Fig S1; Table S3). We identified a significant non-linear relationship between tomato bee abundance and the percentage of forest within a 5 km radius (F = 8.82, d.f. = 9, p < 0.000) (Table S3). Bee abundance decreased until forest patches reached 8-10%, then increased. *Paratrigona lineata* abundance showed a similar response (F = 6.02, d.f. = 9, p = 0.002), but only the initial phase of this curve was statistically significant, showing a clear decline in abundance as forest cover increased towards 10% (Table S3). *Exomalopsis* spp. abundance has a significant non-linear relationship with the percentage of forest (F = 9.53, d.f. = 9, p = 0.000) and grassland (F = 6.37, d.f. = 9, p = 0.002) within a 0.5 km radius (Table S3). *Exomalopsis* spp. abundance decreased until forest reached 10-14%, then increased. We observed a significant positive effect of grassland cover, but only above a 10% threshold. Moreover, we found no significant relationship between the percentage of forest and either tomato bee richness or diversity (Shannon index, H') (Table S3). The percentages of savanna and grassland showed no direct effects on tomato-pollinating bee diversity patterns (Table S3).

3.3. Farm-level – role of non-crop plants and natural habitat distance on bee diversity

Bee abundance did not differ between areas within tomato fields (170.75 ± 27.06 individuals per farm; mean ± SE) and areas with non-crop plants adjacent to cultivated areas (177.92 ± 17.63) (F = 0.05, d.f. = 22, p = 0.826). However, non-crop plants supported significantly greater bee richness (15.83 ± 1.40 species per farm) (F = 29.15, d.f. = 22, p < 0.001) and diversity (1.63 ± 0.14 H' per farm) (F = 9.03, d.f. = 22, p = 0.007) than tomato plants (richness = 8.25 ± 0.76; diversity = 1.02 ± 0.15). Among the non-crop plants, *Bidens pilosa*, *Bidens alba*, *Emilia fosbergii* and *Galinsoga parviflora* were visited by a more diverse and abundant set of bees than other species (Table S2). Nevertheless, model results showed no significant effects of non-crop plant richness on bee diversity patterns (Table S3). Furthermore, the mean distance from tomato crops to the nearest natural vegetation area was 89.82 ± 21.64 (mean ± SE) meters per farm (range: 0.00 - 249.86 m). The distance from the tomato crops to the nearest natural vegetation area had no detectable effect on bee diversity patterns in our study (Table S3).

3.4. Local-level – plant-bee interaction network in agroecosystems

The plant-bee interaction network exhibited significant structural differences from random null models across most metrics analyzed (Fig. S2). We observed a moderately high modularity (QuanBiMo = 0.43) and degree of specialization (H2' = 0.42), but a low nestedness (NODF = 23,59), suggesting distinct ecological modules and specialized interactions in the bee-plant community. This pattern of segregated interactions was further supported by the low niche overlap observed for both bees (mean NOHL = 0.20) and plants (mean NOLL = 0.27), indicating significant resource partitioning within the community. The network's robustness was high for both bees to the extinction of plants (RHL = 0.75) and plants to the extinction of bees (RLL = 0.64), yet did not differ from random null models (Fig. S2).

The network, considering all farms together, comprised five modules (Fig. 2d). Modules 1 and 2 comprised mostly bee and plant species with interaction specificity and marginal topological positions, exhibiting both weighted-betweenness (WB) and weighted-closeness (WC) values below network averages (Fig. 2, Fig. S3; Table S4). Modules 3 and 4 contained the most interactions and central species, concentrating plant species with the highest centrality values (Fig. 2). The non-crop plants *B. pilosa*, *B. alba*, *E. fosbergii* and *G. parviflora* showed frequent interactions with other plants (high WC) and connections among multiple bee species (high WB) (Fig. 2a-b; Fig. S3). Notably, *B. pilosa* and *G. parviflora* were classified as module hubs (high-z and low-c values), revealing high within-module connectivity (Fig. 3a). Similarly, the bees *A. mellifera*, *T. spinipes*, also allocated in modules 3 and 4, exhibited module hub roles (Fig. 3b). Module 5 was dominated by bee species interacting with tomato and three non-crop plants: *Galinsoga quadriradiata*, *Jaegeria hirta*, and *Tridax procumbens* (Fig. 2d). These non-crop plants predominantly attracted *P. lineata*, the most abundant tomato pollinator in our study system, which is also classified as a module hub (Fig. 3b). *Tetragonisca angustula* was classified as a connector (low-z and high-c values), reflecting its role in connecting modules without within-module dominance (Fig. 3b).

Forest cover significantly influenced nestedness at a 3km buffer ($\beta = -0.088$; F = 9.50, d.f. = 10, p = 0.002), while savanna cover affected modularity at an 8km buffer ($\beta = -2.020$; F = 6.38, d.f. = 10, p = 0.012) (Table S3). We also observed that the distance from the tomato crops to the nearest natural vegetation affected modularity ($\beta = -0.003$; F = 8.07, d.f. = 10, p = 0.004) (Table S3). Furthermore, non-crop plant richness exhibited variable effects on network structural properties, showing significant effects on: 1) nestedness decreases after non-crop plants reach 9-11 species (F = 5.29, d.f. = 9, p = 0.005); 2) specialization increases after non-crop plants reach 8-10 species (F = 13.32, d.f. = 9, p = 0.002); 3) non-crop plant richness has a positive effect on modularity (F = 5.18, d.f. = 10, p = 0.023) (Table S3).

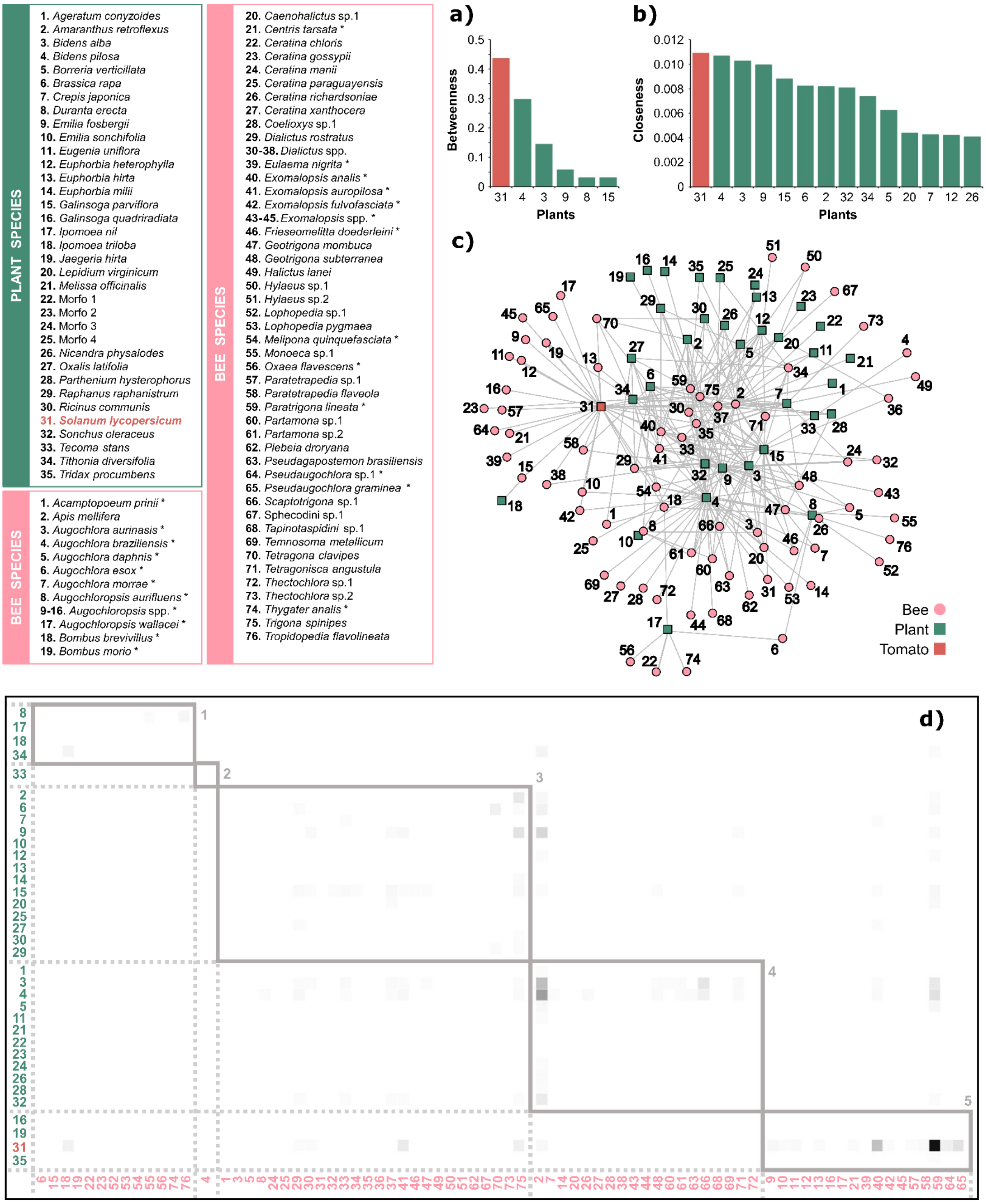

Fig. 2 Plant-bee interaction network from the bee community sampled in 12 farms that adopt the organic management system in the Federal District – Brazil, from July to October 2022 and May to October 2023. On the top left: list of plant species (1-35) and bee species (1-76) recorded in the study. Asterisk indicates bee species considered pollinators of tomato plants. On the top right: key network hubs identified through weighted centrality metrics, only plant species with both weighted-betweenness (a) and weighted-closeness (b) values above the

network-wide mean are shown; and visual representation of the pollination network derived from field sampling data (c). On the base: plot showing classification of the species into different modules according to the analysis of modularity QuanBiMo (d). Pink numbers and circles = bee species, green numbers and squares = plant species, and red numbers and square = tomato plant (Solanum lycopersicum). Gray rectangles represent species groupings (modules, 1-5), and the intensity of the gray squares indicates the relative abundance of each flower-visitor's functional group per species.

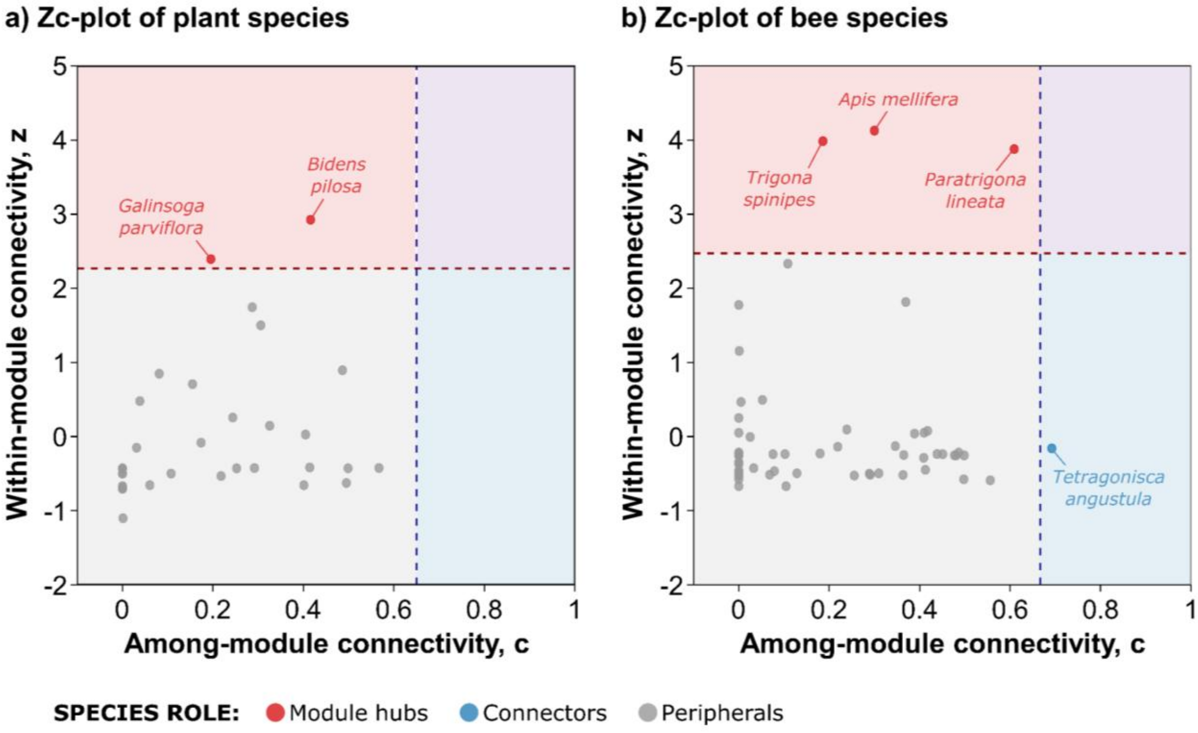

Fig. 3 Roles of plant and bee species in the interaction network based on the Zc-plot. (a) Plant species and (b) bee species are positioned according to their within-module connectivity (z, vertical axis) and among-module connectivity (c, horizontal axis). We classified species into four functional groups by applying z-c values: peripherals (low z, low c – grey circles), poorly connected both within and between modules; connectors (low z, high c – blue circles), bridge modules but lack within-module dominance; module hubs (high z, low c – red circles), highly connected within their module but not beyond; and network hubs (high z, high c – purple area), integrate the entire community.

3.5. Crop-level – pollination effects on tomato fruit quality

Among the 769 tomato fruits harvested from six farms, SP presented lower fruit weight (F = 132.17, d.f. = 764, p < 0.001), size (F = 73.37, d.f. = 764, p < 0.001), and seed number per fruit (F = 228.71, d.f. = 764, $p < 0.001$) compared to OP. Mean OP fruit weight exceeded SP by 22.92%, while fruit length and seed number were 0.64% and 29.46% higher, respectively (Fig. 4). Considering only OP fruits (n = 416) to isolate natural pollination effects, our analysis uncovered that fruit quality was significantly affected by some diversity patterns and network metrics (Table S3). Tomato fruit weight was negatively affected by total bee abundance and *P. lineata* abundance (Table S3). Fruit size followed nonlinear relationships with key pollinator taxa, peaking at approximately 100 *P. lineata* individuals and 45 *Exomalopsis* spp. individuals before declining (Table S3). Tomato fruit weight and size showed a nonlinear response to nestedness, with minimum values at NODOF ≈ 22, followed by an increase (Table S3). We also observed that specialization negatively affects fruit size (Table S3). Moreover, seed number per fruit was positively affected by total bee abundance, *P. lineata* and *Exomalopsis* spp. abundance (Table S3).

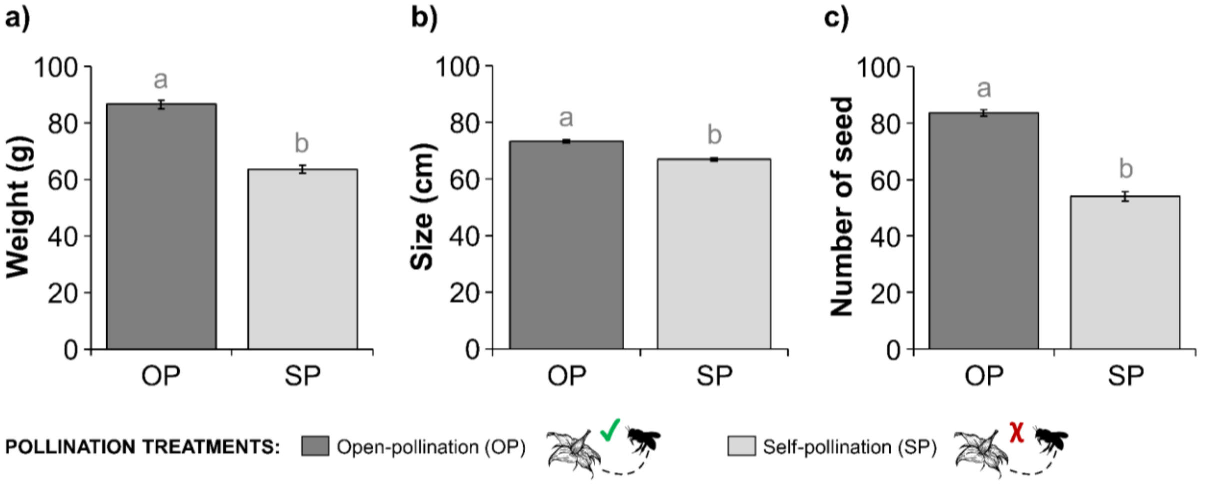

Fig. 4 Quality of tomato fruits harvested from pollination treatments. Quality parameters evaluated: a) weight (g); b) size (cm); and c) number of seeds. Pollination treatments: self-pollination (SP) and open-pollination (OP). We use data of tomato fruits harvested in organic fields in the Federal District – Brazil, from July to October 2022 and May to October 2023. Different gray letters indicate statistically significant differences between pollination treatments ($p < 0.05$).

3.6. Merging spatial scales: Relative contribution of distinct drivers to bee pollination of tomato crops

Model selection based on AICc weights showed that forest cover at different spatial scales was the stronger predictor across all bee diversity parameters, followed by grassland cover (Table S3; Fig. 5). This result is particularly important for tomato bee abundance and *Exomalopsis* spp. abundance, with forest cover explaining over 70% of the variation in both cases (Table S3; Fig. 5). The contribution of non-crop plant richness was moderate but consistent, appearing as a third driver for bee abundance variables (Table S3; Fig. 5). However, for tomato bee richness and diversity, savanna cover at small spatial scales (≤1 km) was the third most important factor (15% and 11% of influence on the model-averaged predictions, respectively).

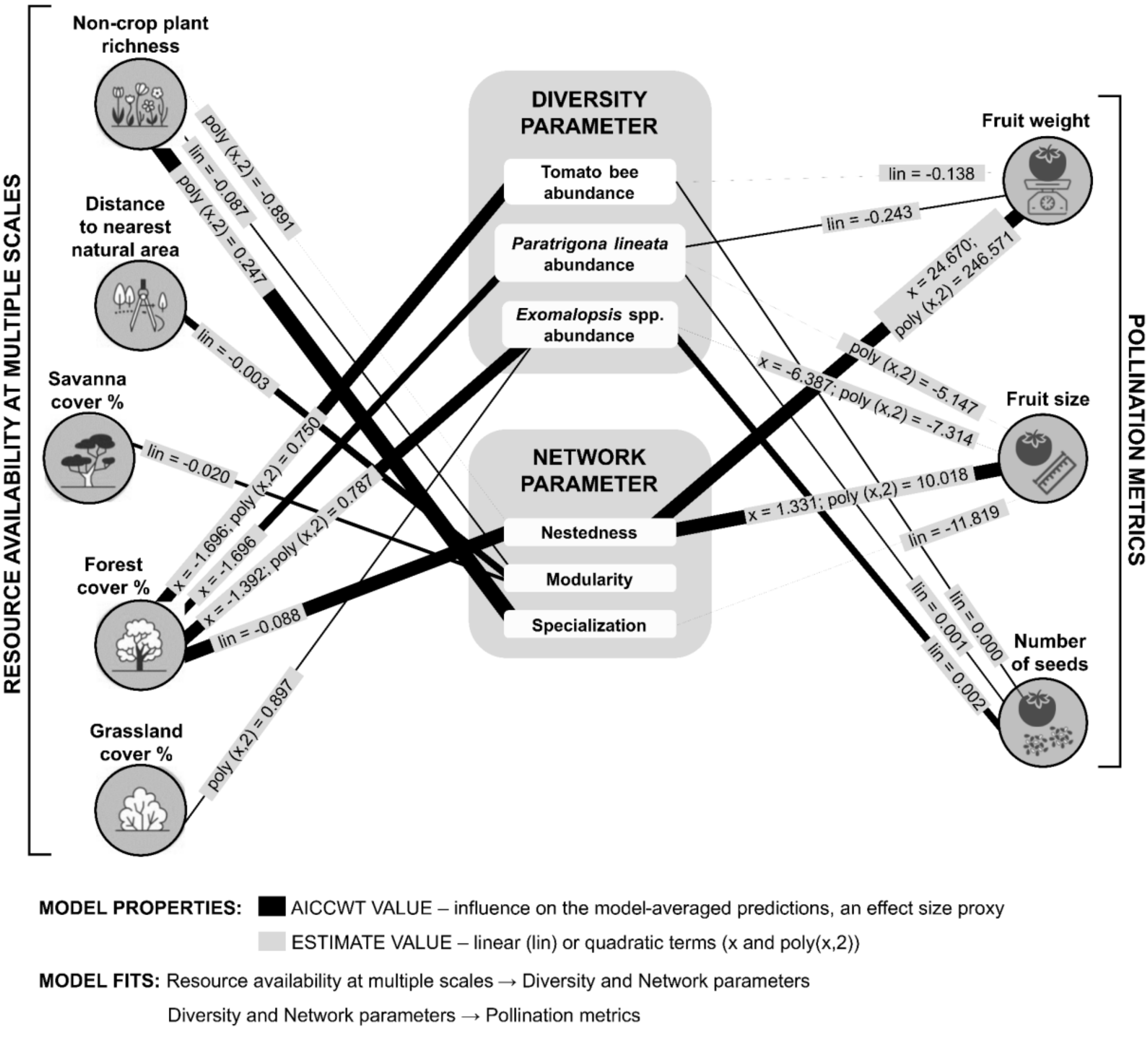

Fig. 5 Contribution of distinct drivers based on Akaike Information Criterion weights (AICcwt) on bee pollinators and their interactions on organic tomato crops. Black lines represent the weight of influence on the model-averaged predictions (an effect size proxy), showing: 1) the influence of resource availability drivers at multiple scales (non-crop plant richness, distance to nearest natural area, and the proportion of savanna, forest, and grassland cover) on (i) bee diversity parameters and (ii) plant-bee interaction network parameters; and 2) the influence of

diversity and plant-bee interaction network parameters on pollination metrics (fruit weight, fruit size and seed number). Values in gray rectangles indicate the coefficients (estimate) of significant model results ($p < 0.05$),

AICcWt values revealed distinct drivers for each network metric (Table S3; Fig. 5). Landscape composition at different spatial scales is more determinant for nestedness, with forest cover explained 81% of variation, and grassland (12% of influence on the model-averaged predictions) and savanna (4%) cover showing secondary importance. On the other hand, modularity and specialization showed stronger responses to farm-level factors, suggesting that local habitat management may be particularly effective for optimizing these network properties. Modularity was primarily influenced by distance to natural areas (47%), savanna cover (25 %), and non-crop plant richness (16%). H2 exhibited near-exclusive dependence on non-crop plant richness (96%), while all other predictors had minimal influence (Table S3; Fig. 5).

Based on AICcWt values, network metrics rather than bee diversity best explained variation in tomato fruit quality parameters. Nestedness was the strongest predictor of fruit weight and size, explaining 83% and 85% of variation in both cases, respectively (Table S3; Fig. 5). Fruit weight had specialization as the second predictor (11% of influence on the model-averaged predictions). *Exomalopsis* spp. abundance showed secondary importance for fruit size (6%). Considering the number of seeds of tomato fruits, *Exomalopsis* spp. (49%) abundance and tomato bee abundance (18%) were the primary predictors, followed by *P. lineata* abundance (18%).

4. Discussion

Our findings demonstrate that wild bee communities, the architecture of plant-bee interaction networks, and tomato pollination outcomes in tropical organic vegetable farms are jointly shaped by interacting habitat features operating at multiple spatial scales. At the landscape level, different types of natural vegetation remnants, particularly forest cover, shape bee abundance in the crop area. By integrating multiple spatial scales, we demonstrated that forest cover acts as the dominant predictor of bee abundance, while savannas play complementary but scale-dependent roles. However, we found that 10% of forest cover in the landscape is a threshold value for sustaining wild bees. The proximity of these habitats to the farms favored crop colonization, while non-crop plant richness structured plant-bee network architecture and increased habitat permeability for wild bees. The high nestedness of bee-plant networks and abundance of specific bee species were the main drivers of tomato fruit quality, highlighting the ecological relevance of functional interactions and specific pollinator groups. Therefore, multiscale interactions favoring bee diversity cascade down to sustain the provision of a key ecosystem service for agriculture.

The high diversity of bee species that we sampled reflects the high biodiversity of this group in the Cerrado biome. Yet, intensive crop fields are less likely to have such a diverse set of species. The less intensive agroecological management practices used in our study sites (e.g., crop diversification, light tillage, non-crop plant management and the use of organic pesticides) are likely the main reason for sustaining such many species within crop fields. Practices focused on enhancing floral resources can bring important benefit to wild bee communities (Kennedy et al., 2013; Tscharntke et al., 2021; Palomo-Campesino et al., 2022). Notably, *P. lineata*, *E. analis*, *E. auropilosa*, and *Pseudaugochlora* spp. dominated tomato flower visits, reinforcing findings from previous studies that show their significance as effective tomato pollinators in the region (Franceschinelli et al., 2017; Assunção et al., 2022).

At the landscape level, forest cover within a 5 km radius explained more than 70% of the variation in tomato bee abundance with a non-linear relationship. Bee abundance declined until a threshold of 8-10% forest cover (approximately 8 km² of forest areas) and increased thereafter. This initial decline was largely driven by *P. lineata*, the dominant species in our study system (>80% of flower visitors), which also showed a negative response at forest cover levels below 10%. *Exomalopsis* spp. Also showed high abundance at high levels of forest cover values, likely reflecting contrasting habitat requirements and foraging strategies within the genus (Cunha et al., 2024; Rabelo et al., 2016). The abundance of these bees increased with grassland cover above 10%, consistent with an open-habitat forager group reported in previous studies. (Arand and Graciolli, 2013; Rabelo et al., 2016; Hautequestt et al., 2020). Interestingly, the responses to the landscape of these *P. lineata* (5 km) and *Exomalopsis* spp. (0,5 km), align with their behavioral traits. *Paratrigona lineata* is a eusocial, generalist, and disturbance-tolerant (Michener, 2007), enabling responses to broader spatial scales (Henle et al., 2004; Williams et al., 2010), despite its small size (Oliveira et al., 2020). In contrast, *Exomalopsis* species present a communal lifestyle (Rozen Jr, 2007), lower population densities, and floral constancy (Michener, 2007; Rabelo et al., 2016; Cunha et al., 2024), making them more dependent on local habitat conditions. Therefore, behavioral and ecological traits can override morphological expectations in shaping species' responses to landscape structure (Williams et al., 2010; Jha and Kremen, 2013; Forrest et al., 2015).

Savanna and grassland cover are the secondary predictors of bee richness and diversity. One explanation is that species richness and community composition can remain relatively stable until habitat cover drops below a critical threshold, typically estimated at 20-30% natural vegetation cover (Fahrig, 1998, 2003; Ferreira et al., 2015; Garibaldi et al., 2021). In our study, only four farms were below this threshold, while the majority of landscapes maintained savanna and grassland vegetation well above it. Another complementary interpretation involves the Intermediate Landscape Hypothesis, which posits that biodiversity is maximized at intermediate levels of habitat complexity and heterogeneity (Priyadarshana et al., 2024). Our findings indicate that different Cerrado vegetation types are functionally complementary rather than functionally equivalent, suggesting that the mosaic of vegetation types is relevant. By moving beyond a single 'natural vegetation' classification to distinguish specific types of natural habitat covers at different spatial scales, we uncovered ecological relationships that conventional broad-scale analyses typically overlook (Bueno et al., 2018; De Brito Freire et al., 2024). This is also evident in other ecosystem services within tropical landscapes (Marins et al., 2024; Machado et al., 2024; Novaes et al., 2024).

This habitat-specific perspective is insightful when examining our farm-level results, where non-crop plants acted as moderate drivers of tomato bee populations. The functional complementarity of non-crop plant traits (floral morphologies, nutritional rewards, and bloom periods) provides the resource heterogeneity to sustain more wild bee species (Rowe et al., 2020; Querejeta et al., 2023; Ribeiro et al., 2024), since tomato plants do not provide continuous or diverse floral resources as they need (Ganser et al., 2018; Nichols et al., 2019). However, a small subset of highly rewarding non-crop plant species (e.g., B. pilosa, B. alba, E. fosbergii and G. parviflora) may disproportionately support bee diversity (Nichols et al., 2019; Assunção et al., 2022), masking richness effects given the relative uniform non-crop plant richness across the farms. Although often non-native or weedy, these plants likely supply essential resources absent in tomato flowers, such as nectar, oils, or floral resins (Hautequestt et al., 2020).

Plant-bee interaction networks at the local level revealed specialized and modular structure, as in other neotropical regions (Fisher et al., 2017; Escobedo-Kenefic et al., 2020, 2022), including the Cerrado (Aguiar et al., 2024). This pattern indicates that non-crop plants play a central role in promoting bee species coexistence by reducing niche overlap because they provide more foraging opportunities within and between modules (Bascompte and Jordano 2007, Olesen et al., 2007; Vizentin-Bugoni et al., 2018). Modules 3 and 4, concentrated most interactions because highly rewarding plants such as *B. pilosa* and *G. parviflora* (module hubs), which attracted several bee species due to their abundant and constant floral resources (Watts et al., 2016; Assunção et al., 2022). These modules also included generalist social species like *A. mellifera* and *T. spinipes* which acted as module hubs due to their social behavior, large colony sizes and generalist foraging behavior (Cortopassi-Laurino and Ramalho, 1988; Aguiar, 2003; Assunção et al., 2025), acting in structuring within-module consolidation rather than solely driving overall nestedness (Giannini et al., 2015b). While other modules might support a broader diversity of bees and plants, Module 5 grouped tomato pollinators, including *P. lineata* (module hub) and *E. analis* (Gaglianone et al., 2015; Franceschinelli et al., 2017), suggesting that the non-crop plants *G. quadriradiata*, *J. hirta* and *T. procumbens* enhance functional complementarity for tomato pollination. These findings highlight the value of non-crop plants as an effective nature-based solution for agroecological intervention in smallholder croplands (Escobedo-Kenefic et al., 2022; Casiá-Ajché et al., 2024).

Nevertheless, the architecture of the plant-bee network is tightly linked to landscape structure. Forest cover at a 3 km buffer was the dominant predictor of nestedness (81% of the observed variation), suggesting that greater forest cover fosters niche differentiation at the community level and reduces reliance on a single core of generalist interactions (Ferreira et al., 2015; Raguse-Quadros et al., 2024). In contrast, savanna cover within an 8 km buffer negatively affected modularity, which contrasts with findings in natural areas (Carstensen et al., 2016; Aguiar et al., 2024). This reversal may arise from reduced habitat connectivity (Steffan-Dewenter, 2003; Rivers-Moore et al., 2020) and from more generalized and opportunistic bee foraging in agricultural landscapes, which weakens modularity (Olesen et al., 2007; Rivers-Moore et al., 2020; Fortuin and Gandhi, 2021; Ferreira et al., 2022). Together with the negative impact of crop-natural habitat distance at the farm-level on modularity (47% of the variation), these results highlight that natural habitat composition and connectivity remain central for sustaining pollination interactions (Mallinger et al., 2016; Ferreira et al., 2020), even when bee diversity patterns remain stable.

Modularity and specialization were mainly driven by farm-level factors, especially non-crop plant richness, which explained 96% of specialization. Networks were dominated by a generalist core when non-crop plant richness was low (< 8-11 species) but shifted toward greater niche partitioning when richness was high, reflecting increased specialization due to resource heterogeneity, availability and continuity (Fontaine et al., 2006; Ghazoul, 2006; Liu et al., 2022). Although generalist foraging is common in plant-pollinator networks (Memmott, 1999), dietary plasticity is an adaptive trait (Vanderplanck et al., 2020; Martins et al., 2023;

Pimenta et al., 2025). Overall landscape structure defines the potential for a complex network (e.g., species pool), but non-crop plants determine how that potential is realized (e.g., niche partitioning).

Tomato fruit quality in organic farms was strongly linked to wild bee foraging behavior and the resulting plant-bee network structure. Open-pollinated fruits were heavier (+22.9%), larger (+0.6%), and had more seeds (+29.5%) than self-pollinated controls, confirming the importance of specialized behaviors on wild, such as "buzz", "scraping" and "milking" (Franceschinelli et al., 2013; Barbosa et al., 2019; Portman et al., 2019; Cooley and Vallejo-Marín, 2021). Nested networks, a pattern indicative of functional redundancy and stability (Memmott et al., 2004; Song et al., 2017), were correlated with heavier (60% of variation explained) and larger (91%) open-pollinated fruits. Such a nested structure suggests that a generalist core ensures consistent pollination services. In contrast, high specialization reduced fruit size, reinforcing the functional importance of the generalist core that defines nested systems. Seed number was best predicted by the abundance of key specific pollinators, particularly *Exomalopsis* spp. and *P. lineata*. This indicates that while broader structural properties like nestedness ensure a consistent pollination service that supports overall fruit development (size and weight), the quantity of pollen delivered by the most abundant and effective tomato pollinators is the primary driver of reproductive output (seed number).

Bee abundance showed a negative relationship with fruit weight, and fruit size exhibited non-linear responses, indicating that high densities of flower visitors can interfere with each other and reduce pollination (Sáez et al., 2014; Cervantes-Loreto et al., 2021; Page and Williams 2022). For example, although *P. lineata* and *Exomalopsis* spp. are effective tomato pollinators (Silva-Neto et al., 2016; Bartelli et al., 2024), high abundance may paradoxically reduce their pollination effectiveness. Unlike open-flower systems, where a high abundance of individuals can promote bee movement (Carvalheiro et al., 2011), we propose that the mechanism in tomato is different due to its specific flower morphology. Bees need to land directly on the poricidal anthers of tomato flowers and cling to them, where excessive visitation may result in aggressive interactions at the flower level. We hypothesize that this can lead to physical stigma damage or inefficient pollen release, a phenomenon also observed in other crops with sensitive floral structures (Sáez et al., 2014, 2018; Ramírez-Mejía et al., 2024). These trade-offs suggest a dissociation between the processes of fertilization and the subsequent somatic development of the fruit and further studies should explicitly test this hypothesis.

Finally, it is important to recognize that the ecological functions observed in our study are likely amplified by the specific context of these organic farms surrounding by close natural remnants of Cerrado (Adhikari et al., 2019; Martínez-Núñez et al., 2019; Rotchés-Ribalta et al., 2023; Cano et al., 2025). The roles of bees extend far beyond the pollination of a single crop; they are dynamic components of the ecosystem, responsible for maintaining native plant diversity, and requiring a full life-cycle's worth of resources that one crop alone cannot provide (Katumo et al., 2022; Requier et al., 2022). Similarly, the plants function as the foundation of this system, offering continuous and nutritionally diverse resources that sustain the pollinator community (Martínez-Núñez et al., 2020; Assunção et al., 2022). This complex functionality would likely be compromised in more intensified agricultural systems (Holzschuh et al. 2008; Pires et al. 2014; Rotchés-Ribalta et al. 2023; Cano et al., 2025). For instance, studies in olive groves show that high-intensity managed farms within simplified landscapes influences bee-plant networks by filtering bee functional traits and changing their trophic niches preferences (Cano et al., 2025). Intensive local management removes large-bodied bees, while landscape-scale habitat loss eliminates specific nesting guilds, leading to functionally homogenized communities and simpler networks (Cano et al., 2025). Similarly, our findings in organic systems highlight a 'best-case scenario', demonstrating the rich functional potential that can be achieve when agricultural management and natural vegetation conservation are aligned.

5. Conclusions

In summary, our findings reveal that pollination services in smallholder tropical organic agroecosystems emerge from a multiscale interplay in which landscape-level features, particularly forest cover, shape the regional species pool of bees, farm-level conditions such as non-crop plant richness and proximity to natural habitats modulate how this pool is expressed locally, and local-level interactions translate structural patterns into ecosystem services at the crop level. This hierarchical perspective suggests that the structural properties of plant-bee interaction networks can be a more powerful predictor of pollination outcomes than species abundance or richness alone, underscoring that ecosystem service resilience and efficiency depend on the integrity of ecological interactions rather than only on species richness and abundance. Distinct components of pollination services (e.g., fruit weight, fruit size, seed number) respond to different drivers, suggesting that agroecological management strategies should adopt a multiscale approach, as no single level is sufficient to guarantee service delivery. Attracting more bees will not compensate for a resource-poor landscape, and healthy landscapes cannot reach their full potential if farms lack the local resources needed to sustain interactions. These results have important implications for how we define and manage ecosystem services, highlighting that maximizing one dimension without considering others may inadvertently compromise the overall positive effects of biodiversity on croplands.

6. Acknowledgements

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7. Supplementary Information

Table S1 Family and species of bees collected on organic farms in the Federal District – Brazil, from July to October 2022 and May to October 2023. The table shows the total number of bees on tomato and non-crop plants (see list on Table 2), as well as the overall total for each bee species.

FAMILY AND SPECIES OF BEES	**PLANTS GROUP**		**TOTAL**
	Tomato	**Non-crop**	
ANDRENIDAE		3	3
*Oxaea flavescens**		1	1
Acamptopoeum prinii		2	2
APIDAE	1883	1902	3785
Apis mellifera	15	1037	1052
*Bombus brevivillus**	27	25	52
*Bombus morio**	2	1	3
*Centris tarsata**	3		3
Ceratina chloris		1	1
Ceratina gossypii		1	1
Ceratina manii		3	3
Ceratina paraguayensis		1	1
Ceratina richardsoniae		13	13
Ceratina xanthocera		1	1
*Eulaema nigrita**	1		1
*Exomalopsis analis**	221	32	253
*Exomalopsis auropilosa**	49	58	107
*Exomalopsis fulvofasciata**	3	1	4
Exomalopsis sp.1*		1	1
Exomalopsis sp.2*		1	1
Exomalopsis sp.3*	1		1
*Frieseomelitta doederleini**		8	8
Geotrigona mombuca		19	19
Geotrigona subterranea		25	25
Lophopedia sp.1		1	1
Lophopedia pygmaea		2	2
*Melipona quinquefasciata**	2	5	7
Monoeca sp.1		3	3
Paratetrapedia flaveola	12	3	15
Paratetrapedia sp.1	4		4
*Paratrigona lineata**	1507	230	1737
Partamona sp.1		9	9
Partamona sp.2		12	12
Plebeia droryana		1	1
Scaptotrigona sp.1		107	107
Tapinotaspidini sp.1		2	2
Tetragona clavipes	1	40	41
Tetragonisca angustula		42	42
*Thygater analis**		2	2

Trigona spinipes	35	198	233
Tropidopedia flavolineata		17	17
COLLETIDAE		3	3
Hylaeus sp.1		2	2
Hylaeus sp.2		1	1
HALICTIDAE	166	273	439
*Augochlora aurinasis**		7	7
*Augochlora braziliensis**		1	1
*Augochlora daphnis**		3	3
*Augochlora esox**		2	2
*Augochlora morrae**		5	5
*Augochloropsis aurifluens**		6	6
Augochloropsis sp.2*	17		17
Augochloropsis sp.3*	4	1	5
Augochloropsis sp.4*	12		12
Augochloropsis sp.5*	2		2
Augochloropsis sp.6*	5	1	6
Augochloropsis sp.7*		1	1
Augochloropsis sp.8*	1	2	3
Augochloropsis sp.9*	1		1
*Augochloropsis wallacei**	2		2
Caenohalictus sp.1		4	4
Dialictus rostratus	5	43	48
Dialictus sp.1	6	39	45
Dialictus sp.10		2	2
Dialictus sp.11		5	5
Dialictus sp.2	1	47	48
Dialictus sp.3		12	12
Dialictus sp.5	1	12	13
Dialictus sp.6		3	3
Dialictus sp.7	2	59	61
Dialictus sp.9	1	1	2
Halictus lanei		1	1
Pseudagapostemon brasiliensis		12	12
Pseudaugochlora sp.1*	35		35
*Pseudaugochlora graminea**	71		71
Sphecodini sp.1		1	1
Temnosoma metallicum		1	1
Thectochlora sp.1		1	1
Thectochlora sp.2		1	1
MEGACHILIDAE		1	1
Coelioxys sp.1		1	1
TOTAL	2045	2186	4231

**Bee species considered pollinators of tomato plants.*

Table S2 Family and species of plant occurring in tomato crop areas, categorized by functional group (tomato, non-crop, fruit, and ornamental plants) and number of bee interactions and visiting bee species for each plant. Data were collected across 12 organic farms in the Federal District – Brazil, from July to October 2022 and May to October 2023.

FAMILY AND SPECIES OF PLANTS	NUMBER OF INTERACTIONS	LIST OF VISITING-BEES
AMARANTHACEAE	82	
Amaranthus retroflexus	82	*Apis mellifera, Dialictus* sp.1, *Exomalopsis analis, Exomalopsis auropilosa, Paratrigona lineata, Tetragona clavipes, Tetragonisca angustula, Trigona spinipes*
ASTERACEAE	1816	
Ageratum conyzoides	4	*A. mellifera, Dialictus* sp.7
Bidens alba	455	*A. mellifera, Augochlora aurinasis, A. morrae Bombus brevivillus, Caenohalictus* sp.1, *Ceratina manii, Dialictus rostratus, Dialictus sp.1, Dialictus sp.11, Dialictus sp.2, Dialictus sp.3, Dialictus sp.5, Dialictus sp.7, E. analis, E. auropilosa, Exomalopsis sp.1, Frieseomelitta doederleini, Geotrigona mombuca, Geotrigona subterranea, Melipona quinquefasciata, P. lineata, Partamona sp.1, Partamona sp.2, Pseudagapostemon brasiliensis, Scaptotrigona postica, T. angustula, T. spinipes*
Bidens pilosa	763	*Acamptopoeum prinii, A. mellifera, A. aurinasis, A.morrae, Augochloropsis aurifluens, Augochloropsis* sp.3, *Augochloropsis* sp.7, *Caenohalictus* sp.1, *Ceratina richardsoniae, Ceratina xanthocera, Coelioxys* sp.1, *D. rostratus, Dialictus* sp.1, *Dialictus* sp.10, *Dialictus sp.2, Dialictus* sp.5, *Dialictus* sp.7, *E. analis, E. auropilosa, Exomalopsis fulvofasciata, Exomalopsis* sp.2, *F. doederleini, G. mombuca, G. subterranea, Lophopedia* sp.2, *M. quinquefasciata, P. lineata, Partamona* sp.1, *Partamona sp.2, P. brasiliensis, S. postica, Tapinotaspis* sp.1, *Temnosoma metallicum, T. angustula, Thectochlora* sp.1, *T. spinipes*
Crepis japonica	16	*A. mellifera, C. manii, Dialictus* sp.1, *Dialictus* sp.2, *Dialictus* sp.3, *Dialictus* sp.5, *Dialictus* sp.7, *E. analis, E. auropilosa, G. subterranea, Halictus lanei, Thectochlora* sp.2, *T. spinipes*
Emilia fosbergii	265	*A. mellifera, A. aurinasis, Augochlora daphnis, A. aurifluens, Augochloropsis* sp.6, *C. manii, Ceratina paraguayensis, C. richardsoniae, Dialictus* sp.1, *Dialictus* sp.2, *Dialictus sp.5, Dialictus* sp.7, *E. analis, E. auropilosa, G. mombuca, G. subterranea, Paratetrapedia flaveola, P. lineata, Partamona sp.1, Partamona* sp.2, *Plebeia droryana, T. angustula, T. spinipes*

Emilia sonchifolia	4	*A. aurinasis, Dialictus* sp.7*, Partamona* sp.1*, Partamona* sp.2
Galinsoga parviflora	134	*A. mellifera, A. aurinasis, A. daphnis, Caenohalictus* sp.1*, D. rostratus, Dialictus* sp.1*, Dialictus* sp.10*, Dialictus* sp.11*, Dialictus* sp.2*, Dialictus* sp.3*, Dialictus* sp.5*, Dialictus* sp.6*, Dialictus* sp.7*, E. analis, E. auropilosa, F. doederleini, G. mombuca, G. subterranea, P. lineata, S. postica, T. angustula, T. spinipes*
Galinsoga quadriradiata	1	*P. lineata*
Jaegeria hirta	1	*P. lineata*
Parthenium hysterophorus	8	A. *mellifera, Dialictus* sp.7*, G. mombuca, T. angustula*
Sonchus oleraceus	84	*A. mellifera, Caenohalictus* sp.1*, D. rostratus, Dialictus* sp.1*, Dialictus* sp.2*, Dialictus* sp.7*, Dialictus* sp.9*, E. analis, E. auropilosa, G. mombuca, G. subterranea, P. lineata, Partamona* sp.2*, P. brasiliensis, S. postica, T. spinipes*
Tithonia diversifolia	60	*A. mellifera, B. brevivillus, Bombus morio, Ceratina gossypii, Dialictus* sp.2*, E. auropilosa, M. quinquefasciata, P. lineata, T. clavipes, T. spinipes*
Tridax procumbens	7	*P. lineata, T. angustula*
Morfo 1	1	*A. mellifera*
Morfo 2	1	*A. mellifera*
Morfo 3	4	*A. mellifera*
Morfo 4	2	*Dialictus* sp.3*, Dialictus* sp.7
BIGNONIACEAE	3	
Tecoma stans	3	*Augochlora braziliensis, B. brevivillus, P. lineata*
BRASSICACEAE	121	
Brassica rapa	91	*A. mellifera, A. aurifluens, D. rostratus, Dialictus* sp.1*, Dialictus* sp.2*, Dialictus* sp.7*, E. analis, E. auropilosa, Paratetrapedia flaveola, P. lineata, S. postica, T. clavipes, T. spinipes*
Lepidium virginicum	23	*Dialictus* sp.1*, Dialictus* sp.2*, Dialictus* sp.5*, Dialictus* sp.6*, Dialictus* sp.7*, Hylaeus* sp.1*, P. lineata,* Sphecodini sp.1
Raphanus raphanistrum	7	*Dialictus* sp.1*, Dialictus* sp.3*, E. analis, T. spinipes*
CONVOLVULACEAE	8	
Ipomoea nil	6	*Augochlora esox, B. brevivillus, Ceratina chloris, Oxaea flavescens, Thygater analis*
Ipomoea triloba	2	*Augochloropsis* sp.8
EUPHORBIACEAE	49	
Euphorbia heterophylla	20	*A. mellifera, Dialictus* sp.5*, Dialictus* sp.7*, Hylaeus* sp.1*, Hylaeus* sp.2*, P. lineata, T. angustula, T. spinipes*
Euphorbia hirta	3	*Dialictus* sp.7*, T. spinipes*
Euphorbia milii	9	*T. spinipes*
Ricinus communis	17	*A. mellifera, P. lineata, T. clavipes, T. angustula, T. spinipes*

LAMIACEAE	1	
Melissa officinalis	1	*A. mellifera*
MYRTACEAE	6	
Eugenia uniflora	6	*A. mellifera, T. spinipes*
OXALIDACEAE	13	
Oxalis latifolia	13	*A. prinii, D. rostratus, Dialictus sp.3, E. analis, E. auropilosa, P. lineata*
SOLANACEAE	2066	
Nicandra physalodes	17	*A. mellifera, Dialictus* sp.1, *Dialictus* sp.7, *P. lineata*
Solanum lycopersicum	2049	*A. mellifera, Augochloropsis* sp.2, *Augochloropsis* sp.3, *Augochloropsis* sp.4, *Augochloropsis* sp.5, *Augochloropsis* sp.6, *Augochloropsis* sp.8, *Augochloropsis* sp.9, *Augochloropsis wallacei, B. brevivillus, B. morio, Centris tarsata, D. rostratus, Dialictus* sp.1, *Dialictus* sp.2, *Dialictus* sp.5, *Dialictus* sp.7, *Dialictus* sp.9, *Eulaema nigrita, E. analis, E. auropilosa, E. fulvofasciata, Exomalopsis* sp.3, *M. quinquefasciata, Paratetrapedia* sp.1, *Paratetrapedia flaveola, P. lineata, Pseudaugochlora* sp.1, *Pseudaugochlora graminea, T. clavipes, T. spinipes*
SPERMACOCEAE	38	
Borreria verticillata	38	*A. mellifera, Dialictus* sp.1, *Dialictus* sp.7, *P. lineata*
VERBENACEAE	28	
Duranta erecta	28	*A. mellifera, A. daphnis, A. esox, B. brevivillus, Lophopedia sp.1, Lophopedia pygmaea, M. quinquefasciata, Monoeca sp.1, Tropidopedia flavolineata*

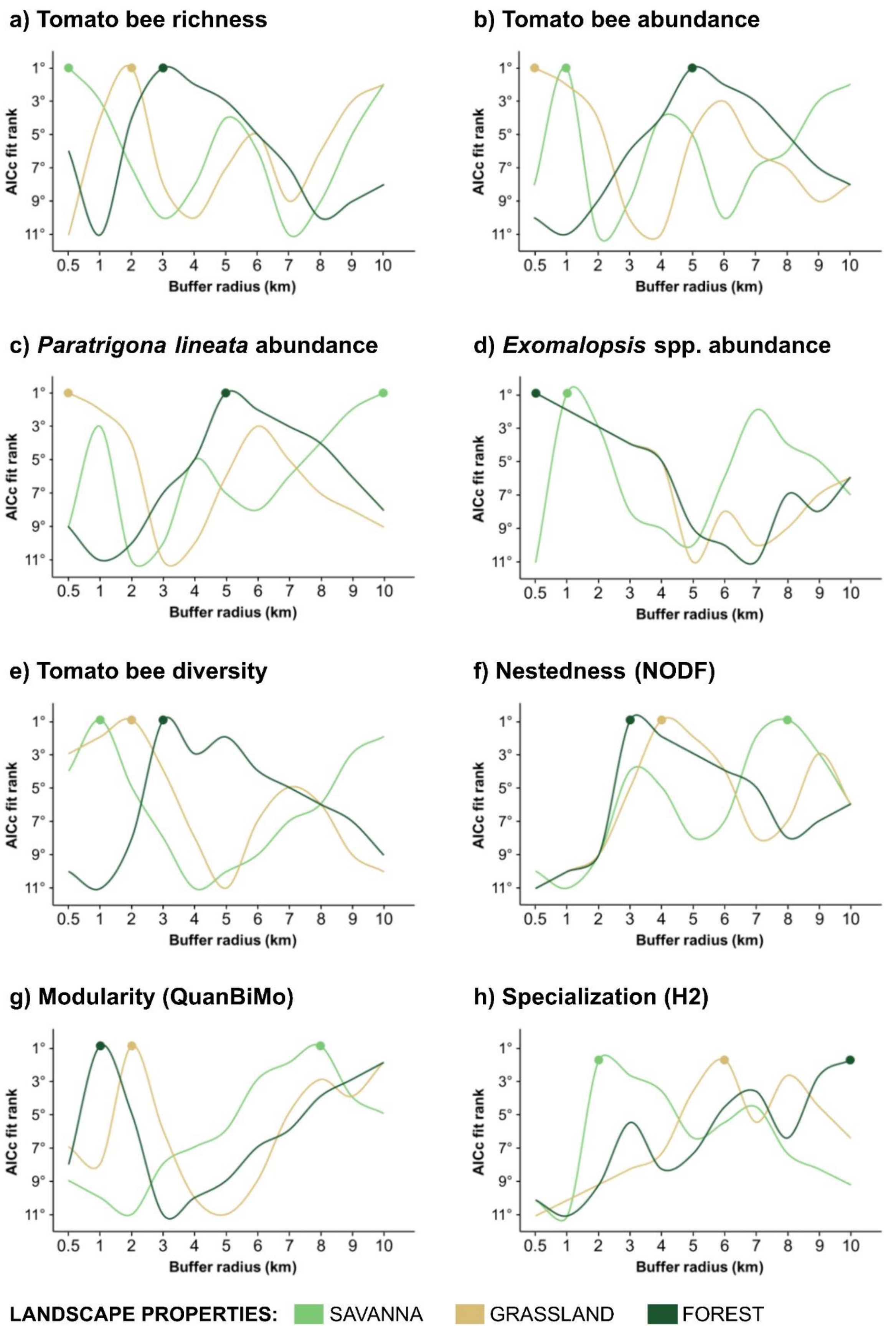

Fig. S1 Effects of Cerrado natural vegetation cover (percentage of savanna, grassland and forest) at multiple spatial scales on: (a) tomato bee richness; (b) tomato bee abundance; (c) *Paratrigona lineata* abundance; (d) *Exomalopsis* spp. abundance; (e) tomato bee diversity (Shannon index); and network-level metrics including (f) weighted nestedness (NODF), (g) modularity (QuanBiMo), and (h) specialization (H_2'). Lines represent top-ranked models selected via AICc (Akaike Information Criterion corrected for small sample sizes). Data refer to samples collected in 12 tomato producing organic agricultural farms located in the Federal District – Brazil, from July to October 2022 and May to October 2023. The landscape composition was characterized based on the map of Cerrado biome from Collection 5 of Brazilian Annual Land Use and Land Cover Mapping Project – MapBiomas (Souza et al. 2020).

Table S3 Effect of landscape and local variables on bee communities, bee foraging patterns, and tomato fruit quality parameters. For each response variables, model coefficients and fit statistics are shown for each explanatory variable. Linear (lin) and quadratic terms (x and [poly(x,2)]) are considered. Separate models were fitted for each response and explanatory variables. We then compared these models using their associated corrected Akaike information criterion weights (AICcWt). The AICcWt is interpreted as the probability that a specific model is the best relative to the others. We use data from bee samples and tomato fruits harvested in 12 organic farms in the Federal District – Brazil. Hyphen (-) represent repeated values.

Response variables	Explanatory variables		Estimate	d.f.	F/χ²	p-value	AICc	AICcWt
Tomato bee richness (poisson)	Non-crop plant richness	lin	-0.004	10	0.01	0.919	69.92	0.04
	Distance to nearest natural area	lin	-0.001	-	0.17	0.683	67.22	0.15
	Savanna % (buffer 0.5 km)	lin	0.002	-	0.25	0.618	67.13	0.15
	Grassland % (buffer 2 km)	lin	-0.015	-	1.36	0.244	66.03	0.26
	Forest % (buffer 3 km)	lin	0.050	-	2.21	0.137	65.20	0.40
Tomato bee abundance (negative binomial)	Non-crop plant richness	lin	0.065	10	0.93	0.334	151.18	0.05
	Distance to nearest natural area	lin	0.001	-	0.07	0.790	152.01	0.04
	Savanna % (buffer 1 km)	lin	0.005	-	0.41	0.521	151.68	0.04
	Grassland % (buffer 0.5 km)	lin	0.016	-	2.06	0.152	150.18	0.09
	Forest % (buffer 5 km)	**x**	**-1.091**	**9**	**8.82**	**0.000**	**145.88**	**0.78**
		poly(x, 2)	**0.806**					
Paratrigona lineata abundance (negative binomial)	Non-crop plant richness	lin	0.144	10	1.97	0.161	149.06	0.19
	Distance to nearest natural area	lin	0.000	-	0.00	0.983	150.90	0.08
	Savanna % (buffer 10 km)	lin	-0.014	-	0.45	0.502	150.46	0.10
	Grassland % (buffer 0.5 km)	lin	0.019	-	1.09	0.298	149.86	0.13
	Forest % (buffer 5 km)	**x**	**-1.696**	**9**	**6.02**	**0.002**	**147.13**	**0.50**
		poly(x, 2)	0.750					
Exomalopsis spp. abundance (negative binomial)	Non-crop plant richness	lin	-0.112	10	2.90	0.089	106.23	0.08
	Distance to nearest natural area	lin	0.001	-	0.34	0.562	108.54	0.02
	Savanna % (buffer 1 km)	lin	0.007	-	0.60	0.437	108.28	0.03
	Grassland % (buffer 0.5 km)	x	0.739	**9**	**6.37**	**0.002**	**105.01**	**0.14**
		poly(x, 2)	**0.897**					
	Forest % (buffer 0.5 km)	**x**	**-1.392**	**-**	**9.53**	**0.000**	**101.67**	**0.74**
		poly(x, 2)	**0.787**					

Tomato bee diversity (gaussian)	Non-crop plant richness	lin	-0.037	10	0.33	0.581	26.75	0.09
	Distance to nearest natural area	lin	0.001	-	0.43	0.528	26.64	0.09
	Savanna % (buffer 1 km)	lin	-0.007	-	0.80	0.391	26.21	0.11
	Grassland % (buffer 2 km)	lin	-0.025	-	1.55	0.242	25.41	0.17
	Forest % (buffer 3 km)	lin	0.100	-	4.03	0.072	23.07	0.54
Nestedness (NODF) (negative binomial)	**Non-crop plant richness**	x	0.309	**9**	**5.29**	**0.005**	**89.17**	**0.03**
		poly(x, 2)	**-0.891**					
	Distance to nearest natural area	x	-0.366	-	2.95	0.052	92.01	0.01
		poly(x, 2)	-0.756					
	Savanna % (buffer 8 km)	lin	-0.011	10	1.48	0.224	88.21	0.04
	Grassland % (buffer 4 km)	lin	0.023	-	3.37	0.067	86.09	0.12
	Forest % (buffer 3 km)	**lin**	**-0.088**	**-**	**9.50**	**0.002**	**82.27**	**0.81**
Modularity (QuanBiMo) (beta)	**Non-crop plant richness**	**lin**	**0.087**	**10**	**5.18**	**0.023**	**-18.24**	**0.16**
	Distance to nearest natural area	**lin**	**-0.003**	**-**	**8.07**	**0.004**	**-20.40**	**0.47**
	Savanna % (buffer 8 km)	**lin**	**-0.020**	**-**	**6.38**	**0.012**	**-19.16**	**0.25**
	Grassland % (buffer 2 km)	lin	-0.008	-	0.32	0.574	-14.10	0.02
	Forest % (buffer 1 km)	lin	0.047	-	3.98	0.046	-17.26	0.10
Specialization (H2) (gaussian)	**Non-crop plant richness**	x	-0.008	**9**	**13.32**	**0.002**	**-28.58**	**0.96**
		poly(x, 2)	**0.247**					
	Distance to nearest natural area	x	0.054	-	2.38	0.148	-17.17	0.00
		poly(x, 2)	0.159					
	Savanna % (buffer 2 km)	lin	0.002	10	1.09	0.322	-18.02	0.00
	Grassland % (buffer 6 km)	lin	-0.007	-	3.78	0.081	-20.63	0.02
	Forest % (buffer 10 km)	lin	0.014	-	2.24	0.165	-19.20	0.01
Tomato fruit weight (gaussian)	Tomato bee richness	lin	-83.757	410	1.30	0.523	3909.42	0.06
		poly(x, 2)	-127.668					
	Tomato bee abundance	**lin**	**-0.138**	**411**	**9.45**	**0.002**	**3930.51**	**0.00**
	***P. lineata* abundance**	**lin**	**-0.243**	**-**	**93.62**	**0.000**	**3920.75**	**0.00**
	Exomalopsis spp. abundance	lin	-0.266	-	2.03	0.156	3934.31	0.00
	Tomato bee diversity (H')	lin	15.831	-	0.79	0.374	3922.37	0.00
	NODOF	**x**	**24.670**	**410**	**14.82**	**0.000**	**3904.17**	**0.83**
		poly(x, 2)	**246.571**					

	QuanBiMo	lin	-18.856	411	0.01	0.931	3918.27	0.00
	H2	x poly(x, 2)	-171.781 -3.812	410	2.34	0.311	3908.19	0.11
Tomato fruit size (gaussian)	Tomato bee richness	x poly(x, 2)	-1.523 -6.359	410	2.02	0.364	1109.71	0.03
	Tomato bee abundance	lin	-0.004	411	1.70	0.193	1126.76	0.00
	***P. lineata* abundance**	x **poly(x, 2)**	-6.492 **-5.147**	**410**	**13.47**	**0.001**	**1124.14**	**0.01**
	***Exomalopsis* spp. abundance**	**x** **poly(x, 2)**	**-6.387** **-7.314**	**-**	**35.74**	**0.000**	**1119.25**	**0.06**
	Tomato bee diversity (H')	lin	0.630	411	0.79	0.375	1116.58	0.00
	NODOF	**x** **poly(x, 2)**	**1.331** **10.018**	**410**	**29.06**	**0.000**	**1103.29**	**0.85**
	QuanBiMo	lin	2.753	411	0.10	0.747	1112.38	0.01
	H2	**lin**	**-11.819**	**-**	**4.89**	**0.027**	**1109.46**	**0.04**
Number of seeds in tomato fruit (negative binomial)	Tomato bee richness	lin	0.002	411	0.07	0.801	3466.85	0.03
	Tomato bee abundance	lin	**0.000**	**-**	**4.75**	**0.030**	**3463.42**	**0.18**
	***P. lineata* abundance**	lin	**0.001**	**-**	**12.28**	**0.000**	**3464.53**	**0.10**
	***Exomalopsis* spp. abundance**	lin	**0.002**	**-**	**6.77**	**0.009**	**3461.41**	**0.49**
	Tomato bee diversity (H')	lin	-0.009	-	0.02	0.897	3466.90	0.03
	NODOF	lin	-0.001	-	0.04	0.834	3466.87	0.03
	QuanBiMo	lin	-0.944	-	2.02	0.155	3465.05	0.08
	H2	lin	0.585	-	0.95	0.329	3466.06	0.05

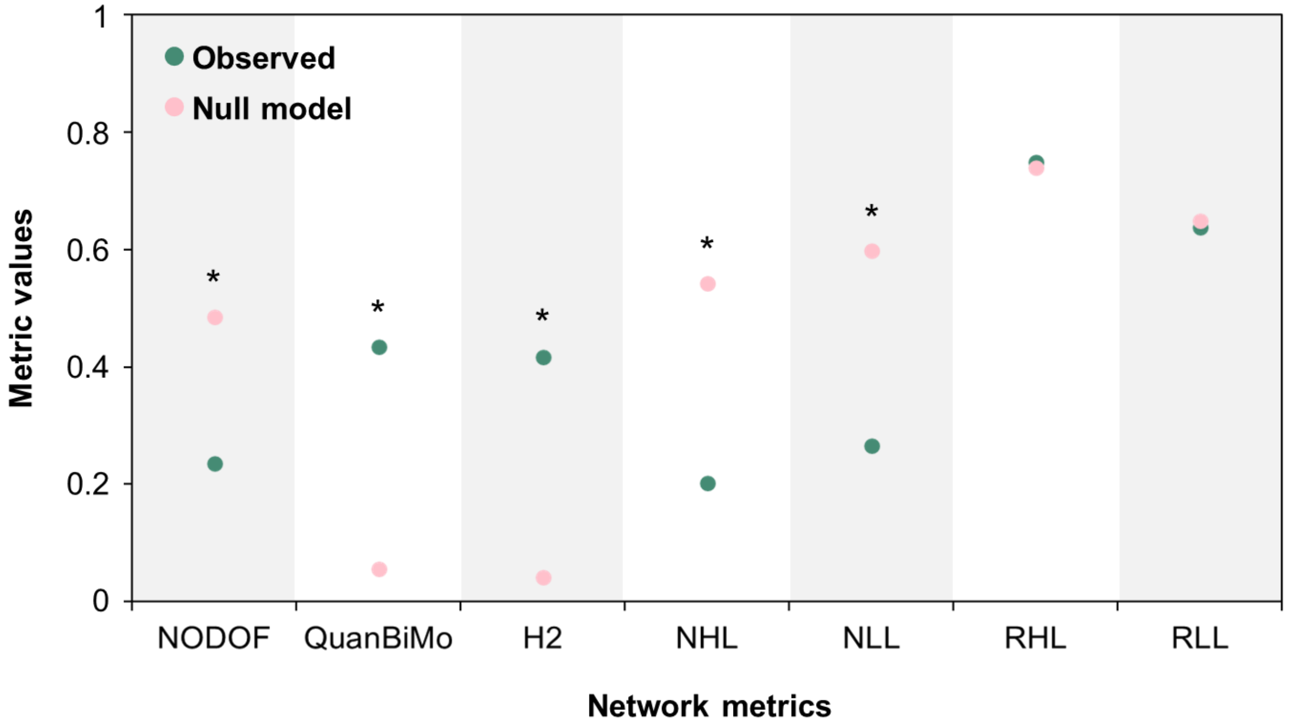

Fig. S2 Observed and expected values of network metrics of the bee community in non-crop plants associated with tomato crop areas. Green circles indicate the average observed values for network metrics. Pink circles indicate the average expected values for random network metrics. Metrics: NODOF – nestedness (value divided by 100 for scaling purposes); QuanBiMo – modularity; H2 – specialization; NOHL – niche overlap high level (bees); NOLL – niche overlap low level (plants); RHL – robustness high level (bees); and RLL – robustness low level (plants). Asterisk indicates metrics that significantly differed from null model expectations ($p < 0.05$).

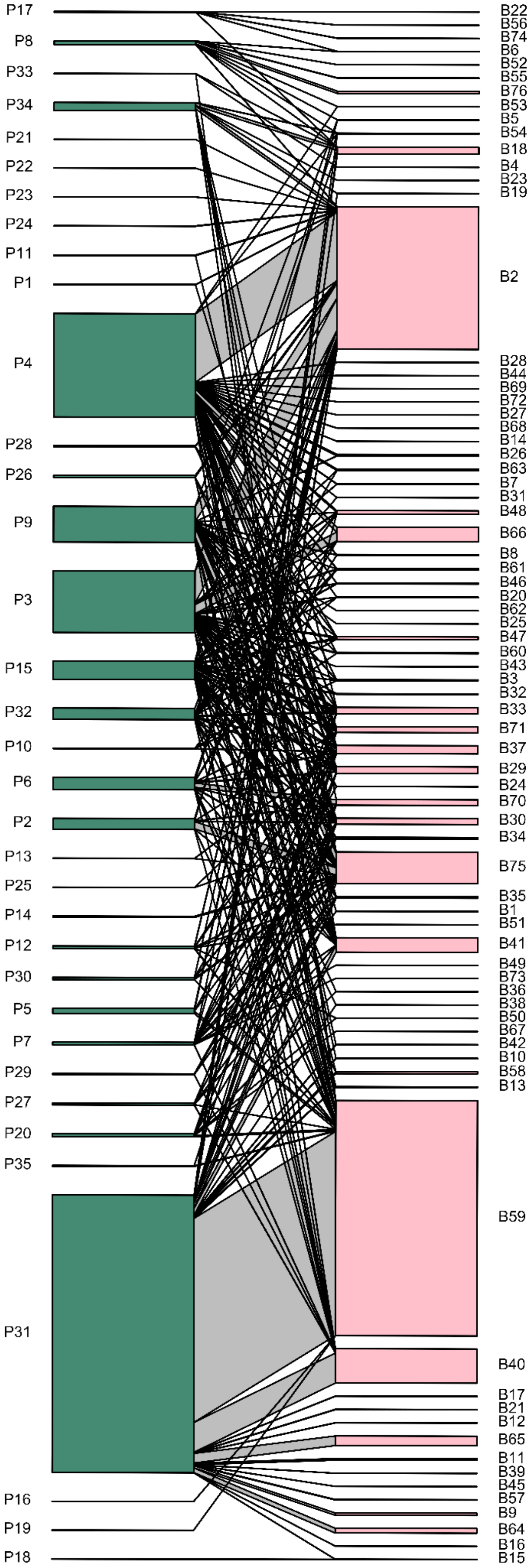

Fig. S3 Bipartite network representing interactions between bee species (pink nodes, right) and plant species (green nodes, left). Node size is proportional to species interaction frequency, and links (gray) indicate recorded interactions between bees and plants. The width of the links reflects interaction strength, based on the number of visitation events observed. Bee community was sampled in 12 farms that adopt the organic management system in the Federal District – Brazil, from July to October 2022 and May to October 2023.

Table S4 Centrality metrics for bee (left) and plant (right) species, reflecting their relative importance within the pollination network. The calculated metrics were degree (D), weighted-betweenness (WB), and weighted-closeness (WC). Mean and standard error of centrality metrics are shown for all species within each functional group. Data were derived from plant-bee interaction network sampled across 12 organic farms in the Federal District – Brazil, from July to October 2022 and May to October 2023.

BEE SPECIES			
	D	**WB**	**WC**
Acamptopoeum prinii	2	0.000	0.001
Apis mellifera	22	0.491	0.003
Augochlora aurinasis	5	0.000	0.001
Augochlora braziliensis	1	0.000	0.000
Augochlora daphnis	3	0.000	0.001
Augochlora esox	2	0.001	0.001
Augochlora morrae	2	0.000	0.001
Augochloropsis aurifluens	3	0.000	0.001
Augochloropsis sp.2	1	0.000	0.002
Augochloropsis sp.3	2	0.000	0.001
Augochloropsis sp.4	1	0.000	0.002
Augochloropsis sp.5	1	0.000	0.001
Augochloropsis sp.6	2	0.000	0.001
Augochloropsis sp.7	1	0.000	0.000
Augochloropsis sp.8	2	0.000	0.000
Augochloropsis sp.9	1	0.000	0.000
Augochloropsis wallacei	1	0.000	0.001
Bombus brevivillus	6	0.090	0.003
Bombus morio	2	0.000	0.001
Caenohalictus sp.1	4	0.000	0.001
Centris tarsata	1	0.000	0.001
Ceratina chloris	1	0.000	0.000
Ceratina gossypii	1	0.000	0.000
Ceratina manii	3	0.000	0.001
Ceratina paraguayensis	1	0.000	0.000
Ceratina richardsoniae	2	0.000	0.002
Ceratina xanthocera	1	0.000	0.000
Coelioxys sp.1	1	0.000	0.000
Dialictus rostratus	7	0.000	0.003
Dialictus sp.1	13	0.000	0.003
Dialictus sp.10	2	0.000	0.001
Dialictus sp.11	2	0.000	0.001
Dialictus sp.2	10	0.026	0.003
Dialictus sp.3	6	0.000	0.002
Dialictus sp.5	8	0.000	0.002
Dialictus sp.6	2	0.000	0.001
Dialictus sp.7	17	0.013	0.003
Dialictus sp.9	2	0.000	0.001
Eulaema nigrita	1	0.000	0.000
Exomalopsis analis	11	0.013	0.003
Exomalopsis auropilosa	11	0.000	0.003
Exomalopsis fulvofasciata	2	0.000	0.001

PLANT SPECIES			
	D	**WB**	**WC**
Ageratum conyzoides	2	0.000	0.001
Amaranthus retroflexus	8	0.002	0.008
Bidens alba	27	0.145	0.010
Bidens pilosa	36	0.297	0.011
Borreria verticillata	4	0.000	0.006
Brassica rapa	13	0.000	0.008
Crepis japonica	13	0.000	0.004
Duranta erecta	9	0.031	0.001
Emilia fosbergii	23	0.057	0.010
Emilia sonchifolia	4	0.000	0.001
Eugenia uniflora	2	0.000	0.002
Euphorbia heterophylla	8	0.000	0.004
Euphorbia hirta	2	0.000	0.001
Euphorbia milii	1	0.000	0.003
Galinsoga parviflora	22	0.031	0.009
Galinsoga quadriradiata	1	0.000	0.000
Ipomoea nil	5	0.000	0.000
Ipomoea triloba	1	0.000	0.001
Jaegeria hirta	1	0.000	0.000
Lepidium virginicum	8	0.000	0.004
Melissa officinalis	1	0.000	0.000
Morfo 1	1	0.000	0.000
Morfo 2	1	0.000	0.000
Morfo 3	1	0.000	0.001
Morfo 4	2	0.000	0.001
Nicandra physalodes	4	0.000	0.004
Oxalis latifolia	6	0.000	0.003
Parthenium hysterophorus	4	0.000	0.002
Raphanus raphanistrum	4	0.000	0.002
Ricinus communis	5	0.000	0.004
Solanum lycopersicum	31	0.437	0.011
Sonchus oleraceus	16	0.000	0.008
Tecoma stans	3	0.000	0.001
Tithonia diversifolia	10	0.000	0.007
Tridax procumbens	2	0.000	0.002
Mean	8.029	0.029	0.004
Standard error	1.565	0.015	0.001

Exomalopsis sp.1	1	0.000	0.000
Exomalopsis sp.2	1	0.000	0.000
Exomalopsis sp.3	1	0.000	0.000
Frieseomelitta doederleini	3	0.000	0.001
Geotrigona mombuca	6	0.000	0.002
Geotrigona subterranea	6	0.000	0.002
Halictus lanei	1	0.000	0.000
Hylaeus sp.1	2	0.000	0.001
Hylaeus sp.2	1	0.000	0.000
Lophopedia pygmaea	2	0.000	0.000
Lophopedia sp.1	1	0.000	0.000
Melipona quinquefasciata	5	0.000	0.001
Monoeca sp.1	1	0.000	0.001
Oxaea flavescens	1	0.000	0.000
Paratetrapedia flaveola	3	0.000	0.001
Paratetrapedia sp.1	1	0.000	0.001
Paratrigona lineata	19	0.366	0.003
Partamona sp.1	4	0.000	0.001
Partamona sp.2	5	0.000	0.002
Plebeia droryana	1	0.000	0.000
Pseudagapostemon brasiliensis	3	0.000	0.002
Pseudaugochlora sp.1	1	0.000	0.003
Pseudaugochlora graminea	1	0.000	0.003
Scaptotrigona sp.1	5	0.000	0.003
Sphecodini sp.1	1	0.000	0.000
Tapinotaspidini sp.1	1	0.000	0.001
Temnosoma metallicum	1	0.000	0.000
Tetragona clavipes	5	0.000	0.003
Tetragonisca angustula	9	0.000	0.003
Thectochlora sp.1	1	0.000	0.000
Thectochlora sp.2	1	0.000	0.000
Thygater analis	1	0.000	0.001
Trigona spinipes	16	0.000	0.003
Tropidopedia flavolineata	1	0.000	0.003
Mean	3.697	0.013	0.001
Standard error	0.514	0.008	0.000

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CHAPTER 2: Past, present, and future of SSR molecular markers in bee studies

Artigo publicado na revista: *Apidologie*

Assunção, R. M., Filgueira, I., de Moura, M. E., Togni, P. H., Pires, C. S., Paula, D. P., & Sujii, P. S. (2025). Past, present, and future of SSR molecular markers in bee studies. Apidologie 56, 91. https://doi.org/10.1007/s13592-025-01220-w

Abstract

Understanding bees, with their extensive morphological, phylogenetic, and behavioral diversity and their significant economic and ecological roles, requires integrating classical and modern scientific methods. Microsatellite (SSR - Simple Sequence Repeat) markers are a low-cost tool useful for various investigations at individual, colony, population, and species levels. However, a comprehensive review on the applications, trends, and limitations of molecular markers in bee studies is lacking. We aimed to systematize the knowledge about microsatellite markers in bee research, characterize research trends, discuss their applications, and present their advantages and limitations to address major knowledge gaps across various research themes. Additionally, we aimed to establish a preliminary database of microsatellite primers and their transferability across related species. We conducted a systematic review of research articles on microsatellites and bees published until 2023. Our review included 576 articles from all biogeographical realms, which covered 173 species from 19 families. Apidae (94.1%) and Halictidae (3.4%) were the most frequent bee families in studies, with a strong dominance of *Apis* and *Bombus* species, followed by Meliponini species. Future research should include more solitary species and additional species from underrepresented tribes. The reviewed articles spanned 11 research themes, ranging from basic bee biology to applied and multi-disciplinary research, with reproduction, conservation, behavior, evolution, breeding, and beekeeping being the most frequent themes. Microsatellite markers are a suitable choice for most research themes and show a promising trend for continued use in future bee studies.

1. Introduction

Bees play a crucial role in natural and human-dominated ecosystems, as they exhibit a wide range of morphological, phylogenetic, and behavioral diversity, with over 20,000 species currently described globally (Packer 2023). Pollination by bees is an important ecosystem service, contributing to plant reproduction in both anthropogenic and natural habitats (Klein et al. 2007). Approximately 35% of global agricultural production depends on diverse communities of bees or key pollinators (Klein et al. 2007; Potts et al. 2016). Consequently, the decline in the functional and taxonomic diversity of bees can disrupt pollination networks, leading to cascading effects on plant communities and food webs, resulting in losses in global food production (Potts et al. 2016; Lemanski et al. 2022).

Understanding bees' biological traits, ecology, behavior, and evolution requires integrating classical and modern scientific methods. For example, combining genetic methods with demographic and reproductive studies allows for a more robust assessment of population dynamics and persistence (Lowe and Allendorf 2010). Genetic data combined with phenotypic, demographic, and spatial data are valuable tools for addressing complex ecological questions and planning effective management of these organisms (Deyoung and Honeycutt 2005).

Among the genetic markers currently in use in bee studies (Online Resource 1), microsatellites, also known as Simple Sequence Repeats (SSRs; Tautz 1989), and here on SSR markers, are particularly noteworthy. They are a class of repetitive DNA of simple sequences (≤ 10 nucleotides) (e.g., CT) repeated directly adjacent to each other (up to a few 100 nucleotides in length) and abundantly (hundreds to millions) scattered throughout the genomes, mainly of eukaryotes (Tautz 1989; Li et al. 2002). Their high variability and the presence in nonfunctional genomic regions make them majorly neutral markers, i.e., not under natural selection pressure, which is a requirement for several analyses in population genetics, such as ancestry, effective population size, migration rates, genetic drift, gene flow, and dispersal (Selkoe and Toonen 2006). Because of the higher mutation rates of repetitive sequences (10^{-2} to 10^{-6} per generation, Li et al. 2002) compared to point mutations, they are highly polymorphic, increasing their discriminatory power in identifying genetic variation over other molecular markers. They are also codominant (i.e., heterozygote genotypes can be inferred), abundant over the genome, and can be genotyped in automated systems (Grover and Sharma 2016). SSR markers require low quantities of DNA, the target regions are usually short fragments (100 to 300 bp), and samples with potentially degraded DNA, such as museum specimens, can be used.

Compared to genomic molecular markers, more specifically SNPs (Single Nucleotide Polymorphism), SSR markers have lower coverage of genomic variation, are less suited for studies on adaptation, have less discriminant power if genetic differences are low, and demand more extensive laboratory work. In contrast, genotyping SSR markers is more cost-effective than genomic markers (Nielsen et al. 2020). These attributes make them useful for a wide range of investigations at the individual level (e.g., individual discrimination), colony level (e.g., sociality, reproduction pattern studies), population level (e.g., demographic, dispersal), and species level (e.g., species delimitation, speciation) (Selkoe and Toonen 2006; Nielsen et al. 2020). One important limitation of the widespread use of SSR markers is the need for prior knowledge of SSR sequences and flanking DNA regions to design taxon-specific primers, which has associated costs and time requirements. To address this issue, ongoing research aims to overcome the shortage of primers for non-model organisms by transferring markers across taxonomically related species (Selkoe and Toonen 2006).

Consulting existing SSR markers developed for a related species may also be challenging due to their scattered distribution in the literature. Moreover, many researchers are unaware of the potential applications of molecular markers and their full utility to advance our understanding of the ecological, behavioral, functional, and taxonomic diversity of species to support their management in natural and anthropized ecosystems. Some reviews have already discussed the application of molecular markers in different contexts. For example, in the early 2000s, articles described trends and perspectives of molecular marker use for insect studies (Behura 2006) and stingless bee studies (Arias et al. 2006). More recently, reviews discussed the applications of genomic tools for insect studies (Singh et al. 2017) and mitochondrial markers for insect ecology (Dong et al. 2021). However, there is no thorough research on molecular markers applications, trends, and limitations in bee studies.

We aimed to systematize the knowledge about SSR markers in bee studies, characterize research trends, discuss their application, and present their advantages and limitations to mitigate the main knowledge gaps in several research themes. To characterize the research trends, we described: (i) temporal trends of publications; (ii) regional distribution of publications and species studied; and (iii) taxonomic representation in the studies. We also compiled all publications on the development and characterization of SSR primers, along with their transferability across related species to establish a preliminary database for future bee studies.

2. Materials and Methods

2.1. Literature search

From December 2022 to January 2024, we searched for scientific articles on the identification and characterization of SSR markers used in bee research and articles describing studies using SSR markers as tools. The search was performed in Web of Science and Google Scholar using as keywords: “bee” AND “microsatellite” OR “SSR” OR “characterization of microsatellites” OR “isolation and characterization of microsatellites” OR “microsatellites developed for”. All the 1780 occurrences (780 retrieved in Web of Science and the first 1000 from Google Scholar) were evaluated. Complementary searches were also conducted in Microsoft Academic and The National Center for Biotechnology Information (NCBI) databases, as well as in the listed literature (Reference section) in the retrieved articles as sources to identify others.

2.2. Literature screening

The selection criteria for the inclusion of a retrieved article in our downstream assessment were being an original research article, being written in English, having at least one bee species as the study subject, and being published by December 2023. Therefore, dissertations, theses, conference abstracts, and review articles were not evaluated.

For the articles specifically on the development and characterization of SSR markers, we extracted the target species, the bee sampling sites, the number of polymorphic loci analyzed, and information on the transferability of the primers to other species. We complemented with data on the species' social organization and the degree of the species' threat.

For all articles, we extracted the following: bee taxonomy, year of publication, other molecular markers used, keywords, purpose of using SSR markers, and biogeographic realm where the study was performed. We grouped synonym species and used only the valid names according to ITIS (2024) (Online Resource 2). We complemented this data with worldwide bee species distribution and their native biogeographic realms. For the division of the biogeographic realms, we considered the six established by (Wallace 1876): Palearctic (Europe, North Africa, and most of Asia); Nearctic (most of North America, Greenland, and the high Arctic islands); Neotropical (South and Central America, and southern Mexico); Ethiopian or Afrotropical (sub-Saharan Africa and Madagascar); Oriental or Indo-Malayan (Southeastern Asia and part of the Malay Archipelago); and Australasian (Australia, the remaining Malay Archipelago, and other Indo-Pacific islands). The sources for each species are listed in Online Resources 2 and 3.

We evaluated which taxa were more frequently studied, verifying the bee tribes and families using the traditional classification proposed by Michener (2007), as it reflects the extensive research on the evolutionary relationships and ecological roles of bees. The only exception was that we considered the tribe Andrenini for the genus *Andrena* in the subfamily Andreninae (Hymenoptera: Andrenidae) as proposed by Silveira and collaborators (2002), facilitating the standardization of the data for analysis. We identified the number of species described for each tribe in Packer (2023) to compare with the number of species studied using SSR markers.

The levels of the bee sociability were determined using three categories: 1) solitary: single adult female per nest, no cooperation in brood care, all females are reproductive and no morphological differences among them; 2) social: multiple adult females share the same composite nest, with or without cooperation in brood care, and with or without worker caste (foragers, explorers, feeders, scavengers, guardians, etc); 3) eusocial: multiple adult females share the same composite nest, with cooperation in brood care and worker caste, overlap in generation, and caste division (queen, worker, and drone) (Michener 1969).

To ascertain the degree of the bee species threat, we consulted the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species as a reference (IUCN 2024). We classified each article in 11 categories based on the purpose of use of SSR markers: Bee health, Behavior, Biological invasion, Breeding-bekeeping, Conservation, Evolution-Taxon delimitation, Gene flow, Landscape genetics, Method development and validation, Molecular Biology, Reproduction, Taxon identification, and Other. We defined the categories a posteriori, grouping the most common applications of SSR markers.

2.3. Literature search

We conducted quantitative analyses of temporal publication trends and the frequencies of studies on target species and tribes, occurrence locations, and bee collection sites using R (R Core Team 2023). We estimated the diversity of species studied in each biogeographic realm, identifying the total number of species (richness) and estimating Shannon's (Shannon 1948) and Simpson's (1-D; Simpson 1949) indices using the package Vegan (Dixon 2003) in R (R Core Team 2023). For articles on the development and characterization of markers, we evaluated the frequencies of transferability tests for each tribe.

3. Results and Discussion

3.1. Publication trends

3.1.1. Temporal trends of publications

The literature review retrieved 576 articles in total: 51 exclusively with marker development, validation, and transferability; 510 applying markers previously published to study bees; and 15 with the development and application of new markers. The first SSR primer-pair set for bees was published in 1993; since then, new sets have been published continuously (Fig. 1), with *primer* sets available for 56 species (Online Resource 4). From 1994 onwards, the number of publications involving SSR marker applications increased, peaking in the 2010s. Since 2018, the trend of the number of articles has stabilized (Fig. 1 and Online Resource 3).

In 2020, there was a sharp decline in publication rates (Fig. 1), mostly attributed to the SARS-CoV-2 viral outbreak, primarily due to the widespread disruption of research activities, shutdown of laboratories, and the reallocation of funding and resources towards pandemic-related studies (Korbel and Stegle 2020). These effects were unequal among researchers from Europe and the USA and their research areas but were more pronounced in studies in molecular biology (Myers et al. 2020).

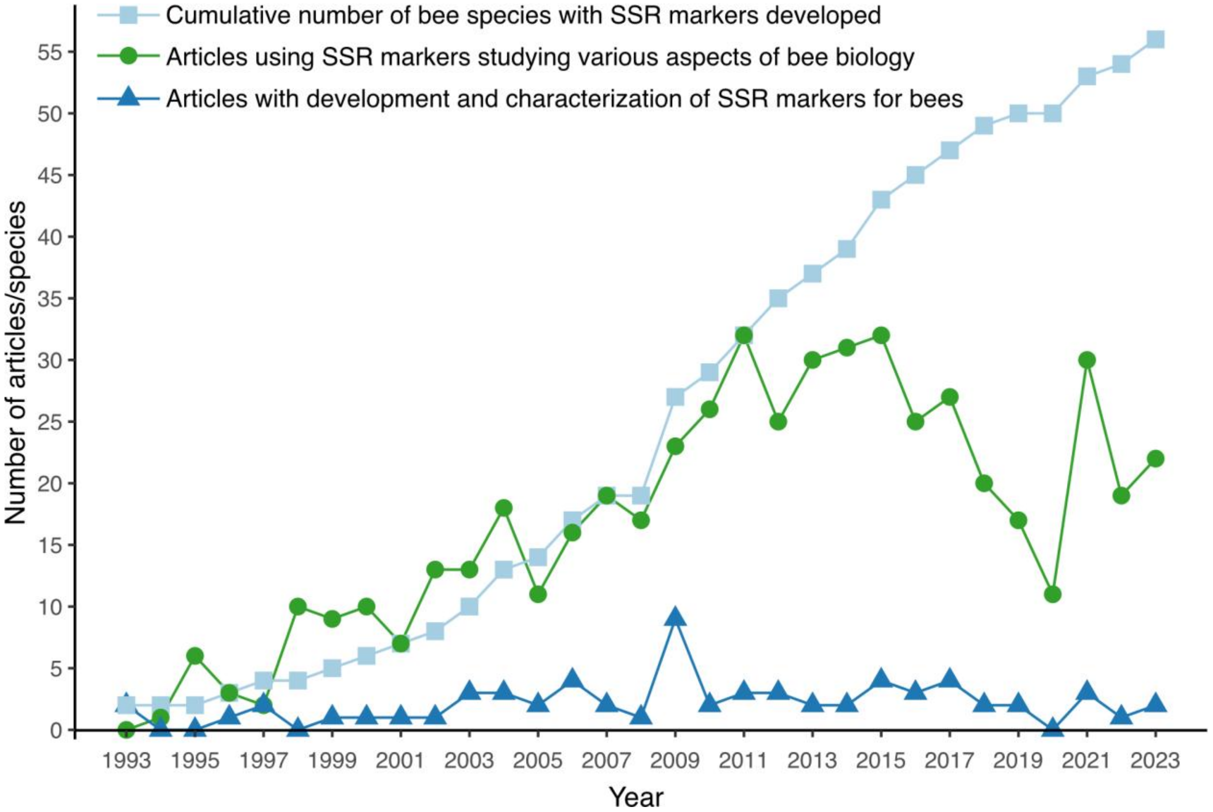

Fig. 1 Annual number of articles published on the use of SSR markers in bee studies (circles), on the development and characterization of SSR markers (triangles), and the cumulative number of bee species with SSR markers published (squares).

The overall trend indicates that SSR markers are still being used as tools even after the development of recent technologies, probably due to their versatility and effectiveness in providing insights into population studies and lower cost in comparison to more modern markers (Grover and Sharma 2016; Nielsen et al. 2020). Details on the possible applications and comparison with other molecular markers are discussed in the next sections. Also, considering that new primer sets are still being published, we expect to see new research results over the next years.

3.1.2. Regional distribution of publications and species studied

We retrieved studies with bees from all biogeographic realms, however, the number of studies conducted in each realm is highly heterogeneous (Fig 2, Table 1, and Online Resource 2). The biogeographic realm with the highest number of studies was the Palearctic (n = 250), followed by the Neotropical with almost 2.5 times less articles published (n = 102) (Fig. 2). However, the research conducted in the Neotropical realm showed the highest species diversity with the highest total number of species, all similarly represented (higher Shannon's and Simpson's diversity indexes). The Palearctic realm showed the second highest total number of species, but with a strong dominance of a few species (second lowest Simpson's diversity index; Table 1).

In the Palearctic realm, 97.8% of the articles focused on native bee species, reflecting a strong emphasis on local species. The higher number of studies conducted in the Palearctic realm with native species may be related to the native bee taxa, given that *Apis* and *Bombus* species occur naturally in the region and have been extensively studied using SSR markers, representing 72.8% of all studies, and 90.0% of studies conducted in the Palearctic region (ITIS 2024). For instance, 53.1% (n = 279) of all the studies included *Apis* species in their samples, half of them conducted in Palearctic realm with native species (27.2%, n = 143), 20% of all studies (n = 105) included *Bombus* species, 30.9% (n = 77) conducted in Palearctic realm with native species (Online Resources 2 and 3). This trend is expected as these two genera comprise 11 managed pollinator species, which represent most of the managed colonies worldwide (Osterman et al. 2021).

The Neotropical realm showed the highest number of bee species studied, mostly native (97.6%), corresponding to 42.1% of species studied across all six biogeographic realms (Fig. 2). Not surprisingly, the stingless bee species from the tribe Meliponini are the most extensively studied group in the Neotropics. Meliponini bees are not only important to the reproduction of native and cultivated plants but also hold significant economic and cultural value (Cortopassi-Laurino et al. 2006), representing the third most managed bee group worldwide (Osterman et al. 2021) and particularly in countries with extensive agricultural frontiers, such as Brazil (Wolowski et al. 2019).

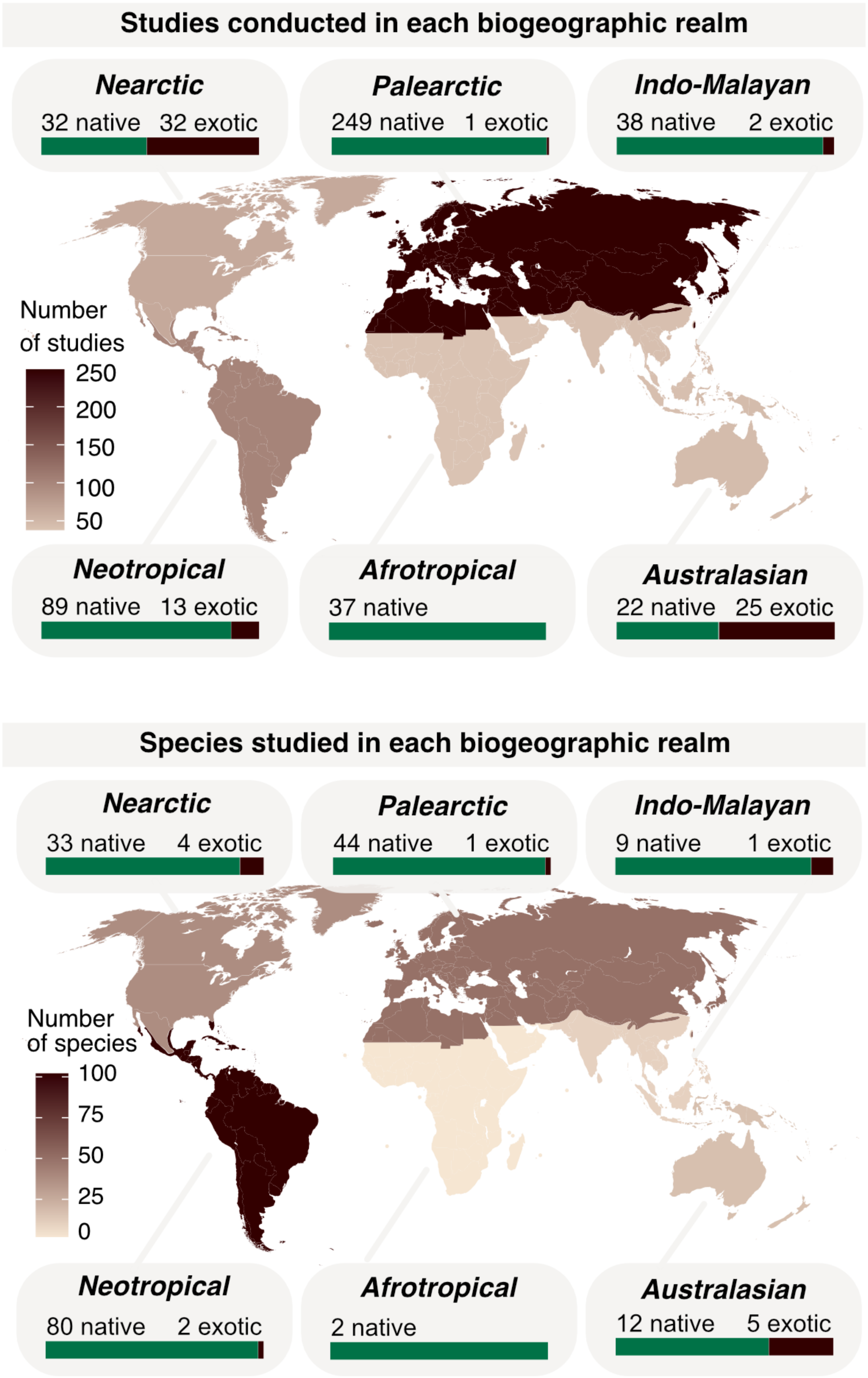

Fig. 2 Heat maps of the number of studies using SSR markers in the six biogeographic realms (Wallace 1876). For each realm, two sets of data are provided: on the top are the number of studies conducted in each realm and the proportion of these related to native or exotic species; on the *bottom* are the number of species studied in each realm and the proportion of these

related to native or exotic species. Articles that included species from more than one realm and both native and exotic species were counted more than once.

Nearctic and Australasian realms had the lowest percentages of articles focused on native bee species (Nearctic = 48.4%; Australasia = 46.8%) (Fig. 2). This pattern is a result of the large number of studies conducted on introduced or invasive species. *Apis mellifera* was introduced from both Palearctic and Afrotropical realms to pollinate crops and is intensively managed (Moritz et al. 1995). In the Nearctic realm, we observed 46.9% (n = 30) of studies involving this species, 17 related to bee breeding, beekeeping, and reproduction. In the Australasian realm, 49.0% of the studies (n = 23) involved *A. mellifera*, seven of them about bee health and reproduction and two about the origin of introduced colonies (Online Resource 3).

Table 1. Diversity indexes of species studied in each biogeographic realm.

	Richness	Shannon's diversity	Simpson's diversity (1-D)
Afrotropical	2	0.119	0.050
Australasian	18	2.186	0.810
Indo-Malayan	10	1.852	0.799
Nearctic	45	3.173	0.908
Neotropical	87	4.196	0.980
Palearctic	45	2.395	0.764

3.1.3. Taxonomic representation in the studies

We found primer sets published for only 56 species (Fig. 3, Online Resource 4), which represent less than 0,3% of the 20,000 bee species described (Packer 2023). However, the 576 articles included in this review described studies with 173 bee species (Online Resource 2). This was possible because primers can be transferred between related species, as long as the genomic region where they bind is conserved (Selkoe and Toonen 2006). Twenty-five sets were successfully tested for transferability to other bee species from the same genus; eight of them were also transferable to other genera from the same tribe. Meliponini was the tribe with the largest number of species with SSR primer sets developed (n = 18), of which 11 were successfully transferable within the tribe (Fig. 3, Online Resource 4). The use of primers transferred between species reduces the time and resources required for genetic analysis and provides a method of rapidly gathering genetic data on under-researched genera, thereby facilitating their inclusion in broader genetic and ecological studies (Selkoe and Toonen 2006). Nevertheless, this approach must be carefully applied, given that cross-species transfer success is highly variable (Barbará et al. 2007) with a decline in allelic diversity and the amplification success of primers observed for insects (Wright et al. 2004), and birds (Primmer et al. 1996).

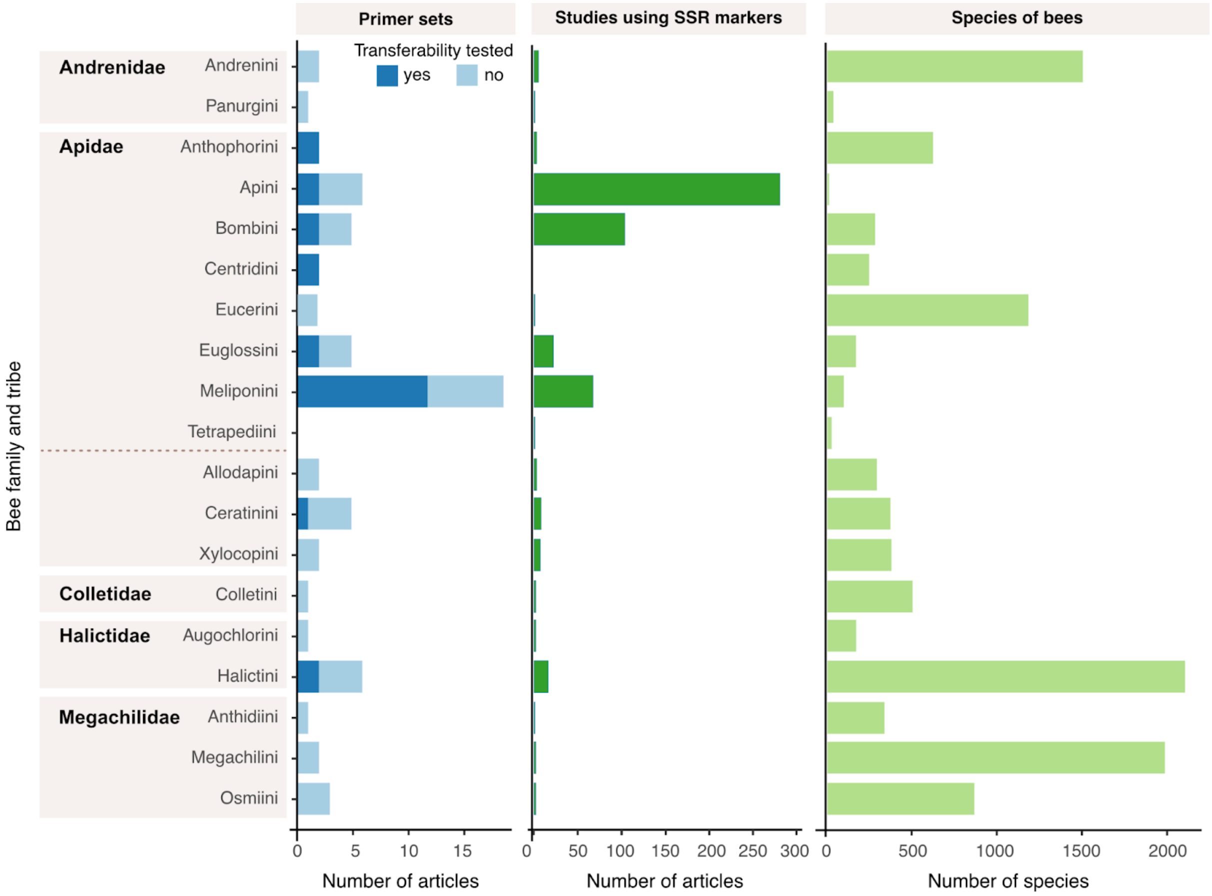

Fig. 3 Number of articles focused on the development, characterization, and transferability of primer sets for SSR markers grouped by bee tribe (*left*), the number of articles using SSR markers to study bees (*center*), and the number of species described from each tribe (*right*) (Packer 2023). Tribes from the Apidae family are grouped in Apinae (above the dotted line) and Xylocopinae (below the dotted line) subfamilies. *Dark blue* indicates that a transferability test was conducted. *Light blue* indicates the absence of a transferability test.

Most of the species with primer sets belonged to the families Apidae (n = 40), Halictidae (n = 6), and Megachilidae (n = 5). Apidae and Halictidae were also the most frequently observed bee families in studies using SSR markers, accounting for 94.1% and 3.4% of the application articles, respectively (Fig. 3 and Online Resource 3). This was expected, as these bee families are the most diverse in terms of the number of described species (Packer 2023) and they contain the most frequently managed species for pollination services (Osterman et al. 2021).

The family Apidae comprises almost 6,000 species, including well-studied bees, such as honeybees (Apini, n > 7) considered a model organism in genetic studies, bumblebees (Bombini, n > 280), stingless bees (Meliponini, n = 605), and orchid bees (Euglossini, n >168) (Packer 2023; Engel et al. 2023). Apini and Bombini were the tribes with the highest number of studies (n = 277 and n = 103, respectively; Fig. 3). Both are single-genus tribes, *Apis* and *Bombus,* with a cosmopolitan distribution (Michener 2007), which provides the opportunity to study the effects of the environmental gradients, geographic barriers, and human activities on the bee genetic variation and gene flow across diverse habitats (Lozier et al. 2013; Nagamitsu et al. 2016; Glück et al. 2022). Species from these tribes hold immense ecological and economic value through honey production and their use in assisted pollination, particularly species of *Bombus,* due to their efficiency in pollinating greenhouse crops and the possibility of artificially rearing colonies in bio factories (Velthuis and Van Doorn 2006). This dual importance in both natural and agricultural ecosystems can also explain the intense use of molecular markers in these two taxa. More specifically, SSR makers have been used since the 1990s to understand the biological features of natural and managed colonies and populations and to subsidize breeding, management, and conservation strategies (Fig. 4 and Online Resource 3).

The majority of studies within stingless bees (Meliponini), the third most frequently studied tribe, focused on the conservation, mating, and population characterization of the genera *Melipona* (n = 22) and *Scaptotrigona* (n = 17) (Fig. 4), with the latter having a higher amount of primer sets developed (*Melipona* – n = 9, and *Scaptotrigona* – n = 2). In addition to their role as pollinators, these species have considerable economic potential, especially for small farmers who engage in the production of honey and other bee-derived products (e.g., propolis, pollen, and cerumen) (Cortopassi-Laurino et al. 2006).

The family Halictidae (~ 3,500 species) also includes many common and widespread bees, such as the sweet bees (Halictini, n > 2,100; and Augochlorini, n ~169) (Packer 2023). Halictini, the fourth most studied group, includes a high number of species and a spectrum of intra and interspecific social organization, which makes them a good model to investigate the mechanisms underlying the transition from solitary living to eusociality (Yagi and Hasegawa 2012), as observed in seven articles in this review (Fig. 4 and Online Resource 3).

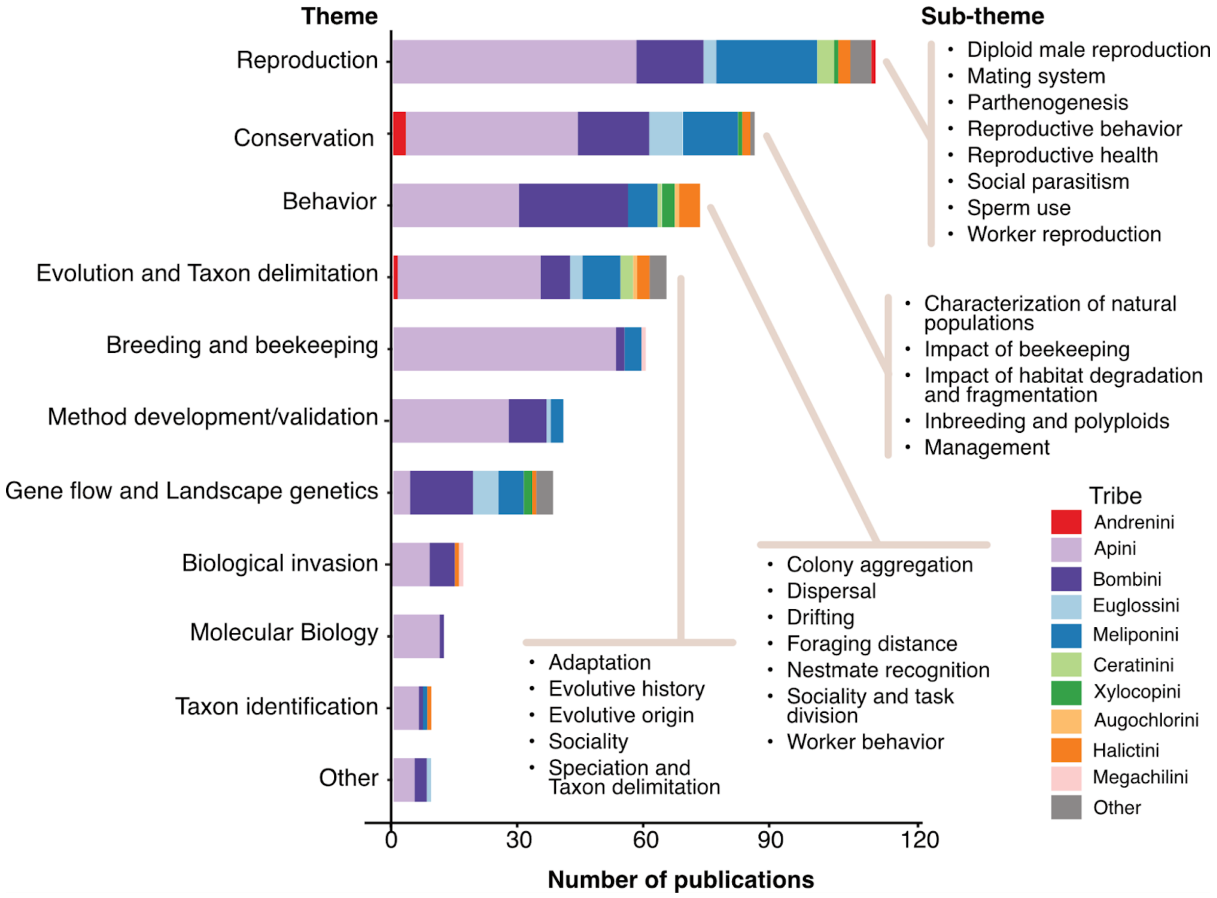

Fig. 4 Number of articles using SSR markers relative to the knowledge areas and bee tribes.

The search for the conservation status of bee species at the IUCN Red List indicated insufficient data for most species. Nine of the species were on the “least concern” list, two were shown as “data deficient” and for the other 45 species for which SSR markers were developed, there was no data available.

Regarding the trend for social organization, 12.1% of the species studied (n = 21) were solitary, although 90% of bee species are solitary (Michener 2007). Social (49.1%, n = 85) and eusocial species (31.2%, n = 54) were more frequently studied, and species with variable sociality were the least frequent (7.5%, n = 13). This discrepancy becomes more pronounced when assessing publication output, with only 5.3% of articles (n = 28) focusing on solitary species. Articles that included eusocial bees were the most frequent (66.8%; n = 325). This group is mainly represented by Apini and Meliponini species (Michener 2007), which are important crop pollinators and intensively managed (Osterman et al. 2021). Social species were present in 24.3% of the articles (n = 128), and articles on species with variable sociality were the least frequent (3.4%, n = 18), with more than half the research on topics related to social behavior (Online Resource 3).

3.2. Applied research themes

In this section, we describe the main applications of SSR markers in bee studies, highlighting some of the articles to illustrate their use. The summary of the most common themes and sub-themes studied is presented in Fig. 4, and the complete list of articles that apply SSR markers to bee studies is presented in Online Resource 3.

3.2.1. Reproduction

Reproduction was the most common theme (22%) among the articles in this review (Fig. 4), probably because elucidating reproductive aspects of a species is essential to understanding evolutionary history and ecological patterns, which are necessary for conservation and population or colony management planning (Adams and Tariq 2024). Also, SSR markers are more suitable for parentage and assignment studies, because they have high polymorphism and have a lower cost per individual compared to genomic tools (Grover and Sharma 2016).

Among social bees, the sociogenetic organization can vary from complete monogamy, as observed in Dawson's burrowing bee (*Amegilla dawsoni* Rayment, 1951) (Beveridge et al. 2006), to extreme polyandry, as observed *Apis dorsata* Fabricius, 1793 (Moritz et al. 1995), with also the possibility of polygyny, as in *Melipona bicolor* Lepeletier, 1836 (Reis et al. 2011). Genotyping workers, queens, and drones was used to identify matrilines and patrilines and infer the mating system (Owen and Whidden 2013), but the queen's genotype is usually inferred from workers' genotypes (DeFelice et al. 2015). The mating system is one of the most common topics studied using SSR markers. Estimates of mating frequencies were used to assess the temporal and geographic variation in polyandry (DeFelice et al. 2015), and the impact of the extreme environmental conditions observed in deserts in the queen's mating frequency (Alattal et al. 2021); to evaluate the effect of relatedness on female reproductive dominance in primitively eusocial species' colonies (Andrade et al. 2016), and to study female nest usage and the tendency to stay in or habitually return to its birthplace or home territory (Santos et al. 2020). Mating frequency is also among the traits assessed to examine the impact of pesticides on bee reproductive health (Milone and Tarpy 2021).

Although queens are responsible for most of the reproduction in a eusocial colony, some workers can have active ovaries and produce males from non-fecundated haploid ovules or females through thelytoky (parthenogenesis with female asexual reproduction). Thelytokously produced queens were identified by genotype comparison with workers' genotypes (Jordan et al. 2008), and their mating capacity can be verified by their progeny patriline identification (Beekman et al. 2011). Queens can also reproduce thelytokously. The frequency of mating and thelytoky (Mikát and Straka 2023), recombination rates, and the mode of gamete fusion (Oldroyd et al. 2008) were estimated by offspring genotyping. It is also possible to infer matriline and patriline from offspring genotypes, which was used to investigate queen-worker reproductive conflict (Tóth et al. 2008), to determine the relative contribution of different subfamilies in queenless colonies to male production (Martin et al. 2004), and to identify intraspecific worker social parasitism (Alves et al. 2009). Worker reproduction by thelytokous parthenogenesis in queenless (Holmes et al. 2015) and queenright colonies (Beekman et al. 2009) was detected by combining genotype information from worker and larvae.

Female bees can store sperm for fertilization throughout their reproductive period. The use of that sperm was investigated to test the sperm-limitation hypothesis, understand if there is any kind of female selection (Kraus et al. 2004), the last or late-mate precedence (Lampert et al. 2014), and sperm competition (Franck et al. 2002; Gençer and Kahya 2020). Stored sperm in spermatheca was used to identify sperm admixture (Franck et al. 2002) and pre-zygotic reproductive barriers (Holmes et al. 2011) that contribute to the evolution of different lineages.

Some bee species have congregation areas or form a kind of drone aggregations, where drones and queens meet to mate. Some studies investigated these behaviors as strategies to reduce inbreeding (Darvill et al. 2007) and maximize genetic diversity (Mueller et al. 2012), which are especially important for species with diploid males and triploid females due to homozygosity in sex-determining loci. Evaluating the matrilines of drones from these aggregations was used to estimate colony number and density, and to calculate drone mating range (Dos Santos et al. 2016; Garcia Bulle Bueno et al. 2022). Also, researchers were able to track diploid male behaviors such as their presence in drone congregations (De Jesús May-Itzá et al. 2021).

Many other aspects of reproductive behavior can be assessed using molecular markers, such as understanding female mate choice criteria (Conrad et al. 2010), analyzing the effects of mating flight behavior on mating success (Simone-Finstrom and Tarpy 2018), identifying interspecific reproductive interference (Remnant et al. 2014), evaluating if the colony's sex ratio is controlled by the queen (Holland et al. 2013) or if it is a result of relatedness and policing of workers (Pennell and Field 2021), and to test what influences queen production (Oliveira et al. 2022).

3.2.2. Conservation

The second most common research topic was conservation (16%), particularly characterizing bee populations in both natural and anthropogenic habitats and evaluating the impact of beekeeping on natural populations (Fig. 4).

Many studies aimed to characterize natural populations as basic research, without direct application of the results. For example, the characterization of *Eufriesea violacea* Blanchard, 1840 populations from natural remnants of tropical rainforests revealed that, despite high genetic diversity across all populations, the gene flow is restricted among the forest remnants (Freiria et al. 2012). In urban areas, characterization of genetic diversity and structure of *Euglossa cordata* Linnaeus, 1758 indicated that the species is capable of long-distance dispersal in anthropic areas, but they detected diploid males, an indication of inbreeding (Cerântola et al. 2011). The analysis of inbreeding levels can indicate threatening risk and potential population viability problems as consequences of low genetic diversity and the presence of diploid males (Soro et al. 2017) and triploid females (Darvill et al. 2012) that commonly have low fertility or are sterile. This type of study is fundamental to understanding population patterns, which can be used to subsidize conservation projects. The current challenge is to use this basic knowledge to effectively guide spatial conservation decision-making (Nielsen et al. 2023).

The impact of habitat degradation and fragmentation on bee species was another common theme in conservation studies (Fig. 4). The use of DNA extracted from the tarsal segment of bumblebees (*Bombus vosnesenskii* Radoszkowski, 1862) and screened at SSR markers transferred from *Bombus terrestris* Linnaeus 1758 genome highlighted that contemporary human land use, rather than geographical, barriers is the primary cause of the genetic differentiation patterns observed in bumblebee populations (Jha 2015). The SSR markers diversity patterns also revealed the geographic origin of specialist solitary bees (*Andrena vaga* Panzer 1799, and *A. fuscipes* Kirby, 1802) and indicated that they remain sufficiently large to maintain a relatively high genetic diversity. However, these studies suggest that fragmentation of natural habitats accounts for a restricted gene flow in bee populations more than geographical distance, even for critically endangered species (Exeler et al. 2010). On the other hand, some abundant bee species show very different levels of gene flow in human-dominated landscapes, and body size isn't always a good proxy of dispersal (Suni and Brosi 2012), especially for highly mobile or managed species (Rattanawannee et al. 2012). These findings emphasized the importance of studying a wide range of species with different characteristics.

SSR markers can subsidize population viability analysis. They were used to estimate extinction risk in bees (Francisco et al. 2016), compare declining and stable populations (Lozier et al. 2011), and monitor genetic diversity over time (Maebe et al. 2019). They were also used to assess the male and female contributions to gene flow and the vulnerability of island populations to extinction by analyzing bee social populations through mitochondrial gene sequencing and SSR genotyping (Francisco et al. 2016). A temporal assessment of genetic diversity in populations of *Bombus pauloensis* Friese, 1912 using museum specimens genotyped with SSR markers revealed a location-specific loss of genetic diversity over time in South America, highlighting the importance of prioritizing local conservation and management efforts across the full distribution range of Neotropical bumblebees in general (Maebe et al. 2019). Such findings contrast with the low representativeness of bees and other insects in the IUCN Red List, and the gaps in effective policies for insect conservation in the Neotropics (Duffus et al. 2023). Therefore, SSR markers may be a valuable tool for rapidly assessing population status to support species inclusion in the IUCN Red List and for long-term monitoring.

Beekeeping activities of exotic and native species, such as species or subspecies introduction and hive movement, can threaten native and feral populations. For example, several stingless bees (*Melipona*, *Nannotrigona,* and *Plebeia* species) are being traded in Brazil outside their native ranges, possibly outcompeting local bee populations due to dominance effects (Dos Santos et al. 2022). The impact of those activities was inferred by investigating genetic diversity, structure, and introgression levels in natural populations (Chapman et al. 2018), by estimating hybridization rates between native and introduced nests (Paul et al. 2023), and by inferring colony densities of populations in areas influenced by beekeeping (Sánchez-Guillén et al. 2018). Furthermore, the health of bees and the colonies' persistence are topics associated with conservation and pollination service studies. Researchers investigated the correlation of different genetic parameters with health and immunity, such as individual heterozygosity and innate immune response (Lee et al. 2013); genetic diversity and parasite load (Baer and Schmid-Hempel 1999); colony density and viral prevalence (Forfert et al. 2016); and patriline and bacterial resistance (Ameline et al. 2023). Consequently, this information can be used to test the efficacy of management and conservation strategies for bee populations (see the subtopic "*Breeding and Beekeeping*" for more details).

3.2.3. Behavior

Implementing molecular techniques has expanded the understanding of behavioral aspects that are difficult to track with classical methods, such as dispersal patterns. One method to infer dispersal distance is to estimate inter-colony relatedness and colony density, identifying queen genotypes and worker sibship, or identifying drone genotypes and estimating their relatedness. This was used to understand the dispersal behavior of queens (Dreier et al. 2014) and to infer foraging distance (Crowther et al. 2019). It is possible to correlate landscape features with colony densities to understand bees' preferences and dispersal barriers. This was used to evaluate if colony numbers were sufficient to provide pollination service to crops in a region (McGrady et al. 2021), to plan for landscape management necessary to increase pollination services (Carvell et al. 2012), and for conservation and management strategies (Conflitti et al. 2022).

Nest-related behavior studies can also benefit from SSR marker use. Queen turnover and gene flow among colonies were inferred by assigning haplotypes from males sampled in drone congregation areas and evaluating nests' genetic diversity and structure (Jaffé et al. 2009). The effect of population density on reproductive female dispersal was evaluated by estimating nestmate relatedness (Vickruck and Richards 2021). In facultatively social species, researchers studied dispersal, nesting, and group formation behaviors by estimating the relatedness of nestmates and non-nestmates (Ostwald et al. 2021). To understand home-site fidelity in seasonal migratory species, queens' genotypes were inferred from workers' genotypes, analyzing nests from various locations over time. They observed that queens migrated seasonally between specific nests over the years (Neumann et al. 2000). Drifting workers (Stephens et al. 2017) and drifters brood (O'Connor et al. 2013) were identified in a colony and at colony aggregations by reconstructing the queen genotype and estimating colony sibship. Identifying drifters (Pradella et al. 2015) and relatedness (Vickruck and Richards 2017b) were used to evaluate how bees recognize nestmates and drifters (Pradella et al. 2015). To identify colony invaders and study nest usurpation within and between species (Lau et al. 2022), and defensive behaviors in invasion events (Gloag et al. 2008), researchers used individual assignment methods at colony and species levels.

SSR markers can be used to investigate intra- and inter-nest social behaviors. For example, researchers evaluated whether genetic factors determine social aspects or if they result from phenotypic plasticity by performing a nest transplant experiment and by estimating transplanted brood relatedness and population of origin using SSR markers. They observed that broods from eusocial bees transplanted to an environment with solitary predominance also showed sociality characteristics (Davison and Field 2018). To evaluate *Halictus scabiosae* Rossi, 1790 reproductive females' inter-nest movement and replacement, they inferred the relatedness of colony members' and brood matrilines and patrilines. In this study, DNA was obtained from the tip of one tarsus using a non-lethal method (Brand and Chapuisat 2016). By identifying individual matriline and patriline, it also possible to investigate maternal manipulation aspects in social plasticity (Kapheim et al. 2011), task specialization (Kryger et al. 2000) and intra-nest genetic structure (Meixner and Moritz 2004), aspects of reproductive dynamics, such as queen-worker conflict in male production (Huth-Schwarz et al. 2011), worker reproduction-related behaviors like policing and nepotism in larval rearing (Nanork et al. 2011), queen execution following diploid male production (Vollet-Neto et al. 2017) and reproductive plasticity in primitively eusocial species (Price and Field 2022).

Many other behavior studies can be complemented with SSR marker data, such as using patriline identification of each group to analyze the trade-off between appetitive and aversive capacities (Brito et al. 2014), biparental care (Mikát et al. 2019), dance communication (Arnold et al. 2002), shift work organization (Kraus et al. 2011), and defensive response (Lenoir et al. 2006). Furthermore, kinship analysis can be used to study aversive learning performances (Junca et al. 2014), aggressiveness (Leonhardt et al. 2011), parasite transmission (Mallon and Schmid-Hempel 2004), and seasonal activity (Hart et al. 2021).

3.2.4. Breeding and Beekeeping

Bees have a profound impact on agriculture, so managing bee colonies can increase pollination in regions with low pollinator diversity and abundance in protected cultivation. *Apis mellifera* and *A. cerana* colonies have been managed for centuries, and more recently, a wide range of taxa can also be managed, such as bumble bees (*Bombus*), stingless bees (Meliponini), and solitary bees (*Megachile* and *Osmia*) (Potts et al. 2016). So, by investing in breeding programs, we can increase species resilience to respond to threats associated with climate change, such as more extreme climatic events, shifts in seasonal patterns, and increases in pest impacts, pesticide use, negative mite impacts, introgression, and hybridization with trade colonies. With research in beekeeping and genetic characterization of managed colonies, we can improve colony health and trade policies (Neumann and Straub 2023, Willcox et al. 2023).

To increase colony quality and longevity and assess the potential of pollination service provision, it is useful to understand the genetic diversity both in stock populations, that are managed populations bred and maintained by beekeepers for various purposes, such as honey production, pollination services, and conservation (Rattanawannee et al. 2020), and natural populations (Rahimi et al. 2023). Identifying the association between genetic markers and phenotypic features can also improve colony management. For traits that have complex inheritance patterns, it is possible to develop association studies between overall genetic diversity and the phenotype, for example, colony health and productivity (Tarpy et al. 2013) or immunocompetence (López-Uribe et al. 2017); between patriline and the characteristic, such as mite resistance (Beaurepaire et al. 2019), or to identify quantitative trait loci (QTL - a set of loci that affect a quantitative trait) associated to the feature, for example, hygienic behavior (Oxley et al. 2010), overwintering success (Döke et al. 2019) and morphology (Maebe et al. 2015). For phenotypes with monogenic inheritance (genetic inheritance pattern of traits determined by a single gene) or epistatic patterns (interaction of genes from multiple loci that collectively influence a particular trait), studies focused on the association between specific molecular markers and disease resistance (Ostroverkhova 2020) or behaviors such as royal jelly production (Parpinelli et al. 2014).

Beekeeping practices, such as nest transport and division, can also be tested or improved by evaluating their effects on genetic diversity (Santiago et al. 2016), population differentiation (Cánovas et al. 2011), and colony health (Jara et al. 2020). It is also possible to test the efficacy of colony management strategies to reduce hybridization by identifying worker patrilines (Mortensen and Ellis 2016); test the success of introducing queen cells into queenright colonies by identifying offspring drones' matrilines (Holmes et al. 2023); and evaluate the consequences of prolonged breeding on population viability (Alves et al. 2011).

Considering the current and potential declines of honeybee populations (https://coloss.org/), cryopreservation can be used for the conservation of species and lineages and to guarantee pollination services. To do so, it is critical to correctly identify species and subspecies using both morphological and genetic data and to assess the genetic diversity of embryos and sperm deposited in biobanks (Gulov et al. 2023).

3.2.5. Biological invasion

The introduction of exotic species threatens native or naturalized species, and hybridization and introgression have the potential to change reproductive behavior or change native populations' persistence (Ottenburghs 2021). Population genetic structure and assignment analyses can be used to track hybridization and introgression levels between native or naturalized and introduced subspecies (McCann and McCormack 2024) and also to evaluate the potential of managed populations to become invasive (Kraus et al. 2011).

The founder population of invasive species can suffer from all the small population problems, i.e., genetic drift and inbreeding depression, with a decrease in genetic diversity and loss of adaptive alleles. Nevertheless, invasive populations can successfully reproduce, spread in the new environment, and outcompete native species, which is known as the genetic paradox of invasion (Allendorf and Lundquist 2003). By assessing the genetic diversity and structure of populations, researchers reconstructed biological invasion history and tested if the invasive population went through a strong bottleneck and if there was one or multiple invasion events (Brock et al. 2021). It is also possible to evaluate the success of invasive species by estimating genetic diversity, colony density, gene flow among established populations, inbreeding levels, and diploid male or triploid female presence (Nagamitsu and Yamagishi 2009).

3.2.6. Evolution and Taxon Delimitation

SSR markers can be used to understand evolution and phylogeny by evaluating the effects of microevolutionary forces on populations with genetic diversity and structure analyses (May-Itzá et al. 2019). The high variability observed in SSR regions allows individual identification and individual measures of relatedness to estimate gene flow and population boundaries (Galindo-Cardona et al. 2013). In bees, the natural selection of heterozygous genotypes (overdominance) was investigated using thelytokous lineages and genotyping of the sex-determining locus (Goudie et al. 2014).

In evolutionary studies, SSR markers are frequently combined with mitochondrial DNA sequences to evaluate phylogeographic patterns and understand the effect of historical and contemporary events on the distribution of diversity of the species. This combination of DNA sequences and SSR genotypes was used to describe geographic patterns of population divergence in *Bombus ephippiatus* Say, 1837 (Duennes et al. 2012), *Partamona rustica* Pedro and Camargo, 2003 (Miranda et al. 2016), and *Euglossa annectans* Dressler, 1982 (Frantine-Silva et al. 2023). This strategy was also used to investigate human historical impacts on the species distribution, such as crop-mediated range expansion (López-Uribe et al. 2016).

It is also possible to investigate the effect of genetic variation and phenotypic plasticity on speciation by associating molecular markers and morphometric studies (Gruber et al. 2013; Austin et al. 2022). As SSR markers require low quantities and integrity of DNA, it is possible to study changes in genetic diversity over generations and evaluate the impact of different events on the population history using museum specimens (Maebe et al. 2013), which can also be used to elaborate conservation plans.

Bees can be insightful models in studies of the Evolution of sociality. Species that show variation in social behavior with solitary and social females, as observed in many Halictidae and Apidae, were studied to investigate patterns of genetic relationship within colonies (Richards et al. 2005), to understand the fitness of social and solitary females (Yagi and Hasegawa 2012; Mikát and Straka 2023), to test for the transition from solitary to social behavior (Soro et al. 2010), and to investigate the conflict between queens and workers over male parentage through policing (Zanette et al. 2012). In these studies, SSR markers were used to estimate kinship, perform maternity tests, and identify matrilines and patrilines within colonies.

In social species, polyandry can increase colony genetic diversity, which can be associated with increased fitness. The evolution of polyandric behavior was investigated by assessing the costs and benefits of multiple nuptial flights and genotyping the offspring to determine the number of patrilines (Schlüns et al. 2005). Polyandric mating behavior was correlated to increased fungal parasite resistance and brood care (Delaplane et al. 2021) in studies that used SSR markers to identify worker patrilines and to estimate the number of queen mates.

Species delimitation should aim to identify independent evolutionary lineages and reconstruct historical processes of the lineages. Therefore, integrating DNA sequence, molecular markers, and morphological and ecological data will likely result in more accurate inferences of evolutionary history and taxon delimitation (Carstens et al. 2013). Researchers aiming to understand cryptic species (McKendrick et al. 2017), species complexes (Brito et al. 2014), and species with incipient speciation (Hurtado-Burillo et al. 2016) may benefit from such integration, as ecological or morphological boundaries are usually blurred. SSR markers can be applied in these studies to estimate intra- and interspecific genetic diversity, measure populations' structure and admixture levels, and identify hybrids. On the other hand, to identify adaptive genes and the effects of natural selection on genes and genomes, thousands of SNP markers or whole genome sequencing are usually required (Nielsen et al. 2020).

3.2.7. Taxon identification

At species and higher levels, morphological features are more commonly used to identify the taxon, but at lower levels, other tools may be necessary. SSR markers have been used to identify subspecies and populations by identifying group-specific alleles (Ostroverkhova et al. 2018), by developing marker panels (Ostroverkhova and Konusova 2022), or by group differentiation analysis (Tavares et al. 2013). Sometimes it is impossible to identify taxa using

SSR markers (Eimanifar et al. 2020), but other markers, such as mitochondrial DNA sequences, may also be useful (Cejas et al. 2018).

The precise number of stingless bee species is difficult to ascertain due to cryptic species, which differ from their relatives by subtle and often undetectable morphological characteristics (Michener 2007). In this context, SSR markers can facilitate the discrimination between stingless bee populations (Brito et al. 2014), even when observable morphological traits fail to provide unambiguous evidence of taxonomic differentiation (Tavares et al. 2013). So, molecular analyses and traditional taxonomy - based on morphological and behavioral characteristics - are complementary approaches. It is crucial to continue employing both methods to define species boundaries, as both approaches are not free of problems (Padial and De La Riva 2021). By integrating morphological analyses with genetic data, researchers can achieve a more comprehensive and robust taxonomy (Quezada-Euán et al. 2007), thereby improving our understanding of biodiversity hotspots, where a high degree of endemism and species richness often coincides with significant conservation challenges.

3.2.8. Gene flow and Landscape genetics

Contemporary gene flow may be affected by factors operating at different spatial scales. To understand how landscape features impact gene flow, relevant data obtained through SSR markers can be utilized, such as natal dispersal estimating relatedness within and among colonies in an aggregation (Friedel et al. 2017). At a broad spatial scale, samples of populations in different habitat types were used to understand species dispersal ability through the landscape (Beveridge and Simmons 2006) or the influence of ecological factors in multiple scales (Paar et al. 2004). Information on gene flow can clarify dispersal patterns, such as the impact of fragmentation on connectivity (Hurtado-Burillo et al. 2016), and male congregations' contribution to population homogenization (Kraus et al. 2008).

Quantifying the effects of landscape features on microevolutionary processes, such as gene flow and genetic drift can contribute to our understanding of evolutionary history and viability, which were used to elaborate landscape management plans to protect and connect natural populations, increasing the potential for pollination services (Jha 2015; Moreira et al. 2015; Pfeiffer et al. 2019), for conservation purposes (Vickruck and Richards 2017a; Martins et al. 2023), and to elucidate taxon delimitation (Lozier et al. 2013). Bees' contemporary genetic diversity and structure have already been explained by current geographic distribution (Suni 2017), natural barriers (Moreira et al. 2015; Da Conceição Lazarino et al. 2023), environmental conditions (Glück et al. 2022), land use and cover patterns (Nagamitsu et al. 2016; Ballare and

Jha 2021), or a combination of multiple factors (Vickruck and Richards 2017a; Pfeiffer et al. 2019). Also, historic niche distribution and stability were evaluated, which helped explain current patterns of genetic diversity distribution (Koch et al. 2018). Therefore, SSR markers may be a valuable and complementary tool for understanding the mechanisms underlying species distribution in heterogeneous landscapes and better subsiding management plans for bee species. Such data may be incorporated with ecological data on resource availability in habitat patches to understand the functional role of these habitats for bees.

3.2.9. Method development and validation

Some articles presented or validated sampling, laboratory, or analysis methods, and used SSR markers to verify the method's efficacy or as the data source for analysis. Museum collections can be a source of material for studies that require ancient samples, but long storage time, handling, and storage conditions can degrade DNA to a point that can affect amplification success. It is also important to use a DNA isolation technique that yields a sufficient amount of DNA for the analysis (Strange et al. 2009). Different researchers developed techniques to isolate DNA from different tissues with different levels of impact. Non-invasive samples can be obtained from larval and pupal exuviae (Su et al. 2007) and faeces (Scriven et al. 2013). Non-lethal or non-destructive samples may come from antennae (Oi et al. 2013), leg tarsus (Hohenlohe et al. 2021), wings (Châline et al. 2004), and stings (Williamson et al. 2019). For reproductive studies, it is possible to obtain DNA from the spermatheca (Moškrič et al. 2023).

When SSR markers were a novelty, target fragment size homoplasy and the mutation process were investigated (Estoup et al. 1995; Viard et al. 1998). Since then, many analysis methods development have been described: estimating colony density from trapped drones (Williamson et al. 2022) or from workers (Nomura et al. 2021); hybrid detection (Excoffier et al. 2005); male ploidy detection (Bortolotti et al. 2022; Nomura and Taniguchi 2023); phenotypic plasticity detection (Radloff et al. 2002); taxon discrimination (Francis et al. 2014); nest-mate identification (Kokuvo et al. 2007); and comparison of morphometrics and molecular data to discriminate subspecies and lineages (Miguel et al. 2011; Oleksa and Tofilski 2015). Also, the association of SSR markers with the analysis of the complementary sex-determiner gene has been proposed to study genetic diversity, reproductive success, and social dynamics (Mroczek et al. 2023).

3.2.10. Molecular biology

Researchers can investigate the genetic bases of behavioral and other traits. However, they are being replaced by whole-genome association studies. Researchers inferred the genes associated with color patterns (Higgs et al. 2009), royal jelly production (Ostroverkhova et al. 2018), worker reproduction (Lattorff et al. 2007), and thelytokous reproduction (Chapman et al. 2015) by identifying offspring parental origins. By tracking parental origins with SSR markers and using complementary tools to investigate methylation patterns, such as bisulfite sequencing, it is possible to analyze imprinting occurrence (Remnant et al. 2016).

SSR markers were also used to construct linkage maps to study recombination rates (Meznar et al. 2010) and understand the organization of the genome (Gadau et al. 2001; Solignac et al. 2007).

3.3. Benefits, limitations, and perspectives of SSR markers

SSR markers have been widely used to evaluate bees' ecological, taxonomic, and evolutionary aspects and for conservation and breeding planning. Most of these applications are still valid, especially for kinship estimation, demographic patterns, taxonomic identification, and other applications that do not require the identification of specific genes or genomic regions (Lozier and Zayed 2017). Comparisons of SSR and SNP markers for genetic species identification and introgression rate analyses indicated that both generated similar overall results at the group level (Browne et al. 2021), but SNPs are more informative at an individual introgression level (Muñoz et al. 2017; Parejo et al. 2018). If DNA is available only in low concentrations or low quality, as in samples from small individuals, from non-lethal collection, from old collections, or that were exposed to conditions that degrade DNA (e.g., samples exposed to environmental conditions in traps), genomic analysis may not be feasible (Lozier and Zayed 2017). SSR markers are more suitable in these situations and can yield robust results, as observed in *Bombus* studies using non-lethal DNA sampling (Jha 2015).

Genomic tools are becoming more accessible with the development of new sequencing technologies, thus more commonly applied in studies of non-model species. Single Nucleotide Polymorphisms (SNPs) are molecular markers based on point mutations. They have lower mutation rates (~10^{-8} per nucleotide per generation) than SSR markers (10^{-2} to 10^{-6} per locus per generation), being more suited to evaluate historical events and less powerful at detecting the effects of the most recent events (Grover and Sharma 2016). SNPs also have fewer alleles per locus, so thousands of loci must be genotyped to have informative data. For this purpose, high-quality DNA at high concentrations is necessary to generate large amounts of sequence reads, which is expensive, and a reference genome is required, which has yet to be available to most species (Theissinger et al. 2023). Given that thousands of loci can be genotyped in a study, SNPs surpass genetic tools like SSR markers in detecting fine-scale or weak genetic structures (Lozier et al. 2016; Jaffé et al. 2019). They are also superior for studying functional and adaptive genetic variation, genetic load, and genomic regions associated with inbreeding depression, as well as for applying genome-editing tools (Lozier and Zayed 2017; Hohenlohe et al. 2021). However, for studies requiring large sample sizes, the cost of genomic tools remains prohibitively expensive for most research groups. In these cases, using SSR markers or a combination of genomic and genetic tools may be necessary (Hohenlohe et al. 2021).

The SSR-GBS method (simple sequence repeats - genotyping by sequencing of SSR amplicons), in which DNA SSR regions are amplified by PCR and then sequenced, takes advantage of the high levels of polymorphism in SSR markers and the greater amount of information obtained from next-generation sequencing (Vartia et al. 2016). It can detect variation in SSR regions and SNPs and reduce size homoplasy biases. This technique still has limitations, such as the higher cost when compared to SSR markers, fragment size detection, and the presence of stutter bands, but both may be overcome with current and future improvements in sequencing technologies (Šarhanová and collaborators 2018). This strategy was used to track the invasion history of *Megachile sculpturalis* Smith, 1853 from Asia to Europe (Lanner et al. 2021).

4. Conclusion

SSR markers are versatile and broadly used in bee studies, demonstrated by their successful, global application in 576 bee studies covering 173 species from 19 families, across 11 research themes, ranging from basic bee biology to applied and complex research. As most studies focused on only five tribes, with strong dominance of *Apis* and *Bombus* species, our understanding of bees would be significantly enhanced if future research included more solitary species and additional species from underrepresented tribes. SSR markers continue to be a suitable choice for most research themes, particularly those involving individual identification and parentage analysis, where only small quantities or poor quality of DNA are available, or where large sample sizes and financial constraints are factors. With the development of primer sets for more species, and considering their proven transferability among related species, SSR markers show a promising trend for continued use in future bee studies.

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6. Glossary

Genetic drift: the evolutionary mechanism that changes the allele frequencies within a population over generations because of a random sampling of gametes

Gene flow: the transfer of alleles among populations following the dispersal and subsequent reproduction of individuals, propagules, or gametes

Genotype: the set of alleles in a locus possessed by an individual

Ploidy: the number of complete sets of homologous chromosomes

Inbreeding: mating among relatives

Introgression: the movement of genes or alleles from one species or population to another following hybridization

Phenotype: property of an organism that results from a genotype
Phenotypic plasticity: the ability of a particular genotype to develop into one of several phenotypes, depending on environmental conditions

Point mutations: a type of genetic mutation where a single nucleotide base in the DNA sequence is altered (substitution, insertion, or deletion)

Polymorphic: a locus that has multiple alleles in a population or species

Polyandry: a mating system in which one female mates with multiple males

Polygyny: a mating system in which one male mates with multiple females

Primer: a short segment of single-stranded DNA or RNA that is used as a starting point for copying template sequences

Thelytoky: parthenogenesis with female asexual reproduction

7. References

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CHAPTER 3: Living locally, connecting regionally: Local abundance patterns and regional gene flow of the native bee *Exomalopsis analis* in Cerrado agroecosystems

Abstract

Understanding how landscape structure influences pollinator dispersal is essential for biodiversity conservation and the maintenance of ecosystem services in human-modified environments. In agricultural landscapes, conserving pollinators requires understanding their population dynamics and genetic connectivity, yet this remains poorly understood. The objective of this study was to characterize *Exomalopsis analis* populations' abundance and genetic structure to understand how landscape structure influences their dispersal and population connectivity. This bee species is an important pollinator of tomato crops in the Cerrado biodiversity hotspot. We sampled *E. analis*, quantified landscape composition and developed species-specific microsatellite markers to assess genetic structure. Bee abundance responded strongly to habitat composition at a restricted spatial scale. Savanna and urban cover positively influenced *E. analis* abundance, whereas forest and agricultural cover had negative effects. Genetic analyses revealed low overall genetic diversity but no evidence of population subdivision. All individuals formed a single genetic cluster, with no isolation-by-distance or isolation-by-resistance. Population graph analysis indicated high regional connectivity, with sites of greater betweenness generally located near natural vegetation, suggesting these remnants act as functional 'stepping stones' facilitating gene flow. *E. analis* populations are demographically sensitive to fine-scale habitat conditions yet maintain broad-scale genetic connectivity across the agricultural mosaic. Conserving natural vegetation patches within farming landscapes is therefore critical for sustaining local bee populations while ensuring the long-term genetic connectivity required for resilient pollination services in the Cerrado.

1. Introduction

Plant-pollinator interactions are fundamental for the reproduction of nearly 90% of all plant species (Ollerton et al. 2011), including many agricultural crops (BPBES and REBIP 2019). Wild bees are considered the most important pollinators, as they form a diverse group with complementary functional traits that enable the pollination of a wide range of crops (IPBES 2019; Noriega et al. 2018). However, environmental degradation and human-driven habitat modification threaten the long-term viability of wild bee populations by increasingly isolating them across the landscape, potentially impairing gene flow (Potts et al. 2010). Landscape-level processes, such as natural habitat loss and fragmentation, are critical factors for pollinator communities, as they affect species abundance and richness, and can interfere with the movement of organisms between different habitats (Kremen et al. 2007; Kennedy et al. 2013; Zeller et al. 2020; Togni et al. 2021). When landscape composition restricts individual movement, it can limit gene flow among bee populations, making them more susceptible to genetic drift, inbreeding, and overall loss of genetic diversity (Manel et al. 2003; Suni et al. 2014; Jaffé et al. 2019).

Landscapes dominated by large-scale agriculture must be functionally permeable to allow the dispersal of ecosystem service providers, such as pollinators, because of the resources provided by flowering crops (Tscharntke et al. 2012; Rosa et al. 2015; Machado et al. 2020). Beyond the floral resources within crops, the persistence of bee populations also requires access to nesting sites and alternative forage provided by adjacent natural vegetation (Requier and Leonhard 2020, Rahimi et al. 2022). For example, landscape structure (e.g., forest cover and habitat connectivity) explained patterns of genetic relatedness among native bees better than geographic distance alone (Jaffé et al. 2019). As populations become more isolated, they are more vulnerable to demographic fluctuations and environmental stochasticity due to small population sizes and increased inbreeding (Manel et al. 2003; Jha and Kremen 2013; Landaverde-González et al. 2017). Therefore, connectivity is a key factor in landscape genetics and genomics, as it helps explain how spatially isolated populations can be functionally linked. In fragmented landscapes, this is achieved through a mosaic of natural vegetation remnants that act as 'stepping stones' for dispersal (Da Rocha et al. 2021).

Several techniques can be used to measure connectivity among populations, including mark-recapture, satellite imagery, radar, and even direct observation (Kool et al. 2013). However, these methods are often unsuitable for assessing populations at large spatial scales, particularly for small-bodied organisms like insects (Kool et al. 2013). To overcome this challenge, genetic methods that measure the similarity between individuals using molecular markers can be employed. These approaches allow researchers to examine levels of isolation and track dispersal routes among populations distributed across broader spatial scales (Aledorf et al. 2012; Lozier et al. 2013; Balkenhol et al. 2015; Jaffé et al. 2019). Among available molecular markers, microsatellites (Simple Sequence Repeats, SSRs) are among the most widely used and versatile tools for ecological applications, including in bee studies (Assunção et al., 2025). This is because they are cost-effective, highly reproducible, and require only small amounts of DNA for analysis (Selkoe & Toonen 2006; Garrido-Cardenas et al. 2018). This method has been used to demonstrate that the genetic diversity of native bee populations can be maintained if functional connectivity exists among them (Rosa et al. 2016). This connectivity is primarily sustained by gene flow through high-quality forest fragments and a permeable landscape matrix (Rosa et al. 2015, 2016).

Strategies to conserve wild bee diversity and pollination services must consider landscape-scale functional connectivity and dispersal events (Allendorf 2017). This is particularly critical in agroecosystems, where biodiversity-driven ecosystem services are essential for long-term sustainable agriculture (FAO 2018; Dainese et al. 2019). Our study is set in the Cerrado, a global biodiversity hotspot and a major agricultural frontier in Brazil (Ratter et al. 1997; De Oliveira Santana and Simon 2022; Machado et al. 2024). Understanding how bee populations persist in these human-modified landscapes is thus a conservation priority. To address these issues, we focused on the native bee *Exomalopsis analis* and its host crop, the tomato (*Solanum lycopersicum* L.), as our model study system. In the Cerrado, tomato crops are visited by a high diversity of native bees, with at least 30 species performing pollination by vibrating the flower anthers (buzz-pollination) (Silva-Neto et al. 2017), among which *E. analis* is a key species in our study region (Assunção et al. 2022; Franceschinelli et al. 2017, 2023, 2024).

The aim of our study was to characterize the genetic structure of *E. analis* populations to understand how landscape structure influences its dispersal and population connectivity. We hypothesized that landscape composition would be the primary driver of population density and genetic structure in *E. analis*. Specifically, we predicted that simplified landscapes, such as those dominated by extensive monocultures or urban centers, would act as barriers, restricting gene flow and leading to detectable genetic differentiation among populations. We also hypothesized that these simplified landscapes would lead to reduced population abundance of *E. analis*, reflecting a poor capacity of the environment to support viable populations. Furthermore, we predicted that this reduced genetic connectivity and lower population size would result in lower genetic diversity within isolated populations, potentially increasing their vulnerability to these fragmented agroecosystems.

2. Material and Methods

2.1. Sample sites

We conducted the study in tomato fields across 26 farms in the Federal District (18 farms) (15°46′48″S, 47°55′45″W) and in the state of Goiás (8 farms) (16°40′00″S, 49°15′00″W), Brazil (Figure 1). Sampling was conducted during four periods. Only in the Federal District in May–October 2019, November 2020 and July–October 2022; and in the two locations in May–November 2023. The farms (1 km apart), which cultivated tomatoes and other vegetables in open fields, were located in the Cerrado biome within distinct landscape contexts. We selected farms at least 1 km apart.

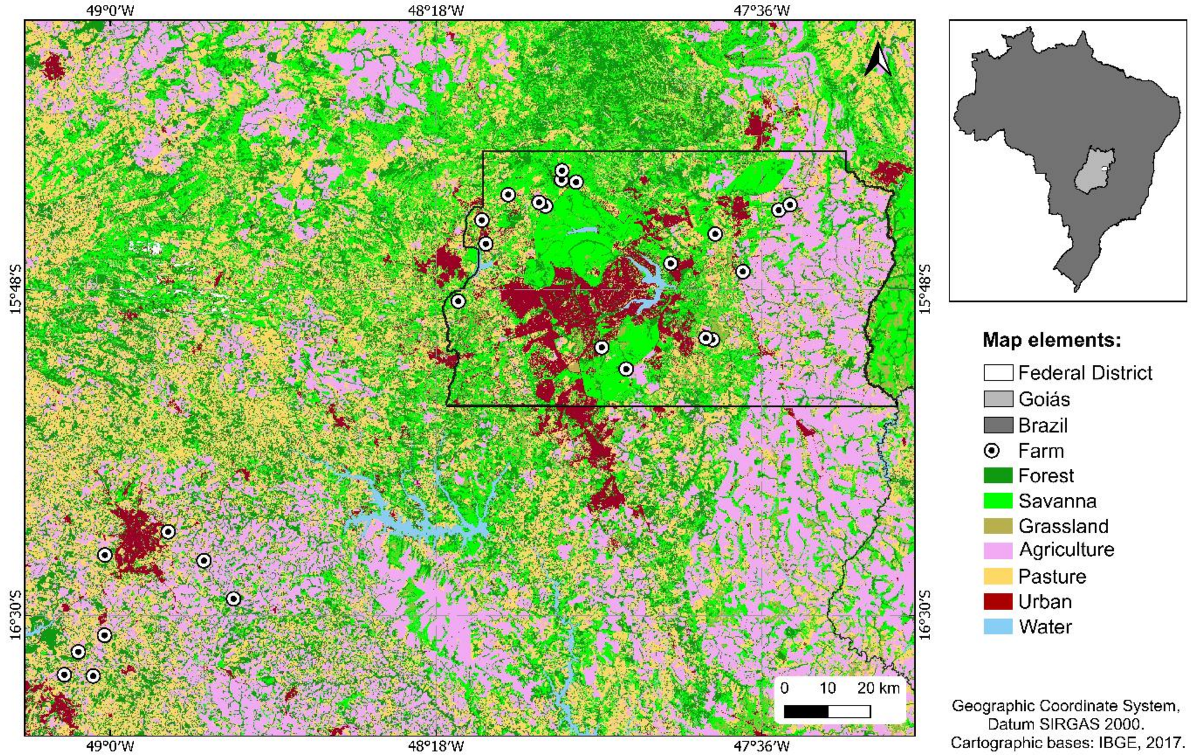

Figure 1. Land cover and geographic distribution of the 26 study farms across Federal District and Goiás, Brazil. The left map shows the landscape composition surrounding the farms, with land cover types: forest, savanna, grassland, agriculture, pasture, and urban areas. Brazilian Federal District and Goiás are showed in the central area of Brazil (up right map). We used the land cover map of the MapBiomas classification for 2023 (Souza et al., 2020).

To understand the influence of landscape structure on bee abundance and genetic structuring, we assessed the landscape composition surrounding the farms. We used the land cover and land use map of the MapBiomas Project Collection 2 land cover classification for 2023 (Souza et al., 2020). This map classifies Sentinel-2 satellite images in a matrixial map (raster format) with a spatial resolution of 10 m (pixels represent areas of 10 x 10 m) using only validated data. We calculated the proportional coverage area for each of the following land cover types: forest formation, savanna formation, grassland formation, agriculture, pasture, and urban areas (Figure 1, 2). Forest is dominated by continuous tree canopies (>80% cover), which typically occurs near river courses. Savanna features 20–70% tree and shrub cover, interspersed with a broad graminoid stratum. Grassland (semi-natural habitat) consists of a graminoid stratum and includes less than 5% tree and shrub cover (Ribeiro and Walter, 2008), but in our region, it also includes degraded areas with a high amount of exotic grasses and abandoned pastures. Agriculture includes areas with annual or perennial crops, often forming mosaics with other land uses. Pasture comprises areas dominated by herbaceous vegetation under grazing management, which may include scattered trees Urban areas are characterized by non-agricultural, human-made structures, including buildings and roads (Souza et al., 2020).

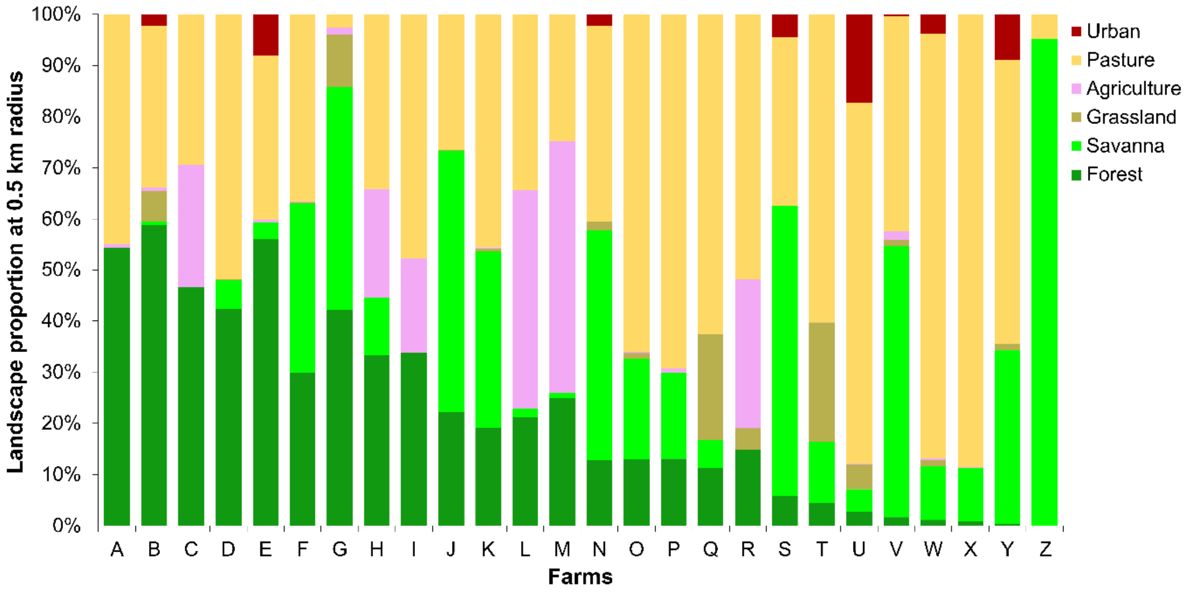

Figure 2. Landscape composition around tomato crops on 26 study farms across Federal District and Goiás, Brazil. The landscape proportion data reflects the coverage area of each land-use type within a 0.5 km radius buffer from the center of the cultivated area. Data refers to the land use map of the MapBiomas classification for 2023 (Souza et al. 2020).

The extent of the buffers used for landscape characterization was selected using the ‘Scalescape’ R package, which avoids problems related to a priori extent and multiple-buffer methods for selecting the extent of the characterized landscape (Lowe et al., 2022). In summary, Scalescape identifies the buffer size that improves model fit for each response variable (i.e., abundance data) weighted by the distance from the sampling point until there are no more detectable effects in the response variable, as well as the direction and magnitude of the landscape effect. Given our focus on a single bee species, we chose the Scalescape method to empirically identify the exact scale of effect for *E. analis*. This avoids assumptions about the species' dispersal range (as seen in chapter 1) and ensures that our landscape analysis is tailored to the specific ecological interactions of our target species with landscape features.

2.2. Bee sampling

To assess how landscape composition affects both the abundance and genetic structure of *E. analis*, we sampled individuals across selected tomato farms. We used a combination of two methods: active collection on flowers and pan traps to complement direct sampling. In the Federal District, we assessed bee visitation to both tomato and non-crop plants, whereas in Goiás, sampling was restricted exclusively to tomato flowers. For active bee collection, the samplers walked through the area (within 10 m radius of the tomato crop), actively collecting bees using plastic pots, from 9:00 am to 12:00 pm (peak of bee activity) during the flowering window of tomato crop. Only bees that landed directly on the flowers were collected. The daily sampling effort was quantified in minutes of collection and multiplied by the number of collectors. The sampling effort in the Federal District ranged from 9 to 20 hours per farm, totaling 305 hours. In Goiás, the sampling effort was 6 hours per farm, totaling 48 hours. The pan-trap sampling method was carried out exclusively on farms in the Federal District. For this, we used the model suggested by Garibaldi et al. (2021). We used only the blue color as it has been shown to be highly effective for capturing bees (Campbell & Hanula 2007). In each farm, traps were placed in three distinct locations: (1) within the tomato crop fields (PT1), (2) in the immediate surroundings, within a 10-meter radius of the crop edge (PT2), and (3) inside the nearest patch of natural vegetation (PT3). A total of 30 pan traps were deployed per farm, with 10 traps at each of the three locations. This approach aims to maximize the abundance sampling of the selected species. The collected bees were stored frozen (-20°C) until DNA extraction.

2.3. DNA extraction

For individuals sampled for DNA sequencing, we washed the bees' bodies with a 0.5% sodium hypochlorite solution to reduce the pollen and microbial load and removed their abdomen to avoid contamination with intestinal content. We then pulverized the bees' remaining bodily parts using a beadbeating machine (FastPrep® MP Biomedicals) with three 3 mm stainless steel spheres for 30 s, at 4 m/s, twice. We extracted the DNA used for sequencing using a variation of the CTAB extraction protocol with a pre-wash step using a buffered sorbitol solution (Inglis et al. 2018).

For samples used for population genetic analysis, we extracted DNA from the three legs from one side of the body. We pulverized the legs using the same beadbeating method and subsequently followed the Chelex protocol (Lienhard and Schäffer 2019) with incubation for 1 h for DNA extraction. All samples were stored in Tris-HCl solutions (10 mM, pH 8.0).

2.4. Primer set development

We sequenced the whole genomes of three *E. analis* females from one site (Farm D) to develop primer sets. One individual was selected for long-read sequencing (PacBio) with CLR library preparation to generate long reads, as there are no reference genomes for the species, while the other two were used for Illumina sequencing (150 bp pair-end). The combination of both sequencing methods provides a comprehensive and more accurate dataset at a reasonable cost-benefit. The comparison of sequences from the three individuals revealed variability in genomic regions, facilitating the identification of suitable primer sets for further analysis.

Quality control of the raw sequencing data from the three samples was performed using the FastQC v. 0.12.0 program (Andrews 2023). PacBio CRL sequencing generates sequence files without a quality value for each nucleotide; therefore, the LoRDEC v.0.9 program (Salmela 2014) was used to correct long-fragment sequences. Data obtained from Illumina sequencing were filtered using fastp v.0.23.4 software (Chen 2018) to remove adapters and low-quality reads. This data was mapped using bwa v.0.7.18 software (Li, Durbin 2010) using the filtered PacBio sequence set as a reference. Duplicate sequences were removed using Picard v.3.2 software (Broad Institute 2024). By comparing the mapped sequences of the three individuals, sequences containing deletion, duplication, or insertion variations were identified using Manta v.1.6 software (Chen et al. 2016). Finally, a manual curation of the sequences was performed to identify those that presented at least two distinct alleles in microsatellite regions in the analyzed individuals. Sequences containing di- and trinucleotide microsatellite regions with at least four motif repetitions were filtered using the GMATA v.2.3 program (Wang et al. 2016) to develop the primer sets. This approach increases the likelihood of amplification of polymorphic DNA regions.

Primers were designed using Primer 3 Plus (Untergasser et al. 2012), with target fragments ranging from 95 to 350 bp, a minimum GC content of 40%, and a melting temperature between 60 and 65°C. All designed primers were tested for hairpin, self-dimers, and heterodimers to ensure specificity and efficiency in PCR amplification using the OligoAnalyzer^{TM} tool (Owczarzy et al. 2008). We synthesized 10 primer pairs to amplify these microsatellite regions. For Polymerase Chain Reactions (PCR), we used a final concentration of 0.2 ng/µL template DNA, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.25 µM of each primer, and 1 unit of Taq polymerase in a 10 µL reaction volume. The amplification included an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55-60°C for 30 seconds, and extension at 72°C for 1 minute, then a final extension at 72°C for 5 minutes. The primer pairs that amplified fragments of the expected sizes were further tested for polymorphism. The polymorphic primers were synthesized with fluorescent dyes for automatic genotyping on an ABI 3730XL Platform to assess genetic diversity within *E. analis* populations. We included DNA from a single individual on all PCR plates as a positive control. This sample was also used to assess variations across genotyping runs.

2.5. Landscape analysis

We conducted a preliminary landscape analysis to evaluate the effect of landscape composition on *E. analis* abundance for all sampled farms. For this, we used the 'Scalescape' R package, which estimates distance-weighted landscape effects on a response variable using a previously specified null model (Lowe et al., 2022). The null models consisted of generalized linear mixed-effect models (GLMMs) using a negative binomial distribution, with an intercept-only effect on *E. analis* abundance, and the farm as a random variable. For the landscape model, 'Scalescape' identifies: i) the buffer range that improves model fit, ii) the weight loss of landscape predictors as the distance increases, and iii) the direction and magnitude of the landscape effect. Landscape GLMMs were built for each predictor variable, starting from the null model and differing in the included landscape predictors. The landscape predictor was the proportion of each land cover type (forest formation, savanna formation, grassland formation, agriculture, pasture, and urban areas).

2.6. Genetic analysis

For the genetic analysis, we established population clusters by grouping farms. The primary criterion for grouping was geographic proximity, with farms located within a 3 km radius of each other. This approach assumes potential gene flow among nearby sites. Additionally, farms for which DNA extraction was unsuccessful were excluded from subsequent genetic analyses, a common issue resulting from factors like sample degradation or low DNA quality. Then, we estimated: allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity under Hardy-Weinberg equilibrium (H_E), and fixation index (F_{IS}) as an estimator of inbreeding (Weir & Cockerman 1984) using the Hierfstat package (Goudet 2005) in R (R Core Team 2013). Genetic differentiation between these groups was estimated using the fixation index (F_{ST}) (Weir & Cockerman 1984), and discriminant analysis of principal components (DAPC) was performed on all collected samples using the Hierfstat (Goudet 2005) and Adegenet (Jombart 2008) packages, respectively.

We also estimated pairwise Nei's genetic distance (Nei 1972) and the conditional genetic distance (cGD), which is the distance through a population graph topology, measured based on the conditional genetic covariance of the entire data set (Dyer et al. 2010). Therefore, cGD is a measure of genetic distance that considers the allele sharing among the entire set of populations studied, revealing how genetically related populations are within the context of all others. These estimates were used to test for isolation-by-distance, using regression analysis to examine the relationship between geographic distance and each genetic distance estimate. We also used cGD to plot a population graph, with node sizes representing populations' betweenness and edges indicating genetic connectivity among them. In these case, high betweenness values represent key populations for genetic connectivity (central links). These analyses were performed using the gstudio package (Dyer 2014). This approach helps visualize genetic relationships and assess the impact of geographic barriers on gene flow.

3. Results

Across the 26 farms included in this study, we recorded a total of 660 *E. analis* individuals (Table S1). Among them, 481 species were selected for genetic analyses. Although the different land cover types had contrasting effects on *E. analis* abundance in the Federal District, the spatial scale at which these effects operated was highly consistent, peaking at a radius of 0.02 km for most classes (Figure 3). The only exception was grassland and pasture cover, where the strongest effect occurred at 0.03 km radius (Figure 3). Savanna cover positively influenced *E. analis* abundance, while forest cover had a negative effect (Figure 3a,c). About the anthropic landscape composition, agricultural cover negatively affected *E. analis* abundance, while urban cover had a positive effect (Figure 3d,f). Grassland and pasture cover showed no significant effect on *E. analis* abundance (Figure 3b,e).

The long read sequencing yielded 854,350 fragments (5.9 Gbp). Samples sequenced in short fragments (Illumina) generated approximately 40 million sequenced fragments (5.8 Gbp), with sequencing error rates of 0.03%, and most errors occurring in expected regions (primer binding regions and fragment terminal positions). This amount of data was sufficient to generate long, high-quality contigs for primer design of microsatellite markers, but the sequencing did not provide sufficient genome coverage for the assembly of a reference genome.

From the 120 sequences containing microsatellite regions and the polymorphisms detected among individuals with sequenced DNA, 16 primer pairs were designed that met the quality criteria described in the methodology. We selected the 10 primer pairs with the highest quality and best potential for forming multiplex sets for synthesis and validation (Table S2). Six of the ten primer pairs amplified DNA fragments of the expected size; one amplified non-specific DNA fragments (Ean 5); and the other three did not yield consistent amplification.

Primer sequences, amplification conditions, and other related information are provided in the supplementary material (Table S2).

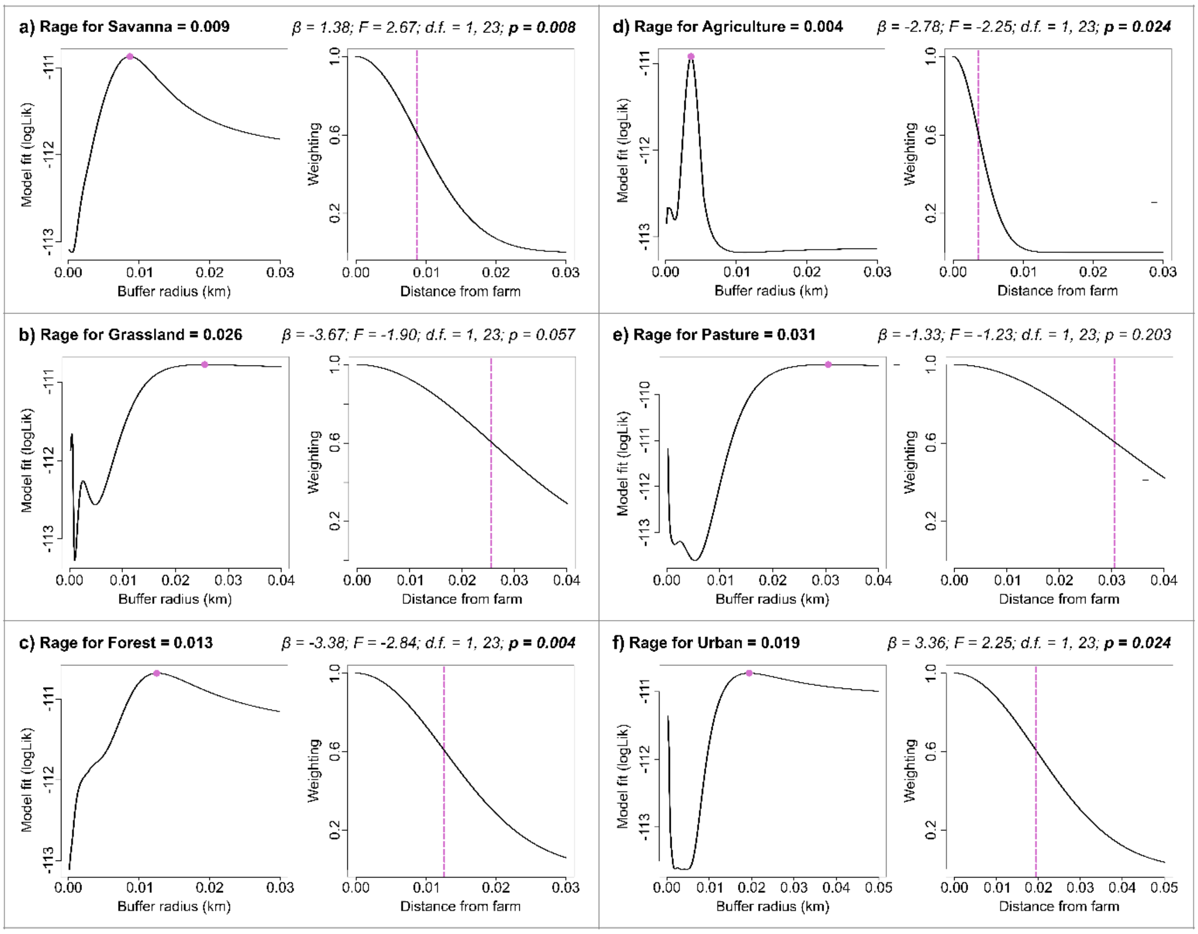

Figure 3. Relationships between landscape composition and *Exomalopsis analis* abundance. For each land use types (a-f) were calculated: the ideal buffer radius to improve model fit, the land cover area weight loss with distance from farm, and the effect of land cover variables on the *E. analis* abundance. Bold p-values indicate statistical significance ($p < 0.05$).

Samples from all sites exhibited low genetic diversity, with low allelic richness and a low proportion of heterozygotes (Table 1). Despite this, no fixation index other than zero was observed for most groups of individuals. Some groups showed a high average fixation index (F_{IS}), but with large variation between loci, resulting in very wide confidence intervals (CIs) that include $F_{IS} = 0$ (Table 1). The analysis of population genetic structure revealed the existence of a single genetic group among all samples. The Bayesian analysis yielded the highest value for the Bayesian Information Criterion (BIC) for the existence of only one genetic group. The overall F_{ST} was 0.06, with only the Farm D property showing values of F_{ST}

significantly different from zero (F_{ST} = 0.455, $CI_{95\%}$ [0.067, 0.916]), indicating migrant limitation.

Table 1. Sample sites, sample size (N), and genetic diversity parameters (allelic richness - A_R, observed heterozigosity - H_O, expected heterozigosity under Hardy-Weinberg Equilibrium - H_E, and fixation index - F_{IS} - with 95% confidence interval - CI) estimated for *Exomalopsis analis* from tomato crop areas distributed across the Federal District and Goiás, Brazil.

Site	**Farms**	**N**	A_R	H_O	H_E	F_{IS} **[CI]**
1	N, W and X	56	1.6	0.219	0.178	0.139 [-0.650, 0.591]
2	H	40	1.3	0.186	0.112	-0.232 [-0.863, -0.010]
3	V and Y	40	1.7	0.223	0.209	0.0842 [-0.698, 0.752]
5	F and K	37	1.6	0.245	0.188	0.032 [-0.723, 0.469]
6	P	31	1.6	0.250	0.189	-0.125 [-0.743, 0.350]
7	G	29	1.6	0.277	0.218	-0.120 [-0.700, 0.457]
8	J	28	1.5	0.220	0.166	0.048 [-0.739, 0.500]
9	Q and T	22	1.8	0.274	0.236	0.158 [-0.627, 0.611]
10	S	54	1.8	0.218	0.246	0.161 [-0.643, 0.847]
11	U	27	1.6	0.216	0.222	0.007 [-0.761, 0.927]
12	O	31	1.5	0.182	0.207	0.121 [-0.787, 0.941]
13	Z	19	1.6	0.149	0.207	0.273 [-0.481, 0.884]
16	U	17	1.5	0.149	0.164	0.242 [-0.500, 0.778]
17	V	25	1.7	0.220	0.198	0.026 [-0.530, 0.452]
18	W	16	1.8	0.164	0.202	0.273 [-0.160, 0.668]
19	X	9	1.4	0.045	0.128	0.333 [0.000, 1.000]

There was no significant correlation between any of the genetic distances (Nei, cGD) and geographic distances, indicating neither isolation-by-distance nor isolation-by-graph-distance. The population graph also showed high connectivity among all sample sites, with samples from seven sites with higher betweenness (Figure 4). In addition, a visual examination of the spatial arrangement of the sampling sites showed that those with higher betweenness were generally situated closer to areas of natural vegetation, including areas within or adjacent to Conservation Units in the Federal District (Figure 4b). This visual association suggests that areas with greater habitat continuity may facilitate gene flow, contributing to the higher connectivity observed in those sites.

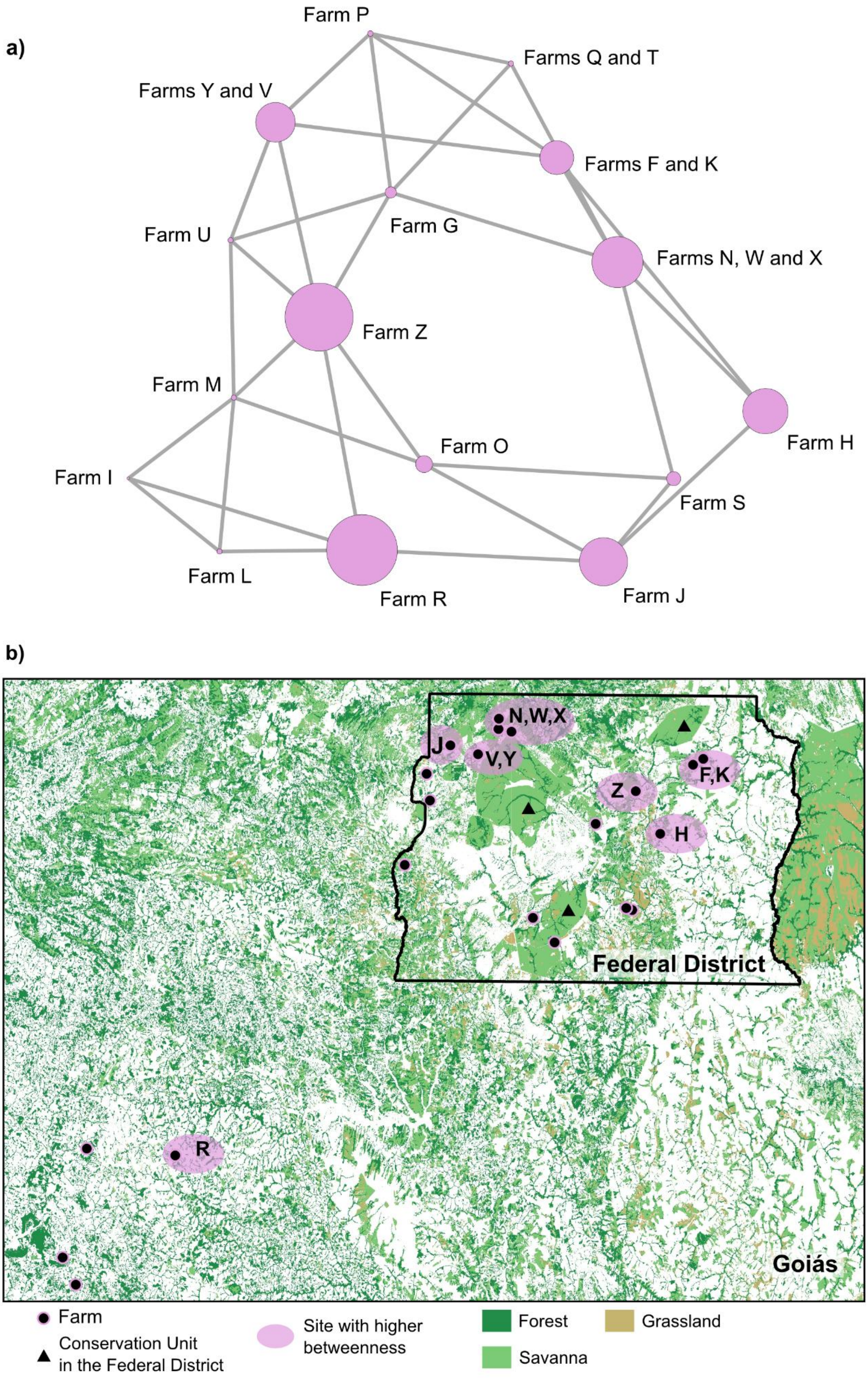

Figure 4. Population graph and spatial distribution of sampled sites. a) Population graph illustrating genetic connectivity among sampling sites. Nodes represent farms, and node size is

proportional to betweenness centrality, a metric that identifies key populations for genetic connectivity (central links). b) Geographic distribution of sampling. Pink shaded areas indicate sites with higher betweenness in the population graph. Background land-cover classes include forest, savanna, and grassland.

4. Discussion

In this study, we investigated how landscape structure shapes the abundance and genetic patterns of the bee *E. analis* in the central area of the Cerrado biome. Our findings demonstrate that while local bee abundance is highly sensitive to fine-scale habitat composition, the population maintains remarkable genetic connectivity across the entire region. We observed that bee abundance responded to specific land cover types within a very local spatial scale (0.02 km radius), with savanna and urban areas supporting higher bee abundance, and forests and agriculture having a negative impact. Despite these local habitat pressures, our genetic analysis revealed a single population with no significant structure or isolation-by-distance, indicating that gene flow is widespread and not impeded by distance or landscape features at the scale of our study. This pattern suggests that the regional population is well-connected, likely using remaining patches of natural vegetation as corridors or 'stepping stones' to move across the landscape.

The abundance of *E. analis* is shaped by the landscape at a remarkably fine spatial scale. For most land cover types, the strongest effects occurred within a 0.02 km radius of the sampling farms. This finding reinforces the idea that these bees respond almost immediately to the resources and conditions in their surroundings (Franceschinelli et al., 2017; Cunha et al., 2024), making them more dependent on local habitat conditions. We found that both savanna and urban areas positively influenced the abundance of *E. analis*. The positive effect of savanna cover is expected, as this biome is a central part of the species' natural distribution range (Silveira and Melo, 2023). These natural savanna areas may offer particularly suitable nesting sites, especially when compared to agricultural fields where frequent soil tilling and other management practices can destroy ground nests (Tschanz et al. 2024). On the other hand, the positive association of *E. analis* abundance with urban areas is a more surprising result. Urbanization has been associated with long-term declines in bees’ ground nest abundance, richness, and phylogenetic diversity driven by the loss of workable soil and the expansion of sealed surfaces (e.g., concrete and pavement) (Pereira et al. 2021). However, the positive effect of urban cover in our study may be explained by growing evidence that active management of urban green spaces can benefit bee populations, turning cities into valuable habitats for pollinators when minimal resources and nesting conditions are provided (Marcacci et al. 2022; Anderson et al. 2023).

In contrast, we found that forest and agriculture cover negatively affected *E. analis* abundance. The negative effect of forest formations might seem counterintuitive, but the species' evolutionary history within the Cerrado may offer a possible explanation. Cerrado is a natural mosaic of vegetation types, ranging from open grasslands to dense forests (Oliveira-Filho and Ratter 2002). It is plausible that populations of *E. analis* in this region have adapted to prefer more open environments, as corroborated by the positive effect of savanna cover on its abundance. Consequently, the characteristics of dense, closed-canopy forests, such as reduced sunlight, varied soil conditions for nesting, and fewer flowering plants, may constitute a less suitable habitat for a species adapted to open, sunlit areas (Odanaka and Rehan 2020). However, these open vegetation types are the first to be removed due to agricultural expansion, since only forests are legally committed to conservation in the Brazilian Cerrado. The negative impact of agriculture on *E. analis* abundance is more straightforward. Although crops can provide floral resources for short periods, agricultural landscapes can become hostile to wild bees without adequate management, due to pesticide use and the removal of natural vegetation (Brown et al. 2024; Shi et al. 2024; Chakraborty and Basu 2025).

While our abundance data revealed a species highly sensitive to its local habitat, our genetic analysis showed that, at the regional scale, *E. analis* exhibits low genetic structure over medium distances (tens of kilometers). Studies conducted with large social and solitary species have found high gene flow between populations. For *B. ignitus*, with populations distributed throughout mainland China, significant differentiation was observed between populations hundreds of kilometers apart and separated by the sea (Shao et al. 2004). In *B. huntii*, high gene flow was observed between populations distributed along almost the entire coast of the United States, with differentiation between the US and Mexican groups being more influenced by environmental factors than by geographic distance (Koch et al. 2018). For *Xylocopa virginica*, high gene flow was also observed, with the restriction being more associated with climatic conditions than with geographic distances (Vickruck, Richards 2017). In a smaller species, *Scaptotrigona xanthotricha*, the same pattern of little or no differentiation between populations separated by tens of kilometers and significant differentiation between populations separated by hundreds of kilometers was found (Duarte et al. 2014). Therefore, the low structuring found for *E. analis* seems to follow the pattern found for bees in general.

The combination of low genetic differentiation among samples and a high proportion of samples with high betweenness indicates that there is a high gene flow among sites, and the system acts as a metapopulation with a stepping-stone instead of a source-sink dynamics (Kimura and Weiss 1964; Dyer and Nason 2004; Dyer 2015). The spatial location of these high-connectivity sites supported this interpretation, as a visual examination revealed that sites with high betweenness were generally located closer to large remnants of natural vegetation, including protected areas. This is consistent with a study on native stingless bees, which found that gene flow was enhanced by the presence of natural areas in a tropical region (Jaffé et al. 2019). Furthermore, this aligns with our results, which show that savanna cover had a strong positive effect on *E. analis* abundance. Taken together, it is possible that savanna remnants not only support larger local populations but also serve as natural stepping stones for maintaining regional genetic flow. A logical next step would be to expand this research to a broader geographic scale and model the potential dispersal routes and corridors through the landscape. Studies are ongoing in this direction. Sampling across a larger area with greater environmental variation would allow us to determine the maximum dispersal capacity of *E. analis* and identify potential barriers to gene flow at a regional level. For instance, similar to dispersal models developed for pest insects (Novaes et al. 2024), we could use a model to predict how *E. analis* population movement changes across different land cover types.

A notable and consistent finding across all sampled sites was the low genetic diversity in *E. analis* populations and a general lack of significant inbreeding. Interpreting this overall low level of genetic diversity is challenging, as this study represents the first genetic assessment for *E. analis* using SRR (Assunção et al. 2025). Therefore, is difficult to ascertain whether this low genetic diversity is indicative of a species facing a decline or if they represent a natural baseline characteristic of its life history past. In Neotropical bees, research on *Tetragonisca angustula* suggests that low genetic diversity can be indicative of recent colonization events (de Matos Barbosa et al. 2022), given that the process of genetic diversity loss often occurs slowly over time and depends on the land-use changes (Kelemen and Rehan 2021). Nevertheless, from a conservation perspective, our results should be considered a potential vulnerability. Low genetic diversity is widely recognized as a factor that can limit a species' adaptive potential, compromising its ability to respond to novel selective pressures. For example, studies on *Apis mellifera* demonstrate that high genetic diversity is associated with enhanced colony health and productivity, improved resistance to mites, and greater immunocompetence (Tarpy et al. 2013; López-Uribe et al. 2017; Beaurepaire et al. 2019). Given that the genetic diversity of tropical bee species is known to be changing (Kelemen and Rehan 2021), our findings underscore an urgent need for further research to establish genetic baselines for Neotropical bees. This is particularly concerning in the context of accelerated environmental changes, such as those driven by climate change and habitat fragmentation in the Neotropical region.

While our results provide a picture of the gene flow in *E. analis* populations, possibly driven by natural habitats, it is important to acknowledge the methodological framework that produced them. The precision of our genetic findings is inherently linked to the markers we developed. Therefore, a brief discussion of the strengths and limitations of our molecular approach is warranted to contextualize our conclusions and guide future research. In this context, the method we employed for primer design proved effective, enabling the development of specific markers within the project's development timeframe, and without the need for financial resources, equipment, or laboratory infrastructure beyond what was planned in the project. If a reference genome of the species or a closely related species already existed, or if financial resources were available to assemble such a genome, it would also be possible to map the markers to the genome and select loci distributed evenly across the chromosomes. An analysis of a larger number of loci or loci with greater polymorphism may be necessary to obtain more precise results. Furthermore, future studies could also utilize a larger number of markers distributed throughout the genome, such as single-nucleotide polymorphisms (SNPs), which allow the detection of more subtle genetic structures and have the potential to identify mutations associated with local adaptations (Lozier and Zayed 2017; Hohenlohe et al. 2021).

5. Conclusion

Exomalopsis analis exists as a well-connected genetic population across a human-modified region in the Cerrado and shows strong ecological responses to local habitat conditions. Bee abundance was shaped by the landscape within just a few meters, highlighting its sensitivity to immediate resources. Our genetic analyses revealed a single population with no significant structure or isolation-by-distance. Additionally, our findings indicate that *E. analis* likely uses the mosaic of natural and semi-natural vegetation as 'stepping stones' to move across the landscape. From a conservation standpoint, our results highlight the critical importance of maintaining natural areas, especially savanna remnants, to sustaining both the abundance and genetic health of native bees associated with open habitats in the Cerrado.

6. Supplementary Information

Table S1. Total abundance of *Exomalopsis analis* collected per farm. The table shows the total number of individuals sampled across 26 farms located in the Federal District (FD) and Goiás (GO), Brazil.

Farm	Local	Bee abundance
A	GO	13
B	GO	13
C	GO	10
D	GO	13
E	FD	18
F	FD	31
G	FD	24
H	FD	25
I	GO	20
J	FD	31
K	FD	20
L	GO	20
M	GO	14
N	FD	20
O	FD	32
P	FD	36
Q	FD	20
R	GO	27
S	FD	91
T	FD	10
U	FD	27
V	FD	16
W	FD	27
X	FD	53
Y	FD	27
Z	FD	22

Table S2. Primer designed for microsatellite region amplification of *Exomalopsis analis*, with their sequences, estimated melting temperatures (T_m), effectively used annealing temperatures (T_A), target fragment size, repetition motif, and whether amplification (Amp.) was successful with polymorphic (P) fragments, or unsuccessful (U).

Name	Sequence (5’- 3')	T_m(°C)	T_A(°C)	Target (bp)	Motif	Amp.
Ean 1 F	CGAATTACAACTGTTTCTGTCGC	62.3	55	84	(GT)7	P
Ean 1 R	CGTCAACACCGAGCAGAATT	62.8				
Ean 2 F	CGTTCATGCTGGTCACAAAC	61.8	-	148	(TAA)12	U
Ean 2 R	CCTTTTACGACACGCAGAGG	62.6				
Ean 3 F	AATTCGCCAAATCGCTCCAG	63.3	-	158	(T)(TA)(T)	P
Ena 3 R	ACTTTCAAAATGGTGCCACAC	62.1				
Ean 4 F	CGGAATCGTGTCAACAAGCC	63.4	-	163	(GA)5	U
Ean 4 R	GTCAGTCCCATGCAACCTG	62.6				
Ean 5 F	CGGGTAATCTCGCCTACTCTG	63.1	61	181	(TA)13	U
Ean 5 R	AGTTCTTCACCTTTTCTCTCAC	62.7				
Ean 6 F	ACTCCTCGGGTGAAAGTTGA	62	53	248	(TA)7(TA)7	P
Ean 6 R	TGTGGATTTGAATAACGACGTGA	62				
Ean 7 F	TTCAGAGAAAGGTTTTAGCGG	60.5	61	281	(AT)6	P
Ean 7 R	TGCCCCACTTCCATCATTTC	62.6				
Ean 8 F	AGATGTGCAAGATGTTCTCAGG	62.4	-	281	(TA)6(TA)4	P

Ean 8 R	TATCATTTGGGGAGTGTGCG	62.2				
Ean 9 F	GGTTGGCTGTACGTATTTTACTG	61.5	-	292	(TA)5	U
Ean 9 R	CCTTGAGGGTCTAGATGAAGGG	63.1				
Ean 10 F	GTAGCTGGGGTTCGAGTCTC	63.3	55	297	(AT)8	P
Ean 10 R	ATTCCGTGTCAGCCGTAGTG	64.0				

7. References

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CHAPTER 4: Wild bees are key pollinators in organic tomato agroecosystems regardless of the presence of a managed stingless bee

Artigo em revisão após major review no periódico: *Apidologie*

Abstract

While managing social bees is a well-documented strategy to enhance pollination services in controlled environments like greenhouses, this approach remains comparatively underexplored in open-field conditions. There is also a lack of empirical data on how managed bees affect wild bee populations and on minimally invasive methods for identifying and selecting healthy colonies for introduction into agroecosystems. This study aimed to evaluate the potential of *Melipona quadrifasciata* for assisted pollination of tomato plants cultivated in open organic fields. We assessed nine *M. quadrifasciata* hives by monitoring foraging activity and qualitative parameters (brood cells, food storage, and pathogens) with environmental conditions (temperature and humidity). Subsequently, we evaluated the bee community and pollination services across seven farms (four with introduced hives). We monitored bee activity on tomato flowers and non-crop plants (commonly referred to as weeds) to assess the impact of introducing hives on wild bees. Pollination services were evaluated through self-pollination and open-pollination treatments, with fruit quality measured by weight, diameter, seed number, and pest damage. Hive evaluations demonstrated that monitoring foraging activity alongside basic environmental data provides a practical, effective, and minimally invasive method for farmers to assess hive health. Open-pollination improved fruit quality compared to self-pollination, confirming that bee pollination enhances tomato production. However, the presence of *M. quadrifasciata* hives did not influence fruit quality, indicating that wild bees primarily drove pollination benefits. Our findings underscore the importance of conserving and promoting wild pollinators in organic agroecosystems by managing non-crop plants, which support diverse pollinator communities with complementary functional traits.

1. Introduction

The demand for assisted pollination has increased, particularly for high-nutrition crops that form the basis of family farming (e.g., fruits and vegetables) and are highly dependent on animal pollination (Giannini et al. 2015; BPBES and REBIP 2019). This demand has also risen in landscapes where habitat changes have reduced the availability of bees for natural pollination (da Silva Carneiro et al. 2024). This trend reflects the growing recognition of pollination's contribution to fruit quality and productivity (BPBES and REBIP 2019), as well as advances in managing social bees for pollination in agroecosystems (Meléndez Ramírez et al. 2018). Although managing social bees can enhance pollination services, most studies focus on the use of *Apis* and *Bombus* species (Iwasaki et al. 2022), which are important for assisted pollination but are exotic to many regions. The introduction of exotic species can directly and indirectly impact interactions with native species, potentially compromising pollination dynamics (Page and Williams 2023; Aguiar et al. 2024; Assunção et al. 2025). This issue is particularly relevant in the Neotropical region, which plays a significant role in global food production and hosts a high diversity of native bees essential for crop pollination (Freitas et al. 2009).

New management strategies of native bees adapted to local contexts have been explored for assisted pollination, showing promising results across various crops (Giannini et al. 2015; Meléndez Ramírez et al. 2018; Maués et al. 2024). This is particularly true for native stingless bees of the tribe Meliponini (Apidae). For instance, *Melipona* species represents the second most economically important group of bees for agricultural pollination in Brazil, after the genus *Apis* (honeybee) (Giannini et al. 2020). Using Meliponini hives for assisted pollination in Brazil offers several advantages: (i) their natural occurrence in the Neotropical region; (ii) the availability of well-established management techniques for many species (Cortopassi-Laurino et al. 2006); (iii) their lack of a functional stinger, which reduces the risk of adverse incidents for farmers (Pedro 2014); (iv) their perennial and expansive colony growth, with a high number of individuals per hive (Roubik et al. 2018); and (v) their generalist foraging habits, floral constancy, and adaptability to diverse environmental conditions (Slaa et al. 2006; Roubik et al. 2018). Additionally, these bees exhibit morphological and behavioural diversity, including the ability to produce body vibrations, which allows them to pollinate a wide variety of plants that rely on buzz pollination (BPBES and REBIP 2019).

The tomato plant (*Solanum lycopersicum* Linnaeus, 1753) is one of the cultivated species that requires specific pollination behaviours, such as licking and buzz behaviour (Gaglianone et al. 2015; Bartelli et al. 2024). Tomato cultivation is widespread across South America and holds significant socioeconomic importance, with countries such as Brazil, Argentina, and Chile progressively increasing both their cultivated areas and yield per unit area (FAO 2024). Although tomato plants are capable of self-pollination due to their hermaphroditic and self-compatible flowers, cross-pollination by bees significantly enhances fruit production (Del

Sarto et al. 2005; Gaglianone et al. 2015). Among Meliponini bees, only species of the genus *Melipona*, such as *Melipona quadrifasciata* Lepeletier, 1836 and *Melipona bicolor* Lepeletier, 1836, can vibrate tomato flowers, making them efficient pollinators of tomato crops (Del Sarto et al. 2005; Gaglianone et al. 2015; de Moura-Moraes et al. 2021). This underscores tomato as an important crop for Brazilian farmers, whose productivity is clearly enhanced by assisted pollination from native bees (Del Sarto et al. 2005; Bartelli et al. 2014; Deprá et al. 2014; Silva-Neto et al. 2019).

To conserve natural resources and sustain farming practices in the long term, it is crucial to explore strategies that enhance tomato production without expanding agricultural land use, particularly in megadiverse countries such as Brazil. In this context, organic production systems promote a more resilient and biologically diverse environment, supporting beneficial organisms (da Silva et al. 2022; Marins et al. 2024), including pollinators (Assunção et al. 2022). Maintaining species diversity is important, as different bees play complementary roles, enhancing the resilience and effectiveness of pollination (Dainese et al. 2019; Assunção et al. 2022). Thus, although introducing beehives can positively impact tomato crops, focusing solely on this approach may limit pollination services on agroecosystems (Garibaldi et al. 2021). The success of managed hives depends on the compatibility between the introduced species and the target crop, as well as the interactions between these managed bees and wild pollinators (Mallinger and Gratton 2015; Mallinger et al. 2017; Garibaldi et al. 2021). Given these complexities, a key concern regarding the introduction of hives into organic agroecosystems is their potential impact on wild pollinator communities due to dominance and competition effects (Mallinger et al. 2017; Page and Williams 2023).

While the potential of managed beehives to mitigate pollination deficits on farms is increasingly recognized, significant knowledge gaps remain about their effective implementation, especially for stingless bees. First, studies on this practice have primarily focused on protected crops, leaving the complex ecological dynamics of open-field systems under-explored (Del Sarto et al. 2005; dos Santos et al. 2009). Second, there is a lack of empirical data on the ecological impacts of introducing managed hives on wild bee populations (Goulson et al. 2015; Geslin et al. 2017). Third, minimally invasive methodologies for identifying and selecting healthy colonies for introduction into agroecosystems are still lacking (Leão et al. 2024). Finally, a particularly problematic gap concerns the widespread practice of translocating native bee species across different habitats based solely on their reputation as effective pollinators elsewhere (Quezada-Euán et al. 2022; Roubik 2023). This practice typically lacks local, empirical validation, raising significant doubts about the efficacy of these bees on new target crops, their foraging preferences, and colony viability in new environments.

Given the need to understand the ecological implications of introducing hives into economically important crops, such as tomatoes, this study aims to evaluate the potential of *M. quadrifasciata* for assisted pollination of tomato plants grown in open organic fields. Specifically, we aimed to (i) assess the quality of *M. quadrifasciata* hives by evaluating bee activity to select healthy hives for assisted pollination strategies; (ii) examine the impact of introducing *M. quadrifasciata* hives on the diversity and abundance of wild bees in organic agroecosystems; (iii) determine whether the introduction of *M. quadrifasciata* hives enhances the productivity and quality parameters of tomato fruits; and (iv) characterize the foraging preferences of the managed *M. quadrifasciata* hives by identifying the floral resources that support their maintenance within the agroecosystem. We chose *M. quadrifasciata* to investigate whether its known efficiency as a tomato pollinator is maintained in an open-field setting in a region outside the species' native range. This approach enables an empirical evaluation of the ecological trade-offs associated with transporting and managing hives in novel environments, including a direct assessment of colony sustainability.

2. Materials and Methods

2.1. Quality parameters of beehives for introduction in open-field organic tomato crop*s*

Melipona quadrifasciata hives were maintained in an experimental area at Embrapa Genetic Resources and Biotechnology, Federal District, Brazil (15°46′48″S, 47°55′45″W), near a natural remnant of riparian vegetation in the Cerrado biome (the Neotropical savanna). The Cerrado is a biodiversity hotspot and the most diverse savanna in the world in terms of plant diversity (Myers et al. 2000; Sano et al. 2019). This biome supports 12% of the bee species in the Neotropical region, comprising approximately 820 species, with a significant proportion of endemic species (Raw 2007). The experimental area provided optimal conditions for hive development. To ensure additional resources for the colonies during the experiments, the hives were supplemented weekly with carbohydrates (50% sucrose solution) (Villas-Bôas 2018). The hives were sourced from beekeepers who maintained these species in modular wooden boxes in the Federal District. Hive acquisition and subsequent management were conducted in strict accordance with Brazilian legislation for the scientific research on native bees (BRASIL 2023). This legislation governs the scientific use of native bees and includes provisions for their transport outside their natural range, specifically for studies like ours that aim to assess the ecological risks and benefits of such introductions.

Approximately one month before the beginning of field experiments in June 2023, we evaluated the behaviour of nine *M. quadrifasciata* hives to establish parameters for selecting healthy hives prior to their introduction into the farms. We assessed the foraging activity of the hives weekly. The number of bees entering and leaving the hives was recorded from 8h to 11h at 30 min intervals. During this period, we observed individuals carrying pollen, resin, or discarding waste as they flew to and landed at the hive entrance, noting the masses of pollen and other materials, such as clay and resin, attached to the bees' corbiculae. Local temperature and relative humidity were recorded at 30-minute intervals using online data from Google's weather forecast service, which provides short-term weather information on mobile devices (Google 2023). By identifying how factors such as temperature and humidity affect bee foraging activity, we aim to propose more effective, easy-to-implement management strategies for agroecosystems.

Qualitative parameters were assessed for all hives, including the presence of food storage pots containing pollen and honey, the absence of phorids or pathogens, and the existence of new and old brood cells. New brood cells are characterised by their lighter colour, smooth texture, and translucent appearance (Villas-Bôas 2018). Due to the invasive nature of this procedure for the colonies, qualitative parameters were measured only twice: on the day the hives were installed on the farms and again when they were returned to the experimental area approximately four months later.

2.2. Bee diversity and impacts of beehive introduction on local fauna

The introduction of *M. quadrifasciata* hives into agroecosystems was evaluated across seven farms from July to October 2023. These farms, located in the Federal District, Brazil (MS1 Figure S1), cultivate tomatoes in open fields. All farmers had been engaged in organic management systems for at least three years, with farms averaging 24000 m^2 in size. Farm management relied predominantly on manual labour. The farms were situated within an agricultural matrix that included natural remnants of Cerrado vegetation, polyculture systems (simultaneous cultivation of multiple crop species, primarily vegetables), non-crop plants (commonly referred to as weeds), and fruit trees (MS1 Figure S2, S3). Farmers allowed non-crop plants to grow between crop rows and along field margins, removing them from cultivated areas as needed. These plants were typically used as live or dead soil cover, contributing to soil protection and nutrient cycling during both cropping seasons and fallow periods. Pest management was conducted using biological and natural products approved by Brazilian legislation, ensuring compliance with organic farming regulations (Togni et al. 2019).

The experiments were conducted using the Italian tomato cultivar (*S. lycopersicum* var. Matinella), widely cultivated by Brazilian farmers, particularly in organic systems (CONAB 2019). A previous study reported a higher frequency of *M. quadrifasciata* visits to this cultivar in greenhouse tomatoes than to cherry tomatoes (Pires et al. 2024). We provided tomato seedlings and fertilisers to the farmers to standardise the number of plants cultivated per area and the nutritional conditions of the tomato plants. Tomato cultivation covered an average of 200 m² per farm, with approximately 380 plants. Soil preparation was carried out before planting, incorporating simple thermophosphate and poultry litter, in accordance with standard agronomic practices for local organic production. The tomato plants were supported using vertical staking and arranged in double rows with 60 cm spacing between rows to optimise light interception and airflow. Farmers used drip irrigation and sprinklers to maintain consistent soil moisture levels throughout the growing season. Seedlings were transplanted to the field 30 days after germination. The experimental design ensured that hives were introduced approximately 10 days after transplanting, allowing the plants to acclimate and reach the early flowering stage, which typically occurs around 40 days after germination.

Pires et al. (2024) estimated that introducing one hive of *M. quadrifasciata* per 100 tomato plants at peak flowering resulted in approximately 90% of the flowers of the Italiano cultivar being visited in greenhouse conditions. Therefore, we established three hives per farm, considering that the cultivation area contained approximately 380 tomato plants. Bee hives were installed on four farms (+BH), while the remaining three farms did not have hives installed near the tomato crops (−BH). Farms with (II, III, V, and VII) and without (I, IV, and VI) hives were paired within the landscape to ensure comparable environmental conditions. The hives were installed in shaded areas adjacent to the field margins, 3–5 m from the tomato crop area. This introduction occurred shortly after the tomato plants were planted (early July), allowing the hives to acclimate before the tomato plants began flowering. Based on foraging activity results, hives with lower activity were excluded from the farms and replaced with new ones. Due to the inability to evaluate foraging activity in these new hives, they were randomly assigned to farms, ensuring that each farm received at least one active colony (previously assessed by foraging activity).

To characterise the community of flower-visiting bees on tomato flowers and assess the potential effects of introducing *M. quadrifasciata* hives, we conducted bee sampling throughout the tomato crop’s flowering period (July to October). We evaluated the occurrence of bees (both introduced and wild species) in the crop area and on non-crop plants around the field margins, within a radius of up to 10m from the cultivated area. Sampling bees on non-crop plants aimed to determine whether *M. quadrifasciata* also foraged on these plants, which are essential for maintaining wild bee interactions within tomato crop areas (Assunção et al. 2022). Each week, collectors walked through the area, actively capturing bees using plastic vials between 9h and 12h. Only bees that landed directly on the flowers were collected. The collected specimens were immediately euthanized and preserved for subsequent identification in the laboratory. The daily sampling effort was quantified in minutes of collection and multiplied by the number of collectors. The total sampling effort was 20h per farm, with 10h spent on tomato plants and 10h on non-crop plants, totalling 140h across the seven sampled farms. The bees were identified to the lowest possible taxonomic level. Bee specimens were deposited in the Entomological Collection of the University of Brasília (DZUB) and the Entomological Collection of Embrapa Genetic Resources and Biotechnology, both located in the Brazilian Federal District.

2.3. Bee pollination in tomato crops

The pollination service provided by wild or introduced bees was evaluated in tomato plants. Two treatments were established in the tomato crops: 1) SP – self-pollination, where tomato inflorescences were bagged during the pre-anthesis stage using material that allows only wind passage, preventing bee visits; and 2) OP – open-pollination, where bees were allowed to visit the flowers (MS1 Figure S4). For each treatment on a given plant, three flowers were selected. Both treatments were applied simultaneously to each tomato plant on branches with flowers at the same developmental stage. A random sample of 100 plants was selected on each farm to implement the treatments, totalling 700 tomato plants per treatment (700 SP + 700 OP). The bags were removed from the SP inflorescences once fruit development began. In the OP treatment, flowers with necrotic marks on the anthers, caused by the insertion of mandibles of buzz-pollinating bees, were considered pollinated, indicating bee visitation (Pires et al. 2024).

The experiments were conducted from the 1st to the 4th flowering cycles to avoid bias related to reproductive investment in flowers at different stages of plant development. The tomato variety used in the experiment exhibits indeterminate growth, with the apical bud exerting dominance over the lateral buds. The plant continuously forms new buds and clusters along the main stem, and fruit development follows an ascending pattern, where the lowest-level clusters produce the most vigorous fruits. To account for these factors in the analysis of fruit quality, we recorded the level of the clusters and buds and marked the flower buds with water-based liquid paper.

We harvested up to two fruits per treatment from each tomato plant. After harvesting, fruit quality was evaluated in the laboratory by measuring fruit weight (mg), diameter (cm), number of seeds per fruit, and pest damage. Pest damage was quantified by counting the number of infestations per fruit, assigning a damage score of 1 for each distinct pest species affecting the fruit. For example, a healthy fruit received a score of 0, while a fruit infested by two pest species received a score of 2, and a fruit infested by three pest species received a score of 3. To identify pests and their respective damage, we followed a technical manual about tomato fruit quality (Michereff Filho et al. 2019).

2.4. Plants used as resources by *M. quadrifasciata*

We sampled pollen from pollen pots of at least one hive from each farm (hives B, C, J, K, L and M - Figure S6). Pollen samples were stored at -20°C. For genomic DNA extraction we used DNeasy Plant Mini Kit (Qiagen) following manufacturer's instructions. We weighted 100 mg of each pollen sample and homogenized in 800 µL of lysis buffer with three stainless steel beads (3 mm) in a FastPrep®-24 (MP Bio) at 4.0 m/s for 30 s three times. We amplified the ITS2 region (internal transcribed spacer 2) using the dual-indexing strategy (Sickel et al. 2015) and primers S2F (Chen et al. 2010) and ITS4R (White et al. 1990). Each sample was amplified in three replicates. For each replicate, the PCR mix contained 1x AmpliTaq Gold™ 360 Master Mix (Applied Biosystems™), each primer (0.25 µM), BSA (0.8 µg/ml), 6.84 µl of ultrapure water, and template DNA (5-20 ng/µL). In addition, a reaction containing ultrapure water instead of template DNA was performed to serve as a negative control (CNpcr) and monitor for possible contamination. The amplification program consisted of an initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, primer annealing at 56 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 10 min. Sequencing was performed using the Illumina MiSeqi100 platform, using the MiSeq i100 series 5M Reagent Kit (600 cycles), following the manufacturer's protocol, to produce paired-end fragments.

The bioinformatic data analysis followed the dada2 pipeline in R (Callaham et al. 2016). We removed primers using cutadapt (Martin 2011), joined paired ends of forward and reverse reads, removed low quality reads (maxEE = 2), removed chimeras and defined (amplicon sequence variants (ASVs). We identified ASVs at species level using BLASTN version 2.17.0 (Camacho et al. 2009), with a minimum threshold of 100% for query cover and 98% of sequence identity. The unidentified ASVs were assigned to the lowest taxonomic level possible using the dada2 algorithm (Callaham et al. 2016) with a custom made ITS2 reference database that included the Cerrado flora species lists (Flora e Funga do Brasil 2025) and all Spermatophyta with voucher entries from NCBI. The reference database was created following the BCdatabaser pipeline (Keller et al. 2020). For each identified taxon, we listed the plant habit based on GBIF.org (2025). We normalized taxa count per sample by rarefaction (Cameron et al. 2021). We subsampled up to 10,000 reads per sample 30 times, calculated the average count and the relative frequency of each taxa in each sample.

2.5. Statistical analysis

To assess the correlation between explanatory variables (temperature, humidity, time of day) and response variables (number of bees leaving and entering the hive), we performed a Spearman correlation analysis, as the data did not follow a normal distribution. We used the Point-Biserial correlation coefficient (rpb) to evaluate the relationship between the binary variable (presence of bees entering the hive with pollen grains) and the continuous variable (number of bees leaving and entering the hive, which was square-root transformed to stabilise variances). Generalised Linear Mixed Effect Models (GLMMs) were fitted to evaluate the impact of explanatory variables on the response variable (Crawley 2012). Variables were selected based on observed correlations to avoid redundancy and multicollinearity. Given the well-documented relationship between temperature and humidity, we selected ‘temperature’ due to its established influence on bee foraging behaviour (Hilário et al. 2000; Ramos et al. 2024). This approach enhances the consistency and comparability of our findings across different seasons and study sites, irrespective of the time of observation. The variable “hive” was included as a random factor in the analysis to account for specific variations among observed hives. This approach was justified by potential natural differences among hives, such as colony size, queen age, brood development rate, and foraging behaviour. To visualise differences among groups (different hives), we performed pairwise comparisons using estimated marginal means. The response variable data were log-transformed ($\log(x + 1)$) to standardise variances.

We fitted Linear Mixed Effects Models (LMMs) and GLMMs to evaluate how richness, abundance, and diversity (Shannon-Wiener Index, H’) of wild bees (response variables) were affected by the introduction of hives in organic farms (+BH and −BH) (Crawley 2012). We included “hive” as a random factor in the analysis to ensure that the observed effects were attributed to hive introduction rather than pre-existing differences among them (as mentioned above). Separate models were fitted for each response variable. The abundance and diversity (H’) data were log-transformed ($\log(x + 1)$) to standardise variances.

To assess whether hive introduction affected pollination effectiveness on the farms, we fitted LMMs and GLMMs with fruit quality parameters (weight, diameter, number of seeds, and pest damage) as response variables, and hive introduction groups (+BH and −BH) and pollination treatments (SP and OP) as explanatory variables (Crawley 2012). We included “hive”, “plants” (replicates), “farms”, “clusters” and “buds” as random factors in the analyses. “Plants”, “clusters” and “buds” were included to control for developmental variation in tomato fruits. Farms were included as random effects to account for environmental differences and bee diversity across study areas. Separate models were fitted for each response variable. Weight data were square-root transformed to standardise variances. One experimental farm (Farm VII) was excluded from the pollination analyses because all tomato plants were affected by disease shortly before fruit harvest. To ensure the validity of our comparisons, we restricted our analyses to the remaining six farms where plants completed their reproductive cycle.

Pairwise comparisons are estimated using package ‘emmeans’ (Russell et al. 2018). Models were fitted using the ‘lmerTest’, ‘lme4’ and ‘glmmTMB’ packages (Bates et al. 2015; Kuznetsova et al. 2016; Brooks et al. 2017). Model fit was evaluated using residual plots and statistical tests from the ‘DHARMa’ package, ensuring the reliability of the fitted models (Hartig and Hartig 2017). The significance of variables in the models was assessed via an ANODEV using an F-test (Crawley 2012). For model comparison and selection, we used the Akaike Information Criterion (AIC). All analyses were performed using the software R (R Core Team 2024).

3. Results

3.1. Quality parameters of beehives for introduction in open-field organic tomato crops

We identified a strong positive correlation between temperature and time of day ($\rho = 0.79$, d.f. = 214, $p < 0.001$); and a strong negative correlation for humidity in relation to both temperature ($\rho = -0.73$, d.f. = 214, $p < 0.001$) and time of day ($\rho = -0.81$, d.f. = 214, $p < 0.001$) (MS1 Figure S5). The data indicated an increasing trend in temperature and a corresponding decline in humidity over the time of day (MS1 Figure S5). We also found a significant correlation between the number of *M. quadrifasciata* individuals entering and leaving the hives ($\rho = 0.96$; d.f. = 214, $p < 0.001$) (Figure S5); and a positive correlation for the presence of individuals entering the hives with pollen grains in relation to both individuals leaving (rpb = 0.79; d.f. = 214, $p < 0.001$) and individuals entering (rpb = 0.73; d.f. = 214, $p < 0.001$) the hives. Given the significant correlations among these variables, we selected only the temperature and the number of bees leaving the hives to analyze the bee foraging activity.

The number of individuals leaving the hives exhibited considerable variation with temperature (β = -0.05, F = 4.32, d.f. = 1, p = 0.038) and among the hives evaluated (F = 416.59, d.f. = 8, p < 0.001) (Figure 1). The highest activity occurred at lower temperatures (18.28 ± 0.25 °C; mean ± SE) and high humidity (63.89 ± 0.91%; mean ± SE), with a gradual decline in foraging activity as the morning progresses and the temperature increases (21.78 ± 0.22 °C) and the humidity decreases (52.56 ± 0.78%). The mean number of individuals leaving the nest was higher in hive-B (110.29 ± 20.41; mean ± SE) and hive-C (105.42 ± 19.77) compared to the other *M. quadrifasciata* hives (Figure 1). In contrast, hive-F (1.75 ± 0.53) and hive-G (2.04 ± 0.48) exhibited considerably lower activity compared to the other hives (Figure 1). Based on these results, hives F and G were not introduced into the farms, and new hives were obtained to replace them.

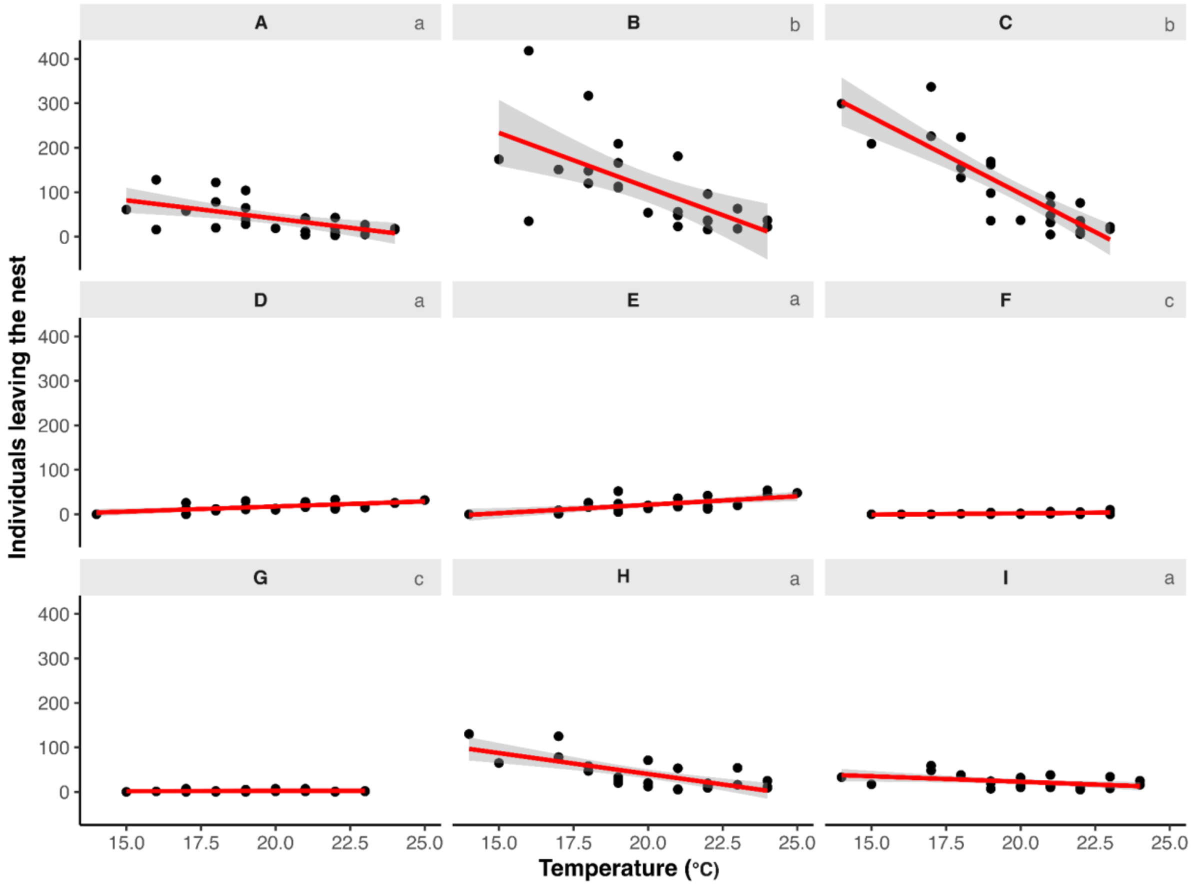


Figure 1 Relationship between temperature and the number of *Melipona quadrifasciata* individuals leaving the hive across different managed hives evaluated (A–I). The scatter plot displays observed data points (black dots), while the red line represents the fitted linear regression model, with the shaded area indicating the 95% confidence interval. Different grey letters indicate statistically significant differences between hives based on a post-hoc test. The

data refer to observations of nine hives of *M. quadrifasciata* made in an experimental area at Embrapa Genetic Resources and Biotechnology, located in the Federal District, Brazil, in June 2023.

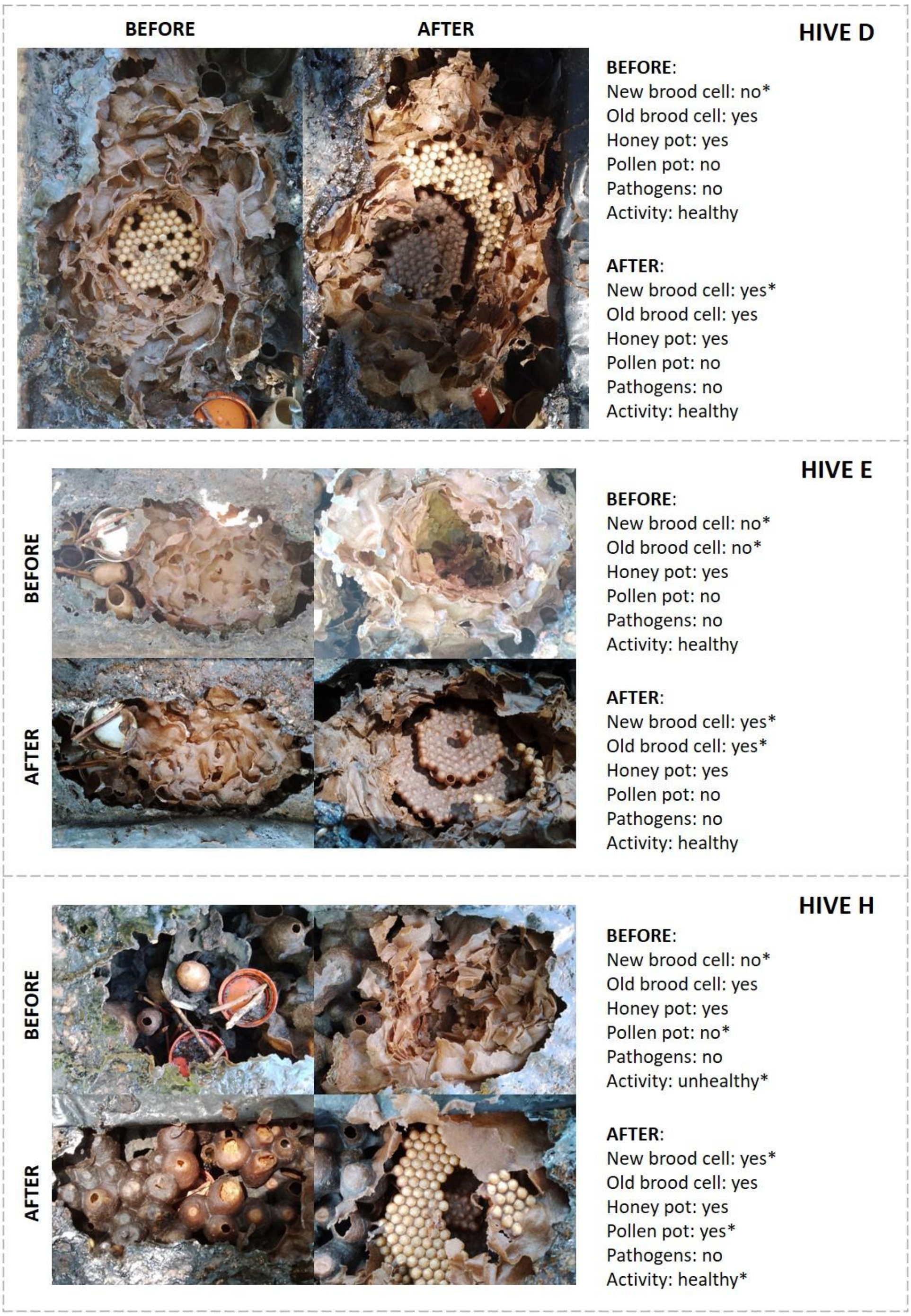
Introducing hives on farms resulted in a general improvement of qualitative parameters related to their health and development. We observed the establishment of new food storage pots (pollen and honey) and brood cells (MS1 Figure S6, S7, S8 and S9), even in hives with less activity such as D and E (Figure 2; MS1 Figure S7). We also observed that the bees continued to forage on the farms, actively collecting pollen from floral resources, indicating that the hives acclimated to the farms within a relatively short period of time.

3.2. Bee diversity and impacts of beehive introduction on local fauna

We collected 2692 bees visiting tomato (1290 individuals) and non-crop (1402) flowers. They were classified into 60 species and five families (Table 1). Overall, we recorded 8 species on tomato plants, 37 on non-crop plants, and 15 in both. The three most abundant species in tomato flowers were *Paratrigona lineata* Lepeletier, 1836 (77.83% of individuals collected), *Exomalopsis analis* Spinola, 1853 (9.84%), and *Pseudaugochlora* sp.2 (4.88%). The most abundant species on non-crop plants were *Apis mellifera* Linnaeus, 1758 (46.22%), *P. lineata* (12.13%), and *Trigona spinipes* Fabricius, 1793 (8.56%). Despite the observations of *M. quadrifasciata* workers leaving and returning to the hives with pollen grains, the species did not visit any tomato flowers or non-crop plants. Furthermore, there were no statistically significant differences between the farms with hives (+BH) and those without hives (-BH) in terms of bee richness, abundance, or diversity on tomato and non-crop plants (Figure 3).

3.3. Bee pollination in tomato crops

A total of 769 tomato fruits were harvested on the six farms. The pollination treatments were the primary factor influencing fruit quality, with OP significantly increasing fruit quality compared to SP (Table 2). However, no significant effect of managed colonies on the assessed fruit quality parameters was observed (Table 2), showing that the benefits of open pollination were consistent across the introduction of hives. The SP had a significant negative effect on the fruit weight, length, and number of seeds produced per fruit (Table 2). On average, the weight of fruits from OP was 22.92% higher than that from SP; the length of OP fruits was 0.64% greater than that of SP fruits; the number of seeds was 29.46% higher in OP fruits. Of the total fruits harvested, 301 (39.15%) exhibited some degree of damage. SP had a slight positive effect on pest damage (Table 2), with pest damage in SP fruits 0.11% higher than in OP. The majority of this damage was attributed to the presence of *Tuta absoluta* Meyrick, 1917 (Lepidoptera: Gelechiidae) (78.41% of fruits with damage), *Neoleucinodes elegantalis* Guenée, 1854 (Lepidoptera: Crambidae) (18.27%), *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae) (5.32%), and other types of damage caused by fungi or bacteria (29.92%).



HIVE D

BEFORE:
New brood cell: no*
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

AFTER:
New brood cell: yes*
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

HIVE E

BEFORE:
New brood cell: no*
Old brood cell: no*
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

AFTER:
New brood cell: yes*
Old brood cell: yes*
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

HIVE H

BEFORE:
New brood cell: no*
Old brood cell: yes
Honey pot: yes
Pollen pot: no*
Pathogens: no
Activity: unhealthy*

AFTER:
New brood cell: yes*
Old brood cell: yes
Honey pot: yes
Pollen pot: yes*
Pathogens: no
Activity: healthy*

Figure 2 Evaluation of qualitative parameters in hives D, E and H before and after their introduction into farms. The images depict internal hive conditions, showing the presence of pathogens, brood cells (both new and old), food storage pots (pollen and honey), and the overall activity of bees into the hives (healthy or unhealthy). The hives were installed on four farms with organic tomato crops located in the Brazilian Federal District, Brazil, from July to October 2023. Asterisks indicate the changes in hives following farm placement.

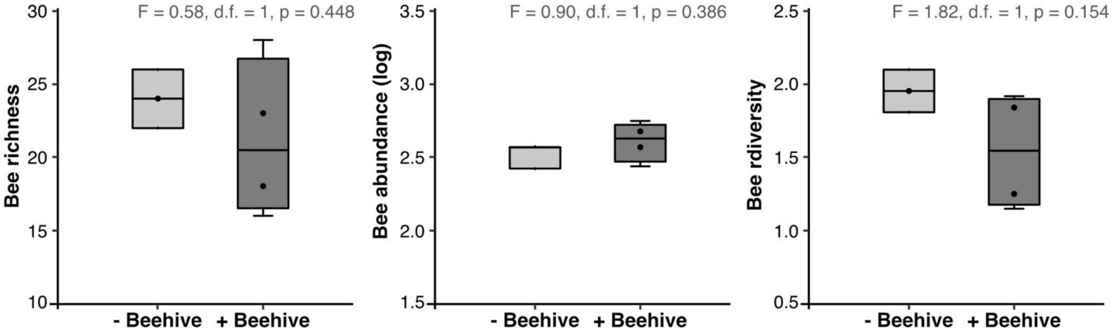
Table 1 Bee species collected from tomato and non-crop plants in organic farms. The table shows the total number of individuals (N) and their relative frequency (fr %) within each plant type (non-crop and tomato plants), as well as the overall total (2,692 individuals) and frequency for each species. Species are listed within each bee family. The samples were made on farms located in the Brazilian Federal District, Brazil, from July to October 2023.

	Non-crop plants		**Tomato**		**Total N**	**Total**
	N (1402)	**fr (%)**	**N (1290)**	**fr (%)**	**(2692)**	**fr (%)**
ANDRENIDAE						
Acamptopoeum prinii	1	0.07	-	-	1	0.04
APIDAE						
Apis mellifera	648	46.22	1	0.08	649	24.11
*Bombus brevivillus***	5	0.36	7	0.54	12	0.45
*Bombus morio***	1	0.07	-	-	1	0.04
Ceratina (*Crewella*) sp.1	1	0.07	-	-	1	0.04
Ceratina fioreseana	4	0.29	-	-	4	0.15
Ceratina richardsoniae	13	0.93	-	-	13	0.48
*Eulaema nigrita***	-	-	1	0.08	1	0.04
*Exomalopsis analis***	23	1.64	127	9.84	150	5.57
*Exomalopsis auropilosa***	52	3.71	42	3.26	94	3.49
*Exomalopsis fulvofasciata***	1	0.07	2	0.16	3	0.11
Exomalopsis sp.2**	1	0.07	-	-	1	0.04
*Frieseomelitta doederleini**	8	0.57	-	-	8	0.30
Geotrigona mombuca	2	0.14	-	-	2	0.07
Geotrigona subterranea	21	1.50	-	-	21	0.78
Lophopedia sp.2	1	0.07	-	-	1	0.04
*Melipona quinquefasciata***	1	0.07	1	0.08	2	0.07
Paratetrapedia connexa	-	-	4	0.31	4	0.15
Paratetrapedia sp.1	3	0.21	12	0.93	15	0.56
*Paratrigona lineata**	170	12.13	1004	77.83	1174	43.61
Partamona sp.1	8	0.57	-	-	8	0.30
Partamona sp.2	11	0.78	-	-	11	0.41
Plebeia droryana	1	0.07	-	-	1	0.04

Scaptotrigona postica	105	7.49	-	-	105	3.90
Tapinotaspis sp.1	1	0.07	-	-	1	0.04
Tetragonisca angustula	38	2.71	-	-	38	1.41
Trigona spinipes	120	8.56	1	0.08	121	4.49
COLLETIDAE						
Hylaeus sp.1	2	0.14	-	-	2	0.07
Hylaeus sp.2	1	0.07	-	-	1	0.04
HALICTIDAE						
*Augochlora aurinasis***	7	0.50	-	-	7	0.26
*Augochlora morrae***	2	0.14	-	-	2	0.07
*Augochloropsis aurifluens***	4	0.29	-	-	4	0.15
Augochloropsis sp.1**	1	0.07	-	-	1	0.04
Augochloropsis sp.2**	-	-	2	0.16	2	0.07
Augochloropsis sp.3**	1	0.07	1	0.08	2	0.07
Augochloropsis sp.4**	-	-	8	0.62	8	0.30
Augochloropsis sp.5**	-	-	2	0.16	2	0.07
Augochloropsis sp.6**	1	0.07	3	0.23	4	0.15
Augochloropsis sp.7**	1	0.07	-	-	1	0.04
Augochloropsis sp.8**	-	-	2	0.16	2	0.07
*Augochloropsis wallacei***	-	-	2	0.16	2	0.07
Caenohalictus sp.1	2	0.14	-	-	2	0.07
Dialictus rostratus	21	1.50	-	-	21	0.78
Dialictus sp.1	14	1.00	1	0.08	15	0.56
Dialictus sp.2	34	2.43	-	-	34	1.26
Dialictus sp.3	2	0.14	-	-	2	0.07
Dialictus sp.5	7	0.50	1	0.08	8	0.30
Dialictus sp.6	3	0.21	-	-	3	0.11
Dialictus sp.7	35	2.50	2	0.16	37	1.37
Dialictus sp.9	1	0.07	1	0.08	2	0.07
Dialictus sp.10	1	0.07	-	-	1	0.04
Dialictus sp.11	4	0.29	-	-	4	0.15
Halictus lanei	1	0.07	-	-	1	0.04
Pseudagapostemon brasiliensis	12	0.86	-	-	12	0.45
Pseudaugochlora sp.2**	-	-	63	4.88	63	2.34
Sphecodini sp.1	1	0.07	-	-	1	0.04
Temnosoma metallicum	1	0.07	-	-	1	0.04
Thectochlora sp.1	1	0.07	-	-	1	0.04
Thectochlora sp.2	1	0.07	-	-	1	0.04
MEGACHILIDAE						
Coelioxys sp.1	1	0.07	-	-	1	0.04

* Bee species considered pollinators of tomato plants; ** Bee species considered pollinators of tomato plants and capable of buzz-pollinating (Deprá et al. 2014; Santos et al. 2014; Silva-Neto et al. 2016; Vinícius-Silva et al. 2017; de Moura-Moraes et al. 2021; Toni et al. 2021).

a) Bees collected on tomato and non-crop plants



b) Bees collected only on tomato plants

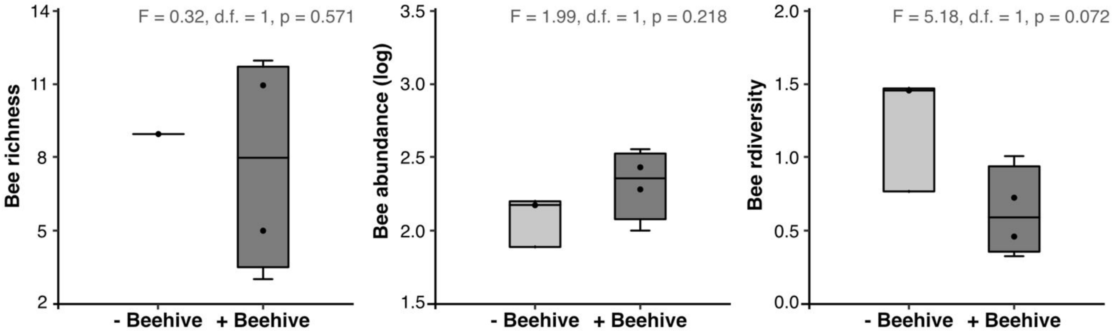


Figure 3 Boxplots showing bee richness, bee abundance and bee diversity (mean ± SE) in farms with (+BH) (dark gray bar) or without (-BH) (light gray bar) *Melipona quadrifasciata* managed hives. (a-c) bee data collected on tomato and non-crop plants; (d-f) bee data collected only on tomato plants. Statistical results from Generalized Linear Models (GLMs) are indicated in each panel. No significant differences were observed in any comparison ($p < 0.05$).

3.4. Plants used as resources by *M. quadrifasciata*

Pollen analysis revealed that the diet of introduced *M. quadrifasciata* hives was composed of ten plant taxa belonging to the families Melastomataceae, Myrtaceae, and Solanaceae (Table 2, MS2). Overall, woody plants, including species with shrub and tree habits, were the primary forage resources, with tomato accounting for a minimal portion of the collected pollen. Melastomataceae was the most important family, with *Miconia* spp. being the dominant pollen type in four of the six hives (C, J, K, and M). Myrtaceae also represented a key resource, particularly for hive L, which foraged heavily on *Psidium* sp. (69.4%), and hive C, which utilized *Siphoneugena* sp. (26.0%). Plants from the Solanaceae family, including the tomato flowers, were detected but at very low frequencies (0.1% in hives L and M) (Table 2, MS2).

Table 2 Quality of tomato fruits harvested from pollination treatments in farms with or without *Melipona quadrifasciata* managed nests. Quality parameters evaluated: weight (Mg), length (cm), number of seeds, and pest infestation damage. Pollination treatments: **SP** - self-pollination; tomato inflorescences were bagged to prevent bees from visiting the flowers; and OP - open-pollination; allowing bees to visit the flowers. Presence of nests: **+BH** - farms with nests installed near the tomato crop; **-BH** - farms without nests installed. Results of models and parameters used to test how the quality of tomato fruits (response variables) is affected by pollination treatments and introduction of nests (explanatory variables) are presented. We use data from tomato fruits harvested in organic fields in the Brazilian Federal District, Brazil. Significant values are indicated in bold ($p < 0.05$).

Fruit quality parameter	**Presence of nest**	**Pollination treatment**	**Mean**	**Standard deviation**	**Sample (n fruits)**	**Effects**				**Model fit**		
						Coefficients	**Est.**	**z**	**p**	**F**	**d.f.**	**p**
Weight (mg)	- BH	OP	95.43	32.12	268							
		SP	69.41	29.28	221	Intercept	9.42	35.83	>**0.001**	-	-	-
	+ BH	OP	70.52	22.72	148	+BH	-1.01	-2.68	0.052	7.18	1	**0.007**
		SP	53.99	17.66	132	SP	-1.23	-12.37	>**0.001**	152.96	1	**>0.001**
Size (cm)	- BH	OP	7.66	1.04	268							
		SP	6.95	1.09	221	Intercept	7.56	30.85	**0.008**	-	-	-
	+ BH	OP	6.72	0.96	148	+BH	-0.84	-1.23	0.347	1.52	1	**0.218**
		SP	6.24	0.90	132	SP	-0.56	-9.05	>**0.001**	81.93	1	**>0.001**
Number of seeds	- BH	OP	81.18	22.50	234							
		SP	51.70	28.70	189	Intercept	80.11	17.81	>**0.001**	-	-	-
	+ BH	OP	87.46	24.53	142	+BH	5.34	0.84	0.452	0.23	1	0.633
		SP	57.76	28.98	123	SP	-28.59	-15.08	>**0.001**	222.70	1	**>0.001**
Pest infestation damage	- BH	OP	0.43	0.62	268							
		SP	0.58	0.71	221	Intercept	-0.92	-2.36	**0.018**	-	-	-
	+ BH	OP	0.45	0.68	148	+BH	0.12	0.22	0.826	0.05	1	0.826
		SP	0.50	0.75	132	SP	0.24	2.25	**0.025**	5.05	1	**0.025**

Table 3 Taxa identified in pollen samples from introduced hives, their habits and normalized occurrence frequencies (%) in each hive. We used data of *M. quadrifasciata* introduced in organic fields in the Brazilian Federal District, Brazil.

Family	**Genus/Species**	**Habits**	**Hives**					
			B	**C**	**J**	**K**	**L**	**M**
Melastomataceae	*Miconia*	Shrub or tree	0.1	73.9	99.5	56.8		81.8
Melastomataceae	*Microlicia*	Subshrub, shrub or tree	88.3					
Melastomataceae	*Trembleya*	Shrub or tree	10.5					
Mirtaceae	*Eucalyptus*	Shrub or tree	0.2			24.9	30.5	17.5
Mirtaceae	*Eugenia*	Subshrub, shrub or tree		0.1		0.4		
Myrtaceae	*Psidium*	Subshrub, shrub or tree				7.2	69.4	0.1
Myrtaceae	*Siphoneugena*	Tree		26.0				
Myrtaceae	*Syzygium*	Tree				7.0		
Solanaceae	*Solanum*	Variable	0.8			3.6		0.6
Solanaceae	*Solanum lycopersicum*	Shrub					0.1	0.1

4. Discussion

Melipona quadrifasciata workers were not observed visiting tomato flowers, with pollen analysis showing a very low foraging preference for this crop. Hive introduction did not influence fruit quality, confirming that wild bees primarily drove the benefits of pollination. This suggests limited effectiveness of *M. quadrifasciata* for assisted pollination in open-field tomato crops. However, the introduction of selected hives into farms demonstrated a general improvement in hive quality parameters, including the establishment of brood cells and storage pots, indicating successful acclimatisation, potential for adaptation even in less active hives, and flower-visiting activity. This foraging success was sustained almost exclusively by pollen from the families Melastomataceae and Myrtaceae, sourced from the adjacent vegetation. On top of that, no differences in bee richness, abundance, or diversity were observed between farms with and without introduced hives, indicating that this practice had no direct effect on the local bee community. Additionally, the evaluation of hive quality confirmed that environmental factors, such as temperature, influenced bee activity.

Our findings reinforce the interaction between environmental conditions, hive quality, and colony performance (Pereboom and Biesmeijer 2003; Hrncir and Maia-Silva 2013). *Melipona quadrifasciata* workers exhibited a preference for foraging early in the morning (between 8h and 10h), when temperatures ranged from 16°C to 20°C and relative humidity ranged from 55% to 70%. This aligns with the thermal foraging window of this species (12–22°C, amplitude: 10°C) and coincides with the peak of pollen collection activity recorded for the species in its natural habitat, the seasonal semi-deciduous forests of southeastern Brazil (Maia-Silva et al. 2014). Relative humidity exhibited the greatest fluctuations during the observation period, with a notable decline over time. Given that *M. quadrifasciata* prefers to forage under high humidity conditions (Maia-Silva et al. 2014; Oliveira-Abreu et al. 2014), we hypothesize that these fluctuations in humidity may explain the decline in bee foraging activity.

From a management perspective, monitoring bees leaving the hive, combined with basic microclimatic data, particularly temperature and humidity, allows farmers to anticipate colony health without the need for frequent hive openings. This approach is essential for optimising hive performance in open-field cropping systems. The low activity levels observed in some hives must be considered critically, as bee foraging activity is essential for colony maintenance and survival through resource collection (Maia-Silva et al. 2014). A previous study demonstrated that foraging activity in stingless bees correlates with colony size, independent of species-specific life history traits (Leão et al. 2024). Furthermore, hives with a larger number of adults were positively correlated with the number of brood cells and food storage pots (Leão et al. 2024), indicating healthy and active colonies (Hilário et al. 2000). We are confident that our approach provides a practical, effective, and minimally invasive alternative for farmers to monitor beehives, as it is directly related to bee foraging behaviour in a time-efficient manner (Leão et al. 2024). Despite the merits, it is necessary to consider that our samples were made after the beginning of bee foraging activity, which is a limitation of our study in this regard. Our study was conducted during the dry season in the Cerrado, when relative humidity can reach low levels (Oliveira and Marquis 2002). Therefore, further evaluations should be performed during other periods of the year and in different ecosystems.

The observed qualitative improvement in hives reinforces the idea that heterogeneous habitats can stimulate bee colony development (Goulson et al. 2015; Timberlake et al. 2021). Although we observed workers returning to hives with pollen grains adhered to their corbiculae and newly formed pollen storage pots, we did not observe *M. quadrifasciata* individuals foraging on tomato plants or nearby non-crop plants. *Melipona* bees can forage up to 2 km away and recruit over 1 km from their nest (Kuhn-Neto et al. 2009). Despite their generalist foraging behaviour, studies indicate that stingless bees prefer forest trees with mass flowering, even when these are available only at long distances (Ramalho 2004; Martins et al. 2023). Our pollen analysis confirms this foraging strategy, revealing a diet dominated by resources from trees and shrubs. Specifically, the high frequency of pollen from the genus *Miconia*, *Microlicia* and *Psidium* demonstrates an apparent reliance on the surrounding native vegetation and fruit trees rather than the agricultural crop. Notably, the study farms were surrounded by natural vegetation patches, located up to 200 m from the tomato crops (MS1 Figure S3).

With 60 bee species identified, encompassing all bee families recorded in Brazil, this study highlights the ecological richness and sustainability of organic agroecosystems. Among these, native species such as *P. lineata*, *E. analis*, *Pseudaugochlora* sp., and *E. auropilosa* were the most abundant in tomato crops. Consistent with previous findings in the Cerrado, *P. lineata* accounted for a substantial proportion of flower visits (77.83%), demonstrating a strong preference for tomato flowers in this region (Assunção et al. 2022). Although this species does not perform buzz-pollination, it was seen using its glossa (bee tongue) to access the poricidal anthers of tomato flowers (MS1 Figure S10). *Paratrigona lineata* can act as an effective pollinator of tomato flowers in the Cerrado, increasing fruit seed production by 30% (Bartelli et al. 2024). This is possible due to additional pollen-gathering behaviours that facilitate tomato pollination (e.g. ‘scraping’ and ‘licking’; Gaglianone et al. 2015; Bartelli et al. 2024), as has also been observed in other stingless bee species, such as *Nannotrigona testaceicornis* Lepeletier, 1836 (de Moura-Moraes et al. 2021).

The composition of bee species differed between the two floral groups. Tomato plants were visited primarily by species with specific behavioural or morphological traits that enhance their interaction with tomato flowers (Gaglianone et al. 2015). In contrast, non-crop plants were predominantly visited by eusocial generalist bee species that prefer plants with mass flowering and abundant nectar, such as *A. mellifera* (46.22%) and *T. spinipes* (8.56%) (Page and Williams 2023; Assunção et al. 2025). However, the species overlap between tomato and non-crop plants suggests that non-crop plants play a crucial role in attracting and sustaining tomato pollinators, as well as pollinators of other crops, in diversified agroecosystems (Assunção et al. 2022). By offering a diverse range of floral shapes, colours, traits, and sizes, non-crop plants support a broader assemblage of pollinator species with complementary functional traits (Bretagnolle and Gaba 2015; Escobedo-Kenefic et al. 2022).

Our findings on *M. quadrifasciata* pollen analysis provide a case study of this dynamic. The species adapted its foraging strategy to local floral availability around each farm, confirming that *Melipona* bees may meet their pollen requirements by foraging on pollen grains from a greater number of flowers (Antonini et al. 2006; Ramalho et al. 2007; da Luz et al. 2018). In addition to this foraging plasticity, our findings show that *M. quadrifasciata* exhibits a strong preference for woody plant families, particularly Melastomataceae and Myrtaceae, suggesting that these families are essential for maintaining colonies in anthropized regions (Barth et al. 2020; Vieira et al. 2022). The near-absence of tomato pollen in their diet, despite its proximity, strongly corroborates this, showing that bees actively chose higher-quality resources from the surrounding native vegetation.

The absence of significant differences in the richness, abundance, or diversity of visitors to tomato and non-crop flowers suggests that the introduced *M. quadrifasciata* hives did not negatively impact wild pollinators. Increased competition for resources is a primary concern when introducing managed bees (Goulson et al. 2015). However, our results indicate that competition may have been avoided due to the distinct foraging preferences of the managed and wild bees in this heterogeneous agroecosystem, where non-crop plants provided high floral availability (MS1 Figure S2). Another key aspect is that studies on hive introduction focused, not surprisingly, on *A. mellifera* and *Bombus* species (Iwasaki et al. 2022). However, the introduction of non-native hives has negatively impacted natural pollinator populations, primarily due to the dominance effects of *A. mellifera* (Garibaldi et al. 2021; Page and Williams 2023; Aguiar et al. 2024).

Although the management of stingless bees has only recently been considered for assisted pollination (Osterman et al. 2021a), the limited available evidence suggests that some could serve as a viable alternative to exotic bees. For example, the introduction of *Tetragonula iridipennis* Smith, 1854 hives did not negatively affect the abundance, visitation rate, or foraging time of wild pollinators in open-field watermelon and fennel crops (Layek et al. 2021, 2022). This study thus represents a pioneering contribution to assessing the impacts of introduced native stingless bee hives on wild pollinators in open-field neotropical agroecosystems. Furthermore, we highlight the need for long-term impact assessments, as our study was limited to a single harvest season.

Managed hives of *M. quadrifasciata* did not directly contribute to tomato pollination in this study. Instead, wild bees provided the primary pollination service. This result highlights a critical distinction between a species' potential to serve as a pollinator and the ecological service it actually provides in a complex landscape with alternative resources. Thus, introducing managed hives in open fields may not necessarily enhance pollination services, particularly when wild pollinators are already established and performing effectively (Winfree et al. 2008; Mallinger and Gratton 2015; Campbell et al. 2023). This is supported by the observed wild bee abundance and richness, as well as the impact of open pollination (OP), which improved fruit quality compared to self-pollination (SP). Pollinators facilitate more effective pollen transfer, promoting uniform fertilisation and seed development, which in turn influences fruit size and weight (Giannini et al. 2015; Khalifa et al. 2021).

We also observed slightly lower pest damage rates in OP fruits compared to SP. *Tuta absoluta*, responsible for substantial tomato yield losses worldwide (Biondi et al. 2018), caused the highest incidence of fruit damage in our study. However, the minor reduction in overall fruit quality may be attributed to *T. absoluta*'s low preference for oviposition in organic tomato plants (Medeiros et al. 2009). Additionally, it is reasonable to hypothesize that bee cross-pollination enhanced tomato plant vigour compared to self-pollination. Future research should therefore investigate the relationship between fruit development and pest infestation.

Assisted pollination may have a limited impact on farmers in organic and diversified agroecosystems, as natural pollination often does not require supplementation (Winfree et al. 2008; Mallinger and Gratton 2015) or introduced bees may forage on other crop and non-crop plants (Bänsch et al. 2020; Osterman et al. 2021b). Given this, rather than focusing solely on the introduction of managed hives, investing in habitat conservation and promoting naturally occurring pollinators may represent a more sustainable and cost-effective strategy for ensuring pollination services in diversified agroecosystems (Garibaldi et al. 2017; Isaacs et al. 2017; Jandt et al. 2025). Moreover, not all bee species are equally efficient at pollinating specific crops in open-field environments, where floral resources are abundant and bees may exhibit preferences for certain plants (Osterman et al. 2021b; Martins et al. 2023), as observed in our study. Therefore, further baseline studies are needed to better understand the foraging dynamics of bees in mass-flowering agroecosystems. Such knowledge will be valuable for designing farm landscapes that minimise competition among bees while maximising species complementarity.

5. Conclusion

While introducing *M. quadrifasciata* hives did not directly enhance tomato fruit quality, it demonstrated the potential for hive acclimatisation and colony development in organic agricultural landscapes. We provided a practical and minimally invasive approach for farmers to assess hive health by monitoring bee foraging activity under appropriate weather conditions.

The primary pollination services in tomato crops were provided by wild bees, particularly *P. lineata*, which exhibited a strong visitation rate for tomato flowers. This finding underscores the importance of conserving and promoting wild pollinator diversity in organic agroecosystems, as they play a critical role in sustaining pollination services. We highlight the need for habitat heterogeneity and the management of non-crop plants, which support a diverse assemblage of pollinators with complementary functional traits. Additionally, our findings point to three directions for future research. First, investigating the floral resources utilised by other stingless bees in agroecosystems and exploring the long-term impacts of hive introduction on wild pollinator communities across different cropping systems and ecosystems. Second, developing management techniques for *P. lineata* should be a promising alternative. Finally, future work should also evaluate the potential of other managed stingless bees for assisted pollination in tomato crops, such as species of *Nannotrigona*, *Frieseomelitta* and *Tetragonula*.

6. Acknowledgements

We are grateful to all farm owners that allowed us to conduct the experiments on their lands. EMATER-DF is acknowledged for its technical support and first contact with farmers. This study was supported by research grants and fellowships provided by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (code 001), Fundação de Apoio à Pesquisa do Distrito Federal – FADF (grant number 00193–00001013/ 2021–82) and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (grant number 405396/2021–0). PHBT is a CNPq fellow. We thank the INCT Pollination (CNPq 406976/2022-8, CAPES 88887.953442/2024-00, and FAPERJ).

7. Supplementary Information

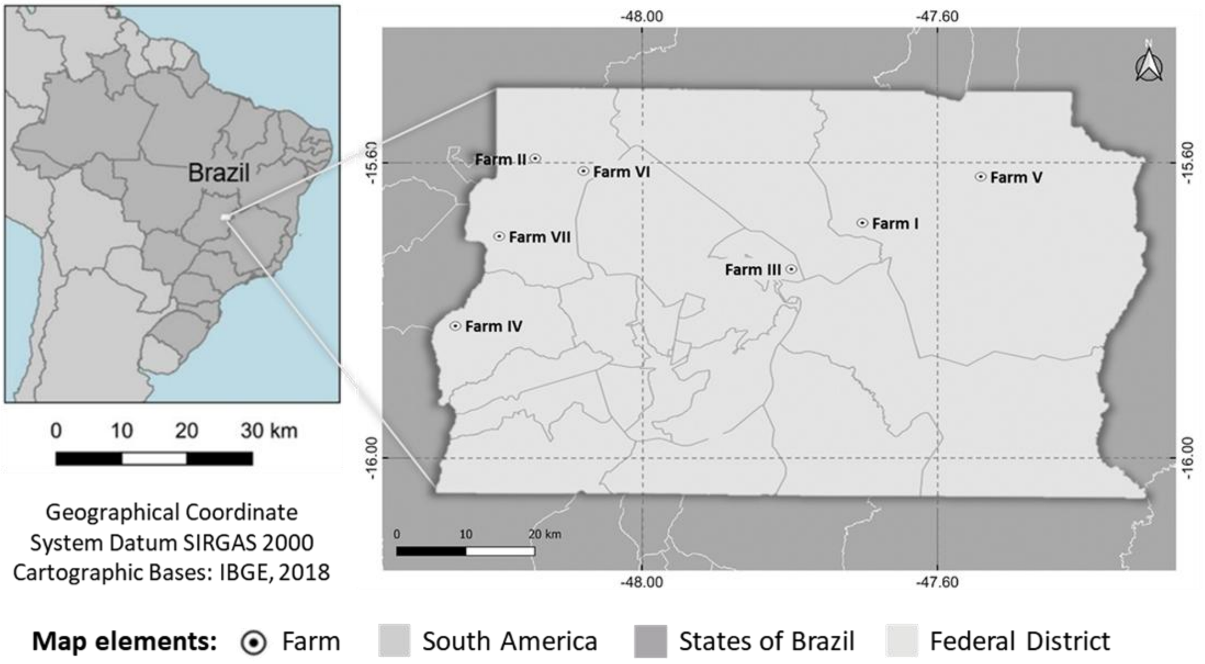


Figure S1 Geographic location of the Brazilian Federal District (15°46′48″S, 47°55′45″W), in the central area of Brazil and distribution of the tomato organic crops where the studies were conducted (circles with black points, Farms I to Farm VII).

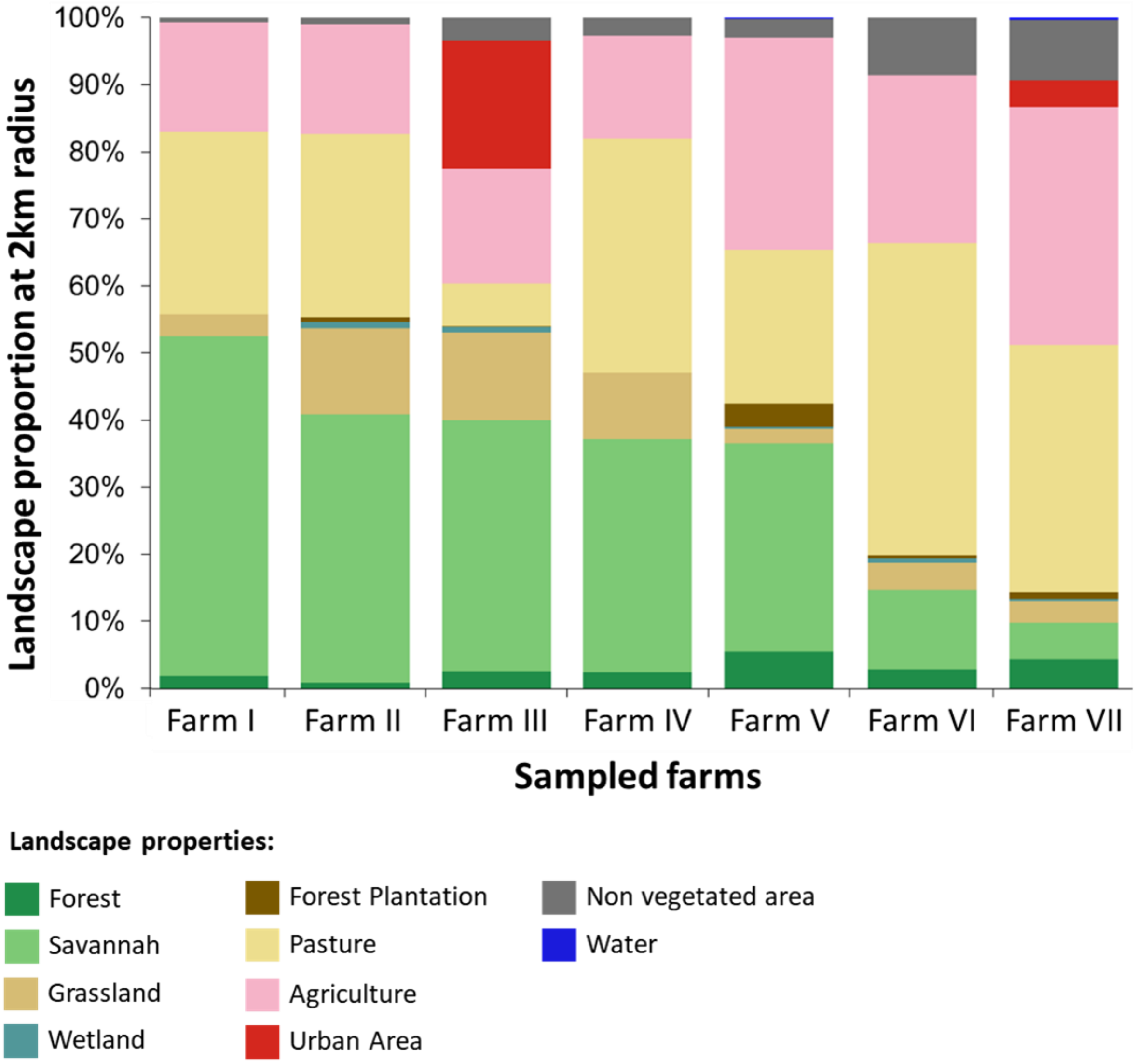


Figure S2 Landscape composition around tomato crops on seven farms in the Federal District, Brazil. The landscape proportion data reflects the coverage area of each land-use type within a 2 km radius buffer from the center of the cultivated area. The colors represent the different land-use types. Data refers to the eighth collection of the 2019 Cerrado biome map from the Annual Mapping of Land Use and Land Cover in Brazil (MapBiomas) project (Souza et al. 2020).

Figure S3 Satellite images of the study farms (Google Earth 2023) showing the location of tomato crops (yellow markmap) and surrounding landscape composition within a 2km radius.

Sketches on the right represent schematic diagrams of cultivated area at a distance of approximately 20 meters from the tomato crop, with colors denoting different types of crops.

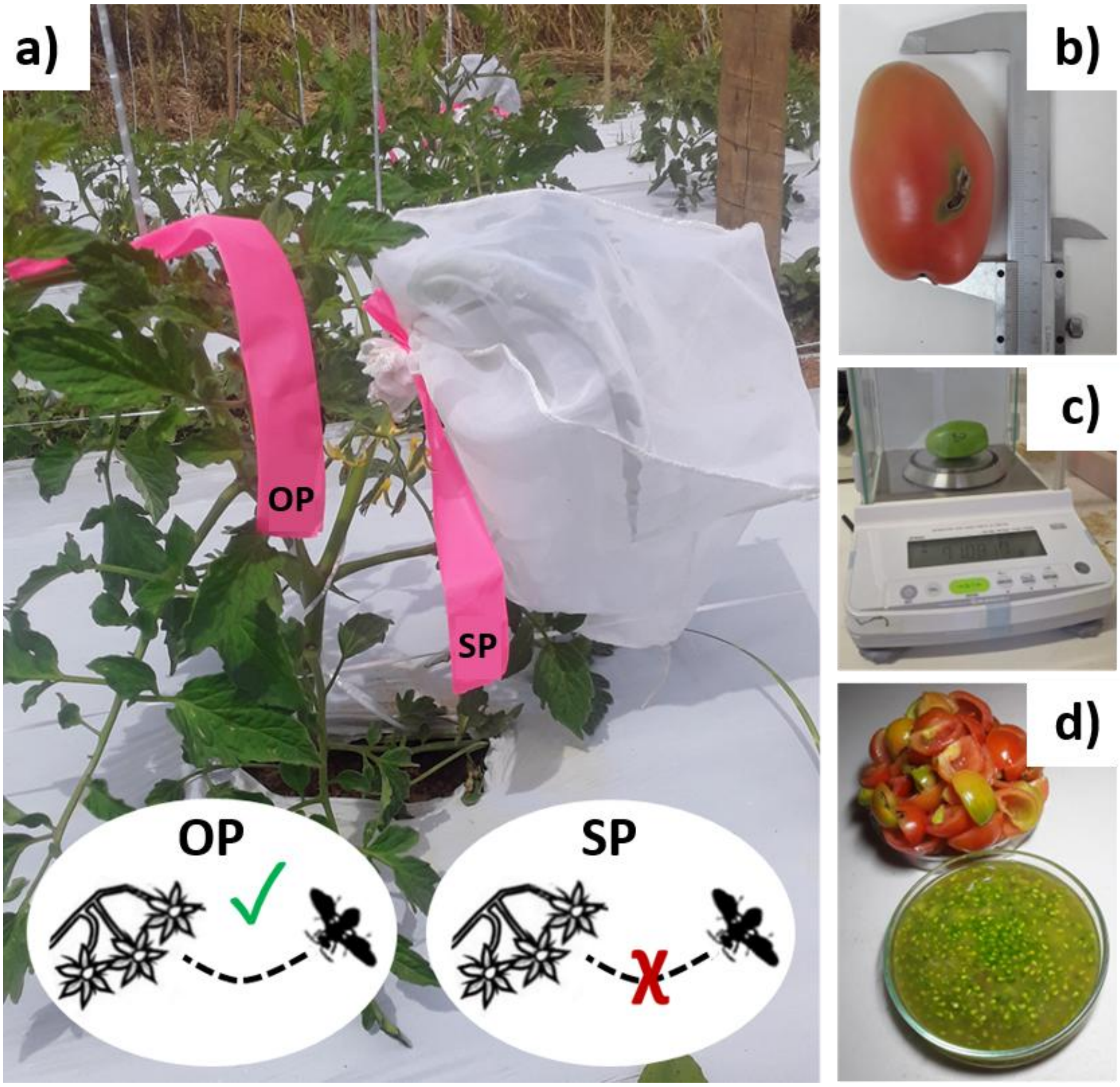


Figure S4 Experimental setup and fruit quality measurements of pollination treatments established in the tomato crops. (a) SP - self-pollination, whereby the tomato inflorescences were bagged in the pre-anthesis stage with material that allows only wind passage, thereby preventing bees from visiting the flower; OP - open-pollination, whereby bees are permitted to visit the flowers. (b) measurement of fruit length using a caliper. (c) assessment of fruit weight using an analytical scale. (d) evaluation of the number of seed production. The treatments were carried out in six tomato-producing organic farms located in the Brazilian Federal District - Brazil, from July to October 2023.

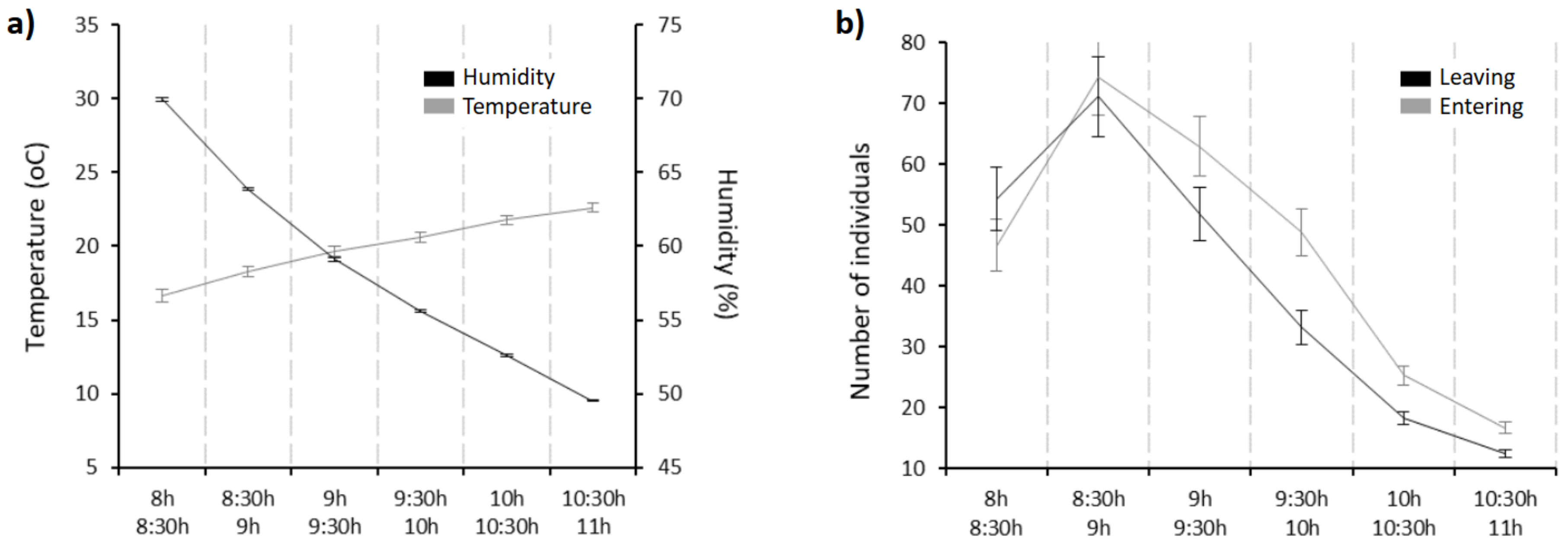
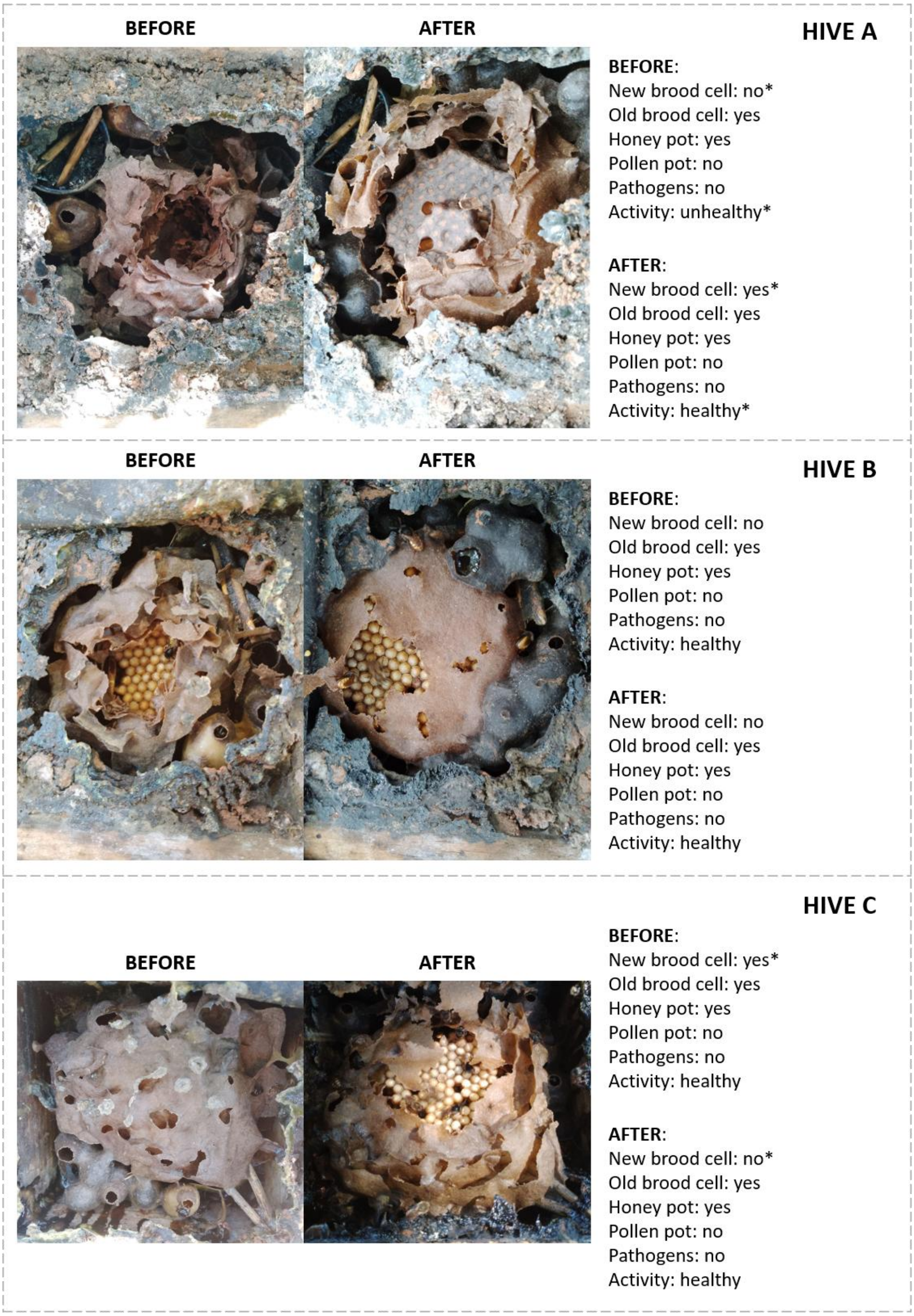


Figure S5 Temperature, humidity, and bee foraging activity over time (mean ± SE). a) variation in temperature (°C) (gray line) and humidity (%) (black line) over intervals of observation, time from 8h to 11h.; b) number of individuals leaving (black line) and entering (gray line) the colonies across the same time intervals.



HIVE A

BEFORE:
New brood cell: no*
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: unhealthy*

AFTER:
New brood cell: yes*
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy*

HIVE B

BEFORE:
New brood cell: no
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

AFTER:
New brood cell: no
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

HIVE C

BEFORE:
New brood cell: yes*
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

AFTER:
New brood cell: no*
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

Figure S6 Evaluation of qualitative parameters in hives A, B and C before and after their introduction into farms. The images depict internal hive conditions, showing the presence of pathogens, brood cells (both new and old), food storage pots (pollen and honey), and the overall activity of bees into the hives (healthy or unhealthy). The hives were installed on four farms with organic tomato crops located in the Brazilian Federal District - Brazil, from July to October 2023. Asterisks indicate the changes in hives following farm placement.

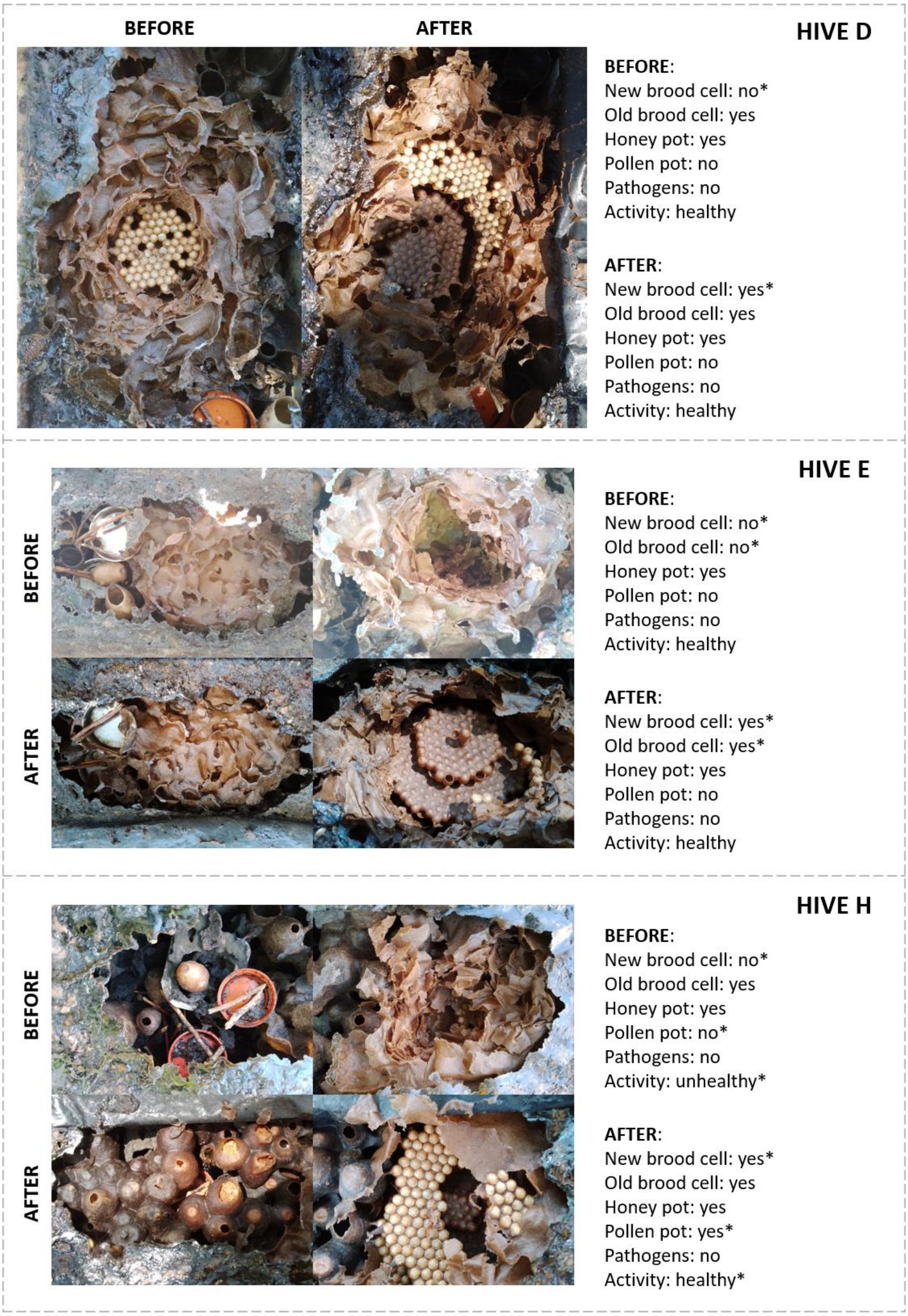
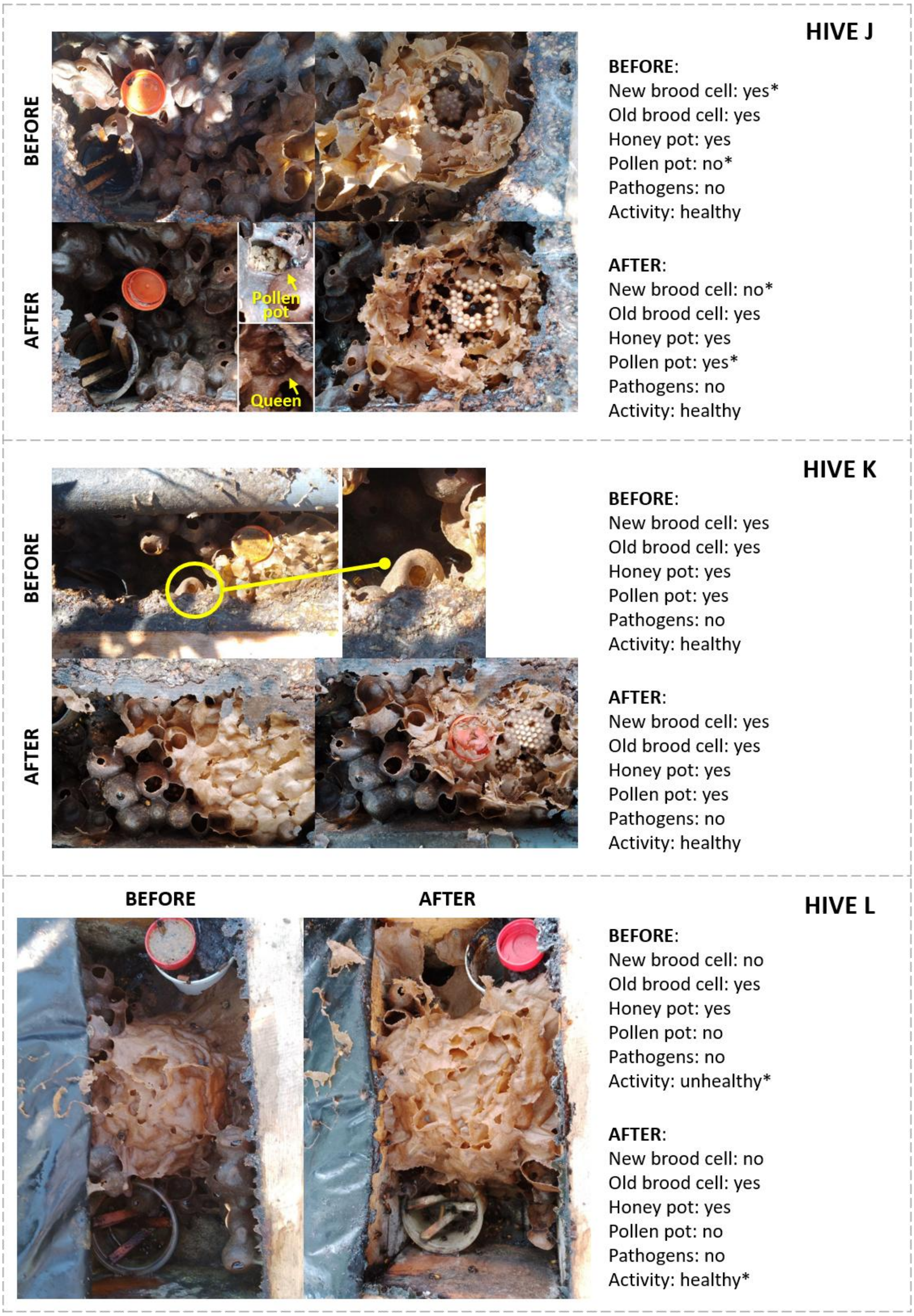


Figure S7 Evaluation of qualitative parameters in hives D, E and H before and after their introduction into farms. The images depict internal hive conditions, showing the presence of pathogens, brood cells (both new and old), food storage pots (pollen and honey), and the overall activity of bees into the hives (healthy or unhealthy). The hives were installed on four farms with organic tomato crops located in the Brazilian Federal District - Brazil, from July to October 2023. Asterisks indicate the changes in hives following farm placement.



HIVE J

BEFORE:
New brood cell: yes*
Old brood cell: yes
Honey pot: yes
Pollen pot: no*
Pathogens: no
Activity: healthy

AFTER:
New brood cell: no*
Old brood cell: yes
Honey pot: yes
Pollen pot: yes*
Pathogens: no
Activity: healthy

HIVE K

BEFORE:
New brood cell: yes
Old brood cell: yes
Honey pot: yes
Pollen pot: yes
Pathogens: no
Activity: healthy

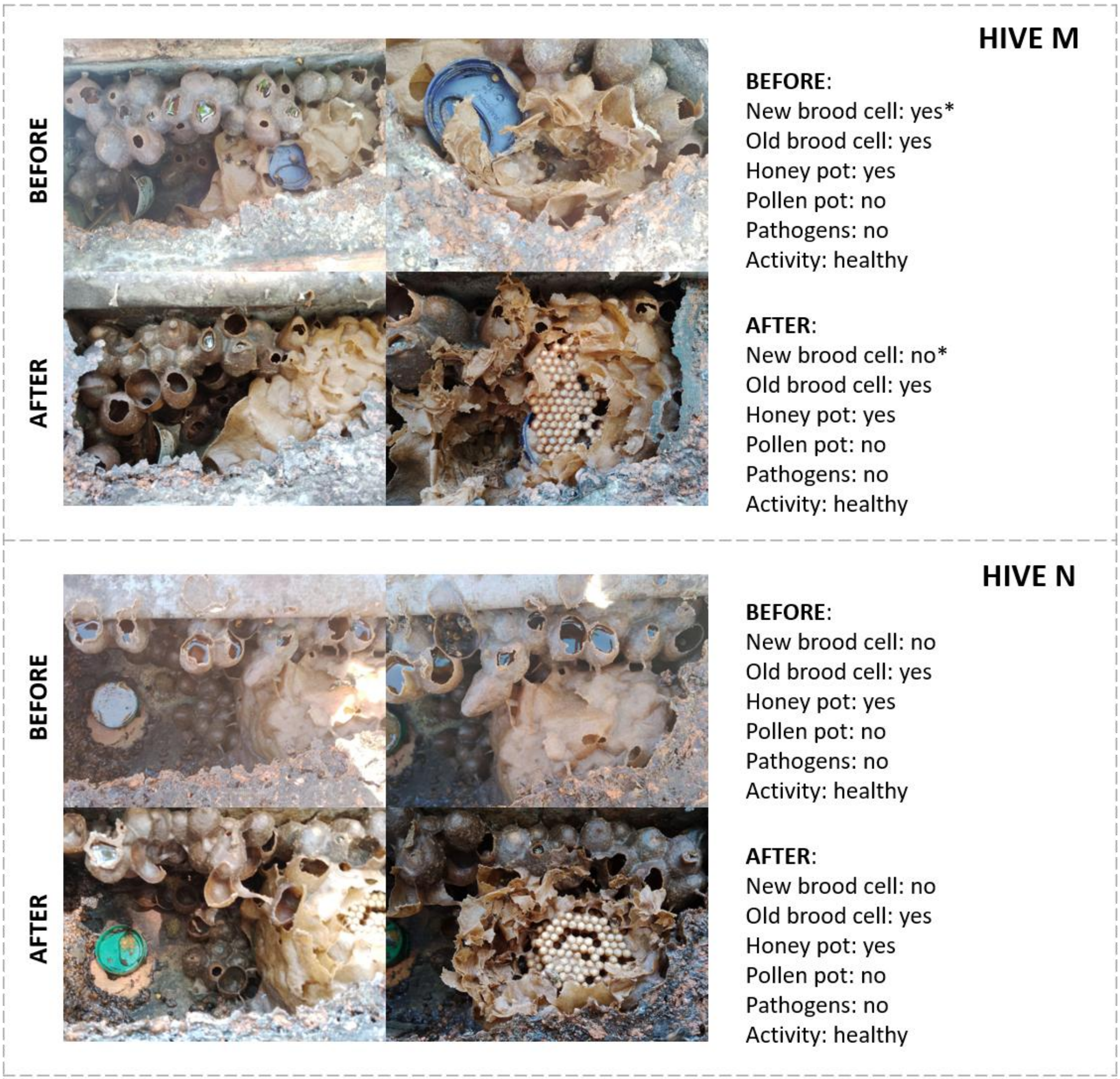
AFTER:
New brood cell: yes
Old brood cell: yes
Honey pot: yes
Pollen pot: yes
Pathogens: no
Activity: healthy

HIVE L

BEFORE:
New brood cell: no
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: unhealthy*

AFTER:
New brood cell: no
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy*

Figure S8 Evaluation of qualitative parameters in hives J, K and L before and after their introduction into farms. The images depict internal hive conditions, showing the presence of pathogens, brood cells (both new and old), food storage pots (pollen and honey), and the overall activity of bees into the hives (healthy or unhealthy). The hives were installed on four farms with organic tomato crops located in the Brazilian Federal District - Brazil, from July to October 2023. Asterisks indicate the changes in hives following farm placement.



HIVE M

BEFORE:
New brood cell: yes*
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

AFTER:
New brood cell: no*
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

HIVE N

BEFORE:
New brood cell: no
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

AFTER:
New brood cell: no
Old brood cell: yes
Honey pot: yes
Pollen pot: no
Pathogens: no
Activity: healthy

Figure S9 Evaluation of qualitative parameters in hives M and N before and after their introduction into farms. The images depict internal hive conditions, showing the presence of pathogens, brood cells (both new and old), food storage pots (pollen and honey), and the overall activity of bees into the hives (healthy or unhealthy). The hives were installed on four farms with organic tomato crops located in the Brazilian Federal District - Brazil, from July to October 2023. Asterisks indicate the changes in hives following farm placement.

Figure S10 *Paratrigona lineata* foraging on tomato flowers in organic crops located in the Brazilian Federal District - Brazil.

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CONCLUSIONS

The central problem addressed by this thesis was the need to move beyond single-scale approach and develop an integrated understanding of how pollination service is maintained in human-modified tropical organic agroecosystems.

Addressing the first objective, the results showed that while landscape-level natural vegetation cover determines the regional species pool of wild bees, farm-level floral diversity modulates local species presence and their interaction patterns. These network patterns, in turn, were also predictors of pollination success than simple bee abundance or richness alone. The second objective was met by a systematic review which confirmed that microsatellite markers are a versatile and broadly used tool in bee research, validating their use in this thesis. Applying this approach to the third objective, the landscape genetics study of *E. analis* revealed that despite its abundance being highly sensitive to local resources, the species maintained a single, well-connected genetic population across the study region, probably using remnants of natural vegetation as 'stepping stones' for dispersal. Finally, the fourth objective was addressed by showing that introducing managed *M. quadrifasciata* hives is not a substitute for a biodiverse and functional ecosystem, as their success also depends on the ecological integrity of the agroecosystem.

Together, these findings point to the main conclusion of this thesis: pollination services in tropical agroecosystems are shaped by a hierarchy of ecological process, where conditions at the large scale determine what is possible at the smaller scale. At the broadest scale at the landscape-level, the composition of natural vegetation acts as the primary source of species, shaping not only the regional pool of potential bee species but also the very pathways that enable their genetic connectivity. Within this landscape context, at the farm-level, a second process operates. On-farm floral diversity and management practices determine the permeability of the agroecosystem, modulating which species from the regional pool are present and whether introduced managed bees can thrive. However, the potential set by the landscape and the farm is only realized at the level of the actual plant-pollinator interactions, where the available biodiversity is translated into the actual ecosystem service. This thesis concludes that the mere presence or abundance of species, wild or managed, is insufficient. The structure of the realized plant-bee interactions is fundamental to predicts pollination service. Therefore, the key to a resilient and functional agroecosystem lies not just in ensuring species availability, but in fostering the local conditions that allow for effective interactions between bees and crop flowers to occur.

This thesis also offers key recommendations for farmers and land managers. The primary management goal should be the conservation of wild pollinators through the maintenance of natural habitats and on-farm floral diversity, a strategy that supports both ecosystem function and genetic connectivity. Additionally, investing solely in assisted pollination cannot compensate for the loss of wild pollinators caused by natural habitat degradation in the Cerrado. The findings also caution against the uncritical adoption of managed hives for pollination, demonstrating that their success is context-dependent and requires local validation. This caution is particularly relevant to the widespread practice of translocating bee colonies across different biomes in Brazil. Such long-distance transportation can compromise hive health by introducing bees to environments with unfamiliar or insufficient floral resources. Finally, this study provides a tangible, minimally invasive method for farmers to monitor hive health, empowering them to make informed management decisions.

In conclusion, this thesis shows that ensuring pollination for sustainable agriculture is not about finding simple solutions, but about understanding and managing its complexity. By addressing ecological interactions across different scales, from landscapes to farms and flower visitors, it is possible to create agroecosystems that are both productive and resilient. Expanding this research is essential for developing effective, evidence-based pollination strategies for different regions.