

UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

Instituto de Biociências – Câmpus de Botucatu

Seção Técnica de Pós-Graduação



PROGRAMA DE PÓS-GRADUAÇÃO ZOOLOGIA

Insights into temporal and spatial dynamics of plant-bee interactions: exploring the complex role of *Apis mellifera*

LEANDRO HACHUY FILHO

**BOTUCATU – SP
2025**

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FELIPE W. AMORIM

Tese apresentada ao Programa de Pós-Graduação em Zoologia, Instituto de Biociências de Botucatu, Universidade Estadual Paulista “Júlio de Mesquita Filho”, como requisito para a futura obtenção do título de Doutor em Ciências (Programa de Pós-Graduação em Zoologia).

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE LEANDRO HACHUY FILHO, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA), DO INSTITUTO DE BIOCIÊNCIAS - CÂMPUS DE BOTUCATU.

Aos 18 de dezembro de 2025, às 9h, no(a) Sala de aula da Pós-graduação - Botânica, realizou-se a defesa de TESE DE DOUTORADO de LEANDRO HACHUY FILHO, intitulada **Insights into temporal and spatial dynamics of plant-bee interactions: exploring the complex role of *Apis mellifera***. A Comissão Examinadora foi constituída pelos seguintes membros: Prof. Dr. FELIPE WANDERLEY DE AMORIM (Orientador(a) - Participação Presencial) do(a) Departamento de Biodiversidade e Bioestatística / Instituto de Biociências - UNESP Botucatu, Profa. Dra. ERIKA MAYUMI SHIMABUKURO (Participação Virtual) do(a) Departamento de Biodiversidade e Bioestatística / Instituto de Biociências - UNESP Botucatu, Profa. Dra. CAMILA SILVEIRA DE SOUZA (Participação Virtual) do(a) FAENG / Universidade Estadual de Montes Claros, Prof. Dr. PEDRO JOAQUIM BERGAMO (Participação Virtual) do(a) Departamento de Ecologia / Instituto de Biociências - UNESP Rio Claro, Pós-doutorando GABRIEL FELLIPE BARROS RODRIGUES (Participação Presencial) do(a) Departamento Biodiversidade e Bioestatística / Universidade Estadual Paulista - UNESP, Após a exposição pelo doutorando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final: ___APROVADO___. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Prof. Dr. FELIPE WANDERLEY DE AMORIM

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Resumo

HACHUY-FILHO, L. **Percepções sobre a dinâmica temporal e espacial das interações planta-abelha: explorando o papel complexo de *Apis mellifera***. 2025. Defesa de Tese de Doutorado – Instituto de Biociências de Botucatu, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Botucatu, 2025.

As interações entre plantas e abelhas dão suporte a reprodução da maioria das angiospermas e conferem estabilidade aos ecossistemas terrestres. Entretanto, esse mutualismo tem sido progressivamente ameaçado por fatores antrópicos como a invasão de espécies. Apesar do crescente interesse em compreender como essas pressões remodelam as redes de interação ecológicas, ainda há escassez de referências históricas e de investigação em múltiplas escalas de organização. Esta tese investiga o papel ecológico da *Apis mellifera* (um polinizador amplamente distribuído, super-generalista) nas dimensões temporal e espacial, a fim de determinar como sua presença influencia a estrutura, o funcionamento e a estabilidade das redes de interação planta-abelha. Utilizando um conjunto de dados históricos singular, o Capítulo 1 reconstrói uma rede planta-abelha de um fragmento de Cerrado já extinto, revelando uma comunidade diversa, altamente compartimentalizada, com forte partição de nicho e numerosas interações especializadas. A presença de *A. mellifera* na ocasião do estudo foi relevante, porém limitada, indicando fases iniciais de sua integração ecológica associadas à expansão de sua distribuição no Brasil. No Capítulo 2, uma análise global comparativa de 80 redes quantitativas de regiões dentro e fora da distribuição nativa da espécie mostra que seus efeitos são dependentes do contexto: propriedades estruturais da rede, como aninhamento e modularidade, permanecem estáveis, enquanto subestruturas em escalas mais finas (incluindo frequências de motivos) indicam crescente homogeneização ecológica em regiões invadidas. O Capítulo 3 apresenta um experimento de campo manipulativo em um fragmento moderno de Cerrado, demonstrando que a introdução de colônias de *A. mellifera* provoca mudanças de curto prazo na equabilidade da comunidade de abelhas, não associadas a perdas imediatas de espécies. Em conjunto, os capítulos mostram que *A. mellifera* exerce efeitos moderados, porém amplos, ao longo de diferentes escalas organizacionais, influenciando a arquitetura das redes, e os papéis das espécies. Os resultados ressaltam que os impactos da abelha *A. mellifera* são intensificados em ambientes simplificados ou degradados, enquanto comunidades diversas, com forte segregação de nicho, amortecem as pressões competitivas. Ao integrar estudos históricos, comparações globais e evidências experimentais, esta tese demonstra a importância da escala e do contexto ecológico na avaliação de invasões biológicas e oferece suporte para conservação das ameaçadas interações entre abelhas e plantas.

Palavras-chave: *Apis mellifera*, Invasão biológica, Ecologia de redes

Abstract

HACHUY-FILHO, L. **Insights into temporal and spatial dynamics of plant-bee interactions: exploring the complex role of *Apis mellifera***. 2025. PhD thesis defense – Institute of Biosciences of Botucatu, São Paulo State University “Júlio de Mesquita Filho,” Botucatu, 2025.

Plant–bee interactions underpin the reproduction of most flowering plants and the stability of terrestrial ecosystems, yet these mutualisms are increasingly threatened by habitat loss, biological invasions, and global pollinator declines. Despite growing interest in understanding how these pressures reshape ecological networks, long-term baselines and multi-scale assessments remain scarce, especially in the Neotropics. This thesis investigates the ecological role of *Apis mellifera* (a globally distributed, highly generalist pollinator) across temporal, spatial, and mechanistic dimensions to determine how its presence influences the structure, function, and stability of plant–bee networks. Using a unique historical dataset, Chapter 1 reconstructs a 50-year-old plant–bee network from a Cerrado fragment, revealing a diverse and highly compartmentalized community with strong niche partitioning, numerous specialized interactions, and a core of native generalist bees. The early presence of *A. mellifera* was detectable but limited, suggesting initial stages of ecological integration during its expansion in Brazil. In Chapter 2, a global comparative analysis of 80 quantitative networks from regions inside and outside the species’ native range shows that honeybee effects are context-dependent: broad network properties such as nestedness and modularity remain largely unchanged, but fine-scale structures (including motif frequencies and species roles) shift in ways consistent with increasing ecological homogenization in non-native ranges. Chapter 3 presents a manipulative field experiment in a modern Cerrado fragment, demonstrating that the introduction of honeybee colonies drives short-term shifts in community diversity and composition. Together, these chapters show that *A. mellifera* exerts subtle yet pervasive effects across organizational scales, influencing network architecture, species roles, and resource partitioning. The findings underscore that honeybee impacts are amplified in simplified or degraded environments, whereas diverse communities with strong niche segregation buffer competitive pressures. By integrating historical baselines, global comparisons, and experimental evidence, this thesis highlights the importance of scale and ecological context when assessing biological invasions and provides essential insights for conserving pollination networks in the increasingly threatened Cerrado biome.

Keywords: Plant–bee networks, *Apis mellifera*, Biological Invasions, Network ecology

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1 GENERAL INTRODUCTION

1.1 INTRODUCTION

1.1.1 Plant–Bee Interactions in a changing world

Pollination is one of the most essential ecological processes on Earth, sustaining the reproduction of most flowering plants and maintaining the structure of terrestrial ecosystems (Benton et al. 2022). Bees, in particular, play a disproportionately important role in this process (Ollerton et al. 2017). With more than 20,000 described species, bees occupy an enormous range of ecological niches and exhibit behavioral and morphological traits that make them highly efficient pollinators (Michener 2007). Their interactions with flowers underpin the reproduction of the majority of angiosperms (Ollerton et al. 2011), contribute to ecosystem resilience, and support agricultural production worldwide (Klein et al. 2007). Yet despite their importance, wild bee populations are facing widespread decline across continents (Panziera et al. 2022). Numerous studies have documented reductions in bee abundance, species richness, or geographic range, particularly over the last four decades (Winfree et al. 2009; Goulson et al. 2015; Zattara & Aizen 2021). These declines are driven by a combination of habitat loss, agricultural intensification, climate change, pesticide exposure, emerging pathogens, and biological invasions (Potts et al. 2010; Sánchez-Bayo & Wyckhuys 2019; MacInnis et al. 2023; Gekière et al. 2025).

Such pressures not only affect individual species but also reverberate throughout ecological communities by altering the patterns of interaction between plants and pollinators (López-Vázquez et al. 2024). Because plant–pollinator systems form complex mutualistic networks, the loss or modification of interactions can reshape community structure, reduce functional redundancy, and weaken ecosystem stability (Memmott et al. 2004; Bascompte & Jordano 2007; Aizen et al. 2012). Understanding how pollination systems respond to rapid anthropogenic change requires analytical tools capable of capturing these interaction-level dynamics.

1.1.2 Ecological networks as tools for detecting change

Ecological network theory provides a powerful framework for analyzing mutualisms such as pollination (Valdovinos & Marsland III 2021). Rather than focusing on species in isolation, network approaches investigate the distribution and organization of interactions across entire communities, revealing patterns of resource partitioning, the structural roles of species, and the ways in which disturbances propagate through systems (Bascompte & Jordano 2007; Ramos-Jiliberto et al. 2020). Metrics such as nestedness, modularity, connectance, and specialization capture complementary aspects of network architecture. For example, nestedness reflects a tendency for specialist species to interact with subsets of the partners used by generalists, a pattern associated with increased robustness to species loss (Bascompte et al. 2003). Modularity captures how interactions are compartmentalized into subsets of species that interact more frequently among themselves than with others (Olesen et al. 2007). This structure may localize the effects of disturbances, enhancing persistence at the community level (Stouffer & Bascompte 2011). Connectance and specialization, in turn, provide insight into the density and exclusivity of interactions, often reflecting the balance between generalist and specialist strategies (Vázquez et al. 2007).

Beyond these macro-scale properties, finer-scale analyses are increasingly recognized as essential for detecting network-level responses to environmental change (Lanuza et al. 2023). Meso-scale metrics, such as motifs, capture recurring sub-structures that are sensitive to competition, invasions, and shifts in interaction strength (Simmons et al. 2019). Micro-scale analyses, such as species-level centrality or within-module degree, reveal the structural importance of individual taxa and can identify keystone pollinators whose loss disproportionately affects community stability (González et al. 2010; Saavedra et al. 2011). Without incorporating these multiple analytical scales, important ecological changes may remain obscured. This complexity is particularly relevant when assessing the impacts of widespread, behaviorally flexible pollinators such as *Apis mellifera*.

1.1.3 *Apis mellifera*: A globally distributed and ecologically transformative species

The western honeybee *Apis mellifera*, native to Africa, Europe, and western Asia (Dogantzis et al. 2021), has become one of the most globally distributed animals due to centuries of human-assisted dispersal for beekeeping and agricultural pollination (Carpenter

& Harpur 2021). Several biological traits underlie its invasive success. Honeybees form perennial colonies with tens of thousands of individuals, enabling them to achieve numerical dominance in many environments. They are extremely efficient at locating and recruiting nestmates to profitable floral resources via complex communication mechanisms such as the waggle dance (Seeley 1995). Their foraging behavior is highly generalist, allowing them to exploit a broad range of floral resources and thrive in diverse habitats (Wcislo et al. 2017). These traits collectively facilitate establishment and proliferation far beyond the species' native range.

However, the same traits that make *A. mellifera* successful also amplify its potential ecological impacts. Studies across different biomes show that honeybees may compete with native pollinators for floral resources, alter visitation frequencies, disrupt plant reproduction, and restructure plant–pollinator networks (Paini 2004; Stout & Morales 2009; Valido et al. 2019). Their super-generalist foraging patterns can lead to ecological homogenization by reducing the relative influence of specialist or functionally unique pollinators (Traveset et al. 2013). Yet, the magnitude of these impacts varies considerably among ecosystems and depends on context-specific factors such as floral resource availability, native bee diversity, and habitat integrity (Roubik & Wolda 2001; Hachuy-Filho et al. 2020; Ballarin et al. 2022; Sponsler et al. 2023). This context dependency suggests that honeybee effects are not uniform, reinforcing the need for studies that integrate temporal, spatial, and experimental perspectives.

1.1.4 The critical but understudied dimension of time: historical baselines

A major limitation in pollination ecology is the scarcity of long-term or historical datasets. Because most ecological networks have been sampled recently (often in already degraded or heavily modified landscapes) it remains difficult to determine whether contemporary patterns reflect long-term ecological dynamics or recent anthropogenic disturbances. Historical datasets are invaluable because they reveal past states of ecosystems, including species interactions that may no longer exist, and they provide baselines for evaluating the trajectory and magnitude of ecological change (Burkle et al. 2013). In particular, they allow researchers to detect shifts in interaction specificity, network

robustness, and functional roles that occur gradually over decades.

In the Neotropics, long-term interaction datasets are exceptionally rare. For the Brazilian Cerrado, a biome that has undergone dramatic transformations since the mid-twentieth century, historical records are almost nonexistent. Thus, the opportunity to reconstruct a half-century-old plant–bee interaction network is unique and scientifically significant. Such reconstruction makes it possible to infer how pollination systems functioned before extensive land-use change, before widespread pesticide use, and during the early phases of honeybee expansion in South America (Kerr 1957; Michener 1975; De Jong 1996). These historical insights are crucial for understanding how Cerrado pollination networks have responded to decades of ecological pressure.

1.1.5 The Cerrado: A Biodiversity Hotspot facing intense anthropogenic pressure

The Brazilian Cerrado is recognized as one of the world’s major biodiversity hotspots (Myers et al. 2000). This tropical savanna harbors extraordinary botanical diversity (including numerous endemic species) and supports one of the richest bee assemblages in the Neotropics (Silberbauer-Gottsberger & Gottsberger 1988; Oliveira & Gibbs 2000). Many Cerrado bees are specialized, such as oil-collecting species that rely exclusively on floral oils from Malpighiaceae, making the biome a reservoir of unique pollination strategies (Gottsberger & Silberbauer-Gottsberger 2006).

However, the Cerrado has experienced extensive and rapid land conversion driven by mechanized agriculture and cattle ranching. More than 50% of its original vegetation cover has been lost (Carvalho et al. 2009), leading to fragmentation, homogenization of plant communities, and reduction of floral resource diversity (Strassburg et al. 2017). These transformations have reshaped pollinator assemblages, often favoring generalist or disturbance-tolerant species and potentially increasing interactions with invasive honeybees (Hachuy-Filho et al. 2020). In this context, understanding how *A. mellifera* integrates into Cerrado interaction network (historically, globally, and experimentally) is critical for predicting future ecological outcomes and informing conservation strategies.

1.1.6 Rationale and integrative framework of this thesis

This thesis adopts a multi-scale, multi-context approach to evaluate the ecological role and impact of *Apis mellifera* on plant–bee interactions across temporal, spatial, and mechanistic dimensions.

Temporal Perspective — Chapter 1

Chapter 1 reconstructs a plant–bee network from the early 1970s using a unique dataset collected in a Cerrado fragment. This historical perspective makes it possible to characterize network architecture (such as nestedness, modularity, and connectance) during a period when the habitat was still relatively intact. The chapter examines niche partitioning among bee species, the functional roles of generalists and specialists, and the early integration of *A. mellifera* soon after its introduction to Brazil. This analysis establishes an essential baseline that helps contextualize contemporary Cerrado communities, which are now more fragmented and floristically impoverished.

Spatial Perspective — Chapter 2

Chapter 2 expands the investigation to a global scale by analyzing 80 quantitative plant–bee networks from both the native and non-native ranges of *A. mellifera*. By comparing networks across biogeographic regions, this chapter tests whether honeybee presence leads to structural convergence (i.e., whether networks become more similar worldwide) or whether unique patterns emerge depending on ecological context. Importantly, the chapter examines network structure at macro-, meso-, and micro-scales, acknowledging that changes in species roles or motif frequencies may occur even when broad properties such as nestedness remain unchanged.

Experimental Perspective — Chapter 3

Chapter 3 presents a manipulative field experiment in which honeybee colonies are introduced into a Cerrado fragment. This experimental approach allows direct assessment of

short-term changes in the structure of native bee communities, demonstrated by shifts in community diversity and composition. By identifying mechanisms underlying competitive interactions, this chapter helps explain broader patterns observed in the historical and global analyses.

1.1.7 Integrative Thesis Argument

Together, these chapters provide a comprehensive understanding of the role of *Apis mellifera* in shaping plant–bee networks. The results indicate that honeybee effects are often subtle in magnitude but widespread in scope, influencing multiple structural components of ecological networks. They also demonstrate that impacts detectable at fine scales (such as species roles or motif participation) may remain obscured when network changes are evaluated solely at broad scales, underscoring the importance of multi-level analytical approaches. Furthermore, the thesis shows that ecological context plays a central role in mediating honeybee effects: communities with high floral resource diversity or strong niche partitioning tend to buffer honeybee influence, whereas more simplified or degraded environments may amplify competitive interactions (Hachuy-Filho et al. 2020; Andena et al. 2012).

Finally, the work highlights that network structure itself provides a sensitive and informative tool for detecting honeybee-driven changes in ecological communities. Nestedness, modularity, species roles, and motif frequencies each reveal distinctive aspects of how interactions are reorganized in the presence of abundant invasive pollinators. By integrating temporal baselines, global scale comparisons, and experimental manipulations, this thesis illustrates that understanding the ecological consequences of biological invasions requires an approach attentive to scale, context, and network-level dynamics.

1.2 OBJECTIVES AND HYPOTHESES

The overarching aim of this thesis is to investigate how *Apis mellifera* influences plant–bee interactions across temporal, spatial, and ecological contexts. By combining historical

reconstruction, global comparative analyses, and manipulative field experiments, the thesis seeks to reveal how honeybee presence shapes network structure, species roles, and resource partitioning at multiple organizational scales. The specific objectives and associated hypotheses of each chapter are presented below.

1.2.1 Objective 1 – Historical Perspective (Chapter 1)

Objective:

To reconstruct a historical plant–bee interaction network from a Cerrado fragment sampled in the early 1970s and characterize its structure, patterns of resource partitioning, and the early integration of *Apis mellifera*.

Hypotheses:

Historical networks exhibit high specialization and strong modularity, reflecting a diverse and functionally rich bee assemblage prior to extensive habitat conversion and ecological homogenization.

Niche partitioning among native bees is well defined, with clear separation between generalist and specialist taxa based on floral resource use.

Early presence of *A. mellifera* shows limited but detectable integration, with honeybees occupying peripheral or connector roles that do not yet dominate network structure.

1.2.2 Objective 2 – Global spatial perspective (Chapter 2)

Objective:

To compare plant–bee network structure inside and outside the native distribution of *Apis mellifera* using 80 quantitative networks, evaluating differences at macro-, meso-, and micro-scales.

Hypotheses:

Macro-scale network metrics (e.g., nestedness, modularity) will show minimal differences between native and non-native ranges, reflecting the general structural robustness of plant–pollinator networks.

Meso-scale patterns, such as motif frequencies, will differ between ranges, with networks outside the native range exhibiting increased motif configurations indicative of competition or dominance by generalist species.

Micro-scale species roles of *A. mellifera* will shift across its range, with honeybees acting as more central and structurally influential nodes outside their native distribution due to higher relative abundance and behavioral dominance.

Overall, networks in the non-native range will show signs of structural homogenization, consistent with ecological convergence driven by widespread honeybee introduction.

1.2.3 Objective 3 – Experimental/Mechanistic Perspective (Chapter 3)

Objective:

To assess the short-term ecological effects of honeybee colony introduction on native bee assemblages and floral resource dynamics in a Cerrado fragment through a manipulative field experiment.

Hypotheses:

Honeybee introduction will lead to increased competition for floral resources, resulting in reduced visitation rates by native bees, especially during periods of limited resource availability.

Native bees will adjust their foraging patterns (either temporally, spatially, or taxonomically) in response to honeybee presence, reflecting behavioral displacement or niche shifts.

Floral resource partitioning will become less pronounced, with honeybees

disproportionately exploiting abundant or high-reward plant species, potentially reducing interaction diversity among native pollinators.

Short-term changes in interaction patterns will mirror fine-scale processes that explain larger spatial and temporal trends identified in Chapters 1 and 2.

1.2.4 Synthesis

Together, these objectives and hypotheses integrate temporal baselines, geographic variability, and experimental mechanisms to evaluate the ecological influence of *Apis mellifera* from multiple complementary perspectives. This integrative framework allows for a deeper understanding of how invasive generalist pollinators reshape mutualistic networks and highlights the importance of considering scale and context when assessing biological invasions in pollination systems.

1.3 REFERENCES

- Aizen MA, Sabatino M & Tylianakis JM (2012) Specialization and rarity predict nonrandom loss of interactions from mutualist networks. *Science* 335: 1486–1489.
- Andena SR, Nascimento FS & Del Lama MA (2012) Africanized honey bees in Brazil: state of the art. *Sociobiology* 59: 1–20.
- Ballarin CS, Arbetman MP, Aizen MA (2022) Invasive honeybees reduce the space for coexistence of native pollinators in a Patagonian forest. *Biol Invasions* 24: 3015–3029.
- Bascompte J & Jordano P (2007) Plant–animal mutualistic networks: the architecture of biodiversity. *Annu Rev Ecol Evol Syst* 38: 567–593.
- Bascompte J, Jordano P, Melián CJ & Olesen JM (2003) The nested assembly of plant–animal mutualistic networks. *Proc Natl Acad Sci USA* 100: 9383–9387.
- Benton MJ, Wilf P, & Sauquet H. (2022) The Angiosperm Terrestrial Revolution and the origins of modern biodiversity. *New Phytologist* 233: 2017–2035.
- Burkle LA, Marlin JC & Knight TM (2013) Plant–pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339: 1611–1615.
- Carpenter J & Harpur BA (2021) Honey bee genetics: diverse origins, conserved traits. *Curr Opin Insect Sci* 46: 81–88.
- Carvalho FMV, Del Rio CM, Ribeiro MC, et al. (2009) The Brazilian Cerrado: a threatened biodiversity hotspot. *Conserv Sci Pract* 1: 1–6.

- De Jong D (1996) Africanized honey bees in Brazil, twenty years of adaptation and success. *Bee World* 77: 67–70.
- Dogantzis KA, Tiwari T, Conflitti IM, et al. (2021) Thrice out of Africa: molecular phylogenetics and biogeography of *Apis mellifera*. *Mol Phylogenet Evol* 155: 107024.
- Garibaldi LA, Pérez-Méndez N, Cordeiro GD, et al (2021) Negative impacts of dominance on bee communities: Does the influence of invasive honey bees differ from native bees? *Ecology* 102: 12.
- Gekière A, Gérard M, Potts SG, et al. (2025) Underlying mechanisms shaping wild bee decline. *Biological Journal of the Linnean Society* 145: blaf043.
- González AMM, Dalsgaard B & Olesen JM (2010) Centrality measures and the importance of generalist species in pollination networks. *Ecol Complex* 7: 36–43.
- Gottsberger G & Silberbauer-Gottsberger I (2006) Life in the Cerrado: Pollination and Seed Dispersal. Vol. 2. *Reta Verlag*, Ulm.
- Goulson D, Nicholls E, Botías C & Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347: 1255957.
- Hachuy-Filho L, Rosas-Guerrero V & da Silva Oliveira FL (2020) Competition between honeybees and native bees: effects on foraging, floral resource use, and plant reproduction. *Apidologie* 51: 947–959.
- Kerr WE (1957) Introdução de abelhas africanas no Brasil. *Brasil Apícola* 3: 211–213.
- Klein AM, Vaissière BE, Cane JH, et al. (2007) Importance of pollinators in changing landscapes for world crops. *Proc R Soc B* 274: 303–313.

- Lanuza JB, Allen-Perkins A, & Bartomeus I (2023) The non-random assembly of network motifs in plant–pollinator networks. *Journal of Animal Ecology* 92: 760-773.
- López-Vázquez K, Lara C, Corcuera P, et al. (2024) The human touch: a meta-analysis of anthropogenic effects on plant-pollinator interaction networks. *PeerJ* 12: e17647.
- MacInnis G, Normandin E, & Ziter CD (2023) Decline in wild bee species richness associated with honey bee (*Apis mellifera* L.) abundance in an urban ecosystem. *PeerJ* 11, e14699.
- Memmott J, Waser NM & Price MV (2004) Tolerance of pollination networks to species extinctions. *Proc R Soc B* 271: 2605–2611.
- Michener CD (1975) The Brazilian bee problem. *Annu Rev Entomol* 20: 399–416.
- Michener CD (2007) The Bees of the World. 2nd ed. Johns Hopkins University Press, Baltimore.
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB & Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.
- Olesen JM, Bascompte J, Dupont YL & Jordano P (2007) The modularity of pollination networks. *Proc Natl Acad Sci USA* 104: 19891–19896.
- Ollerton J, Winfree R & Tarrant S (2011) How many flowering plants are pollinated by animals? *Oikos* 120: 321–326.
- Ollerton J (2017) Pollinator diversity: distribution, ecological function, and conservation. *Annual review of ecology, evolution, and systematics* 48: 353-376.

- Oliveira PE & Gibbs PE (2000) Reproductive biology of woody plants in a cerrado community of Central Brazil. *Flora* 195: 154–159.
- Paini DR (2004) Impact of the introduced honeybee (*Apis mellifera*) (Hymenoptera: Apidae) on native bees: a review. *Aust Ecol* 29: 399–407.
- Panziera D, Requier F, Chantawannakul P et al. (2022) The diversity decline in wild and managed honey bee populations urges for an integrated conservation approach. *Frontiers in Ecology and Evolution* 10: 767950.
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O & Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25: 345–353.
- Ramos-Jiliberto R, Valdovinos FS, Moisset de Espanés P & Flores JD (2020) Topological plasticity increases robustness of mutualistic networks. *Ecol Lett* 23: 181–192.
- Roubik DW & Wolda H (2001) Do competing honey bees matter? Dynamics and abundance of native bees before and after honeybee invasion. *Popul Ecol* 43: 53–62.
- Saavedra S, Stouffer DB, Uzzi B & Bascompte J (2011) Strong contributors to network persistence are the most vulnerable to extinction. *Nature* 478: 233–236.
- Sánchez-Bayo F & Wyckhuys KAG (2019) Worldwide decline of the entomofauna: a review of its drivers. *Biol Conserv* 232: 8–27.
- Seeley TD (1995) *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies*. Harvard University Press, Cambridge.
- Silberbauer-Gottsberger I & Gottsberger G (1988) A polinização no Cerrado. *Rev Bras Entomol* 32: 147–171.

- Simmons BI, Cirtwill AR, Baker NJ, et al. (2019) Motifs in bipartite ecological networks: uncovering indirect interactions. *Oikos* 128: 154–170.
- Sponsler DB, Milbrath M, Lonsdorf EV, Rundlöf M & Smith JP (2023) Honey bee foraging ecology: global synthesis and future directions. *Ann Entomol Soc Am* 116: 211–225.
- Stouffer DB & Bascompte J (2011) Compartmentalization increases food-web persistence. *Proc Natl Acad Sci USA* 108: 3648–3652.
- Stout JC & Morales CL (2009) Ecological impacts of invasive alien species on bees. *Apidologie* 40: 388–409.
- Strassburg BBN, Brooks T, Feltran-Barbieri R, et al. (2017) Moment of truth for the Cerrado hotspot. *Nat Ecol Evol* 1: 0099.
- Traveset A, Heleno R & Nogales M (2013) The ecology of seed dispersal by animals: recent advances and future directions. *Oikos* 122: 1–8.
- Valdovinos FS & Marsland III R (2021) Niche theory for mutualism: A graphical approach to plant-pollinator network dynamics. *The American Naturalist* 197: 393–404.
- Valido A, Rodríguez-Rodríguez MC & Jordano P (2019) Honeybees disrupt the structure and functionality of plant–pollinator networks. *Sci Rep* 9: 4711.
- Vázquez DP, Morris WF & Jordano P (2007) Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecol Lett* 8: 1088–1094.
- Wcislo WT, Arneson L, Roesch K, Gonzalez V & Smith AR (2017) Honey bees and competition for resources: behavioral ecology in human-dominated landscapes. *Biol Rev* 92: 1799–1817.

Winfree R, Aguilar R, Vázquez DP, LeBuhn G & Aizen MA (2009) A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90: 2068–2076.

Zattara EE & Aizen MA (2021) Worldwide occurrence records suggest a global decline in bee species richness. *One Earth* 4: 114–123.

2 CHAPTER I*

Reconstructing a 50-Year-Old Plant–Bee Network in a Brazilian Cerrado: Insights into historical diversity, network structure, and bee functional roles

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Reconstructing a 50-Year-Old Plant–Bee Network in a Brazilian Cerrado: Insights into historical diversity, network structure, and bee functional roles

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Abstract

Bees play an important role as pollinators, and evidence of significant global bee population declines is increasingly common. While most studies of plant-pollinator networks have focused on contemporary ecosystems, there is growing interest in reconstructing historical interaction networks to understand long-term species declines and their consequences for ecological interactions. Here, we revisited a historical dataset of plant-bee interactions from a cleared Cerrado fragment in Brazil. We described the structure of the plant-bee interaction network and bee topological roles, and explored how bee functional traits, such as body size, sociality, and floral resource specialization, were associated with species' positions within the network. The network revealed *i*) a core of generalist bee species, primarily natives; *ii*) peripheral bees exploiting a wide variety of floral resources; *iii*) a highly heterogeneous and compartmentalized organization of interactions; *iv*) a high level of interaction specificity; and *v*) a weak relationship between bee functional traits and network topological roles. The weak correlation between bee functional traits and network roles suggests that factors beyond size, sociality, and floral resource specialization may shape network topological positions. This study provides insights into the dynamics of plant-pollinator interactions and offers a crucial context for understanding the ecological consequences of contemporary bee declines.

Keywords: Floral resources, Functional traits, Historical dataset, Honeybee, Pollination

2.1 INTRODUCTION

Bees play a fundamental role as pollinators across ecosystems worldwide (Klein et al. 2007, 2018; Ollerton et al. 2017; Potts et al. 2016). Despite their importance, evidence of widespread population declines has become increasingly common (Winfree et al. 2009; Goulson et al. 2015). Recent reports highlight the decline of species from functionally important bee families such as Apidae (Sánchez-Bayo & Wyckhuys 2019; Potts et al. 2010b), Megachilidae (Mathiasson & Rehan 2020), and Melittidae (Zattara & Aizen 2021), raising serious concerns about the global conservation of many bee groups (Potts et al. 2010a; Ollerton et al. 2014). For instance, global records of bee species have declined markedly over the past three decades, with a quarter of all known species not reported since the 1990s (Zattara & Aizen 2021). Bee declines are driven by multiple factors, including habitat loss (Weiner et al. 2014), pesticide misuse (Woodcock et al. 2017), climate change (Potts et al. 2010a; Ollerton et al. 2014), and the spread of invasive species (Stout & Morales 2009). These declines affect not only bee populations but also broader ecological interactions, ultimately compromising plant reproduction through pollination (Potts et al. 2010a).

While studies of plant-pollinator networks have primarily focused on contemporary ecosystems, there is growing interest in reconstructing historical networks for a better understanding of long-term ecological changes (Burkle et al. 2013). Yet, such studies remain scarce, particularly in Neotropical regions, where access to long-term data on plant-pollinator interactions is limited (Van Klink et al. 2020). In Brazil, a country renowned for its biodiversity (Raven et al. 2020), the lack of historical datasets represents a major barrier to assessing long-term trends and the impacts of bee decline. This gap is especially critical in the Brazilian Cerrado, a globally threatened biodiversity hotspot for conservation priorities (Myers et al. 2000; Carvalho et al. 2009), where bees constitute the most abundant and frequent pollinators (Silberbauer-Gottsberger & Gottsberger 1988; Oliveira & Gibbs 2000; Gottsberger & Silberbauer-Gottsberger 2006b).

Understanding the structure and dynamics of plant-pollinator networks is essential for guiding conservation strategies, as network structure is often associated with ecosystem stability and can be used as a tool to reveal specific-species roles indicated by their interactions and hierarchy topological position (Bascompte & Jordano 2007; Ramos-Jiliberto et al. 2020; Bascompte & Scheffer 2023). While studies have shown that certain bee functional traits, such as body size, sociality, and diet breadth, are crucial in determining bee species' roles within contemporary plant-pollinator networks (e.g., specialization, centrality, and interaction patterns; Maia et al. 2019a,b; Raiol et al. 2021; Ostwald et al. 2024), how these functional traits influence bees' topological roles in historical networks before the widespread bee declines in a given habitat has yet to be thoroughly explored.

Understanding the functional attributes of bees in relation to their position in the interaction network can provide valuable insights into how ecological roles may have shifted due to bee decline (Elle et al. 2012). For example, bee size and behavior (e.g., solitary vs. social) may influence the capacity of a given species to interact with a wide range of plant species or the ability to occupy a more central or specialized role within the network (Peralta et al. 2024). This information could help explain why some bee species are more resilient to declines than others and could inform conservation priorities for bee species with central network roles.

In this study, we address these knowledge gaps by revisiting a dataset of plant-bee interactions collected between 1971 and 1975 to reconstruct a 50-year-old plant-pollinator network from a converted Cerrado area in São Paulo State, southeastern Brazil. São Paulo has undergone extensive agricultural expansion since the mid-20th century, leading to severe fragmentation of its original Cerrado vegetation. Nevertheless, surveys conducted in the 1970s, by two of the present authors (I. Silberbauer-Gottsberger and G. Gottsberger), provide a rare historical record of plant-bee interactions, captured before the most intensive land-use changes of the past five decades (Silberbauer-Gottsberger & Gottsberger 1988; Gottsberger & Silberbauer-Gottsberger 2018).

Through the analysis of this dataset, we: (1) reconstructed the historical plant-bee network (from 1971 to 1975), providing a unique baseline for understanding network dynamics before the major anthropogenic disturbances of the late 20th century; and (2) examined how bee functional traits, such as body size, sociality, and floral resource specialization, are associated with their roles within the network. Reconstructing such networks offers important baselines for assessing how anthropogenic pressures reshape pollination systems, thereby providing valuable perspectives for conservation of Cerrado biodiversity.

2.2 MATERIAL AND METHODS

2.2.1 Study site

This study was conducted at the natural reserve Fazenda “Treze de Maio” (FTM), located in Botucatu, São Paulo State, Brazil (22°43'24.89"S, 48°26'04.87"W; 550 m a.s.l.). The climate of the Botucatu region has recently shifted from humid subtropical (Cwa, Köppen classification) to tropical savanna climate (Aw, Köppen classification), driven by increasing winter temperatures and declining precipitation over the past three decades (Franco et al. 2023). Climate at FTM presents two well-defined seasons, a cold and dry winter from March to September, and a warm and wet summer from October to April, with a mean annual temperature of 21°C and total annual precipitation of approximately 1,500 mm.

The original area of FTM comprised several patches of gallery forest (*i.e.* forest strips along narrow watercourses). However, its predominant vegetation was a mosaic of two Cerrado physiognomies: Cerradão, distinguished by a dense cover of trees, surrounded by treelets and shrubs, with a canopy reaching up to 12m and Cerrado *sensu stricto*, characterized by sparsely distributed trees, treelets, shrubs, and subshrubs (Oliveira-Filho & Marquis 2002; Gottsberger & Silberbauer-Gottsberger 2006a). During the fieldwork conducted between 1971 and 1975, FTM covered a total of 700 ha. However, following

widespread conversion to sugarcane plantations, only 150 ha of Cerrado remained. The 1-hectare plot where the original sampling was conducted was completely cleared in the early 1980s (see Silberbauer-Gottsberger & Gottsberger 1988).

2.2.2 Bee, plants, and bee-plant interaction samplings

A 1-hectare plot (100 m x 100 m) was delimited in a Cerrado *sensu stricto*. This Cerrado had a woody cover of 50% and a tree canopy height ranging from 3 to 8 m. In a floristic survey conducted in the same plot, we identified 301 angiosperm species, of which 28% were trees, and the remaining were treelets, shrubs or herbaceous plants (Silberbauer-Gottsberger & Eiten 1987).

The data for this study are based on observations of bee pollination conducted in this plot (Gottsberger & Silberbauer-Gottsberger 2006a, 2006b; Silberbauer-Gottsberger, unpublished list). We also recorded the types of floral resources available to visitors, including pollen, nectar, oil, fragrances, resins, and floral tissues. All plant species were collected and identified with the assistance of specialists (see Gottsberger & Silberbauer-Gottsberger 2006a). Only bees acting as pollinators were recorded, and their behavior and use of floral resources (e.g. pollen, nectar) were documented. Bee specimens were identified by specialists (see Gottsberger & Silberbauer-Gottsberger 2006a), and vouchers are deposited at the entomological collection “Prof. J.M.F. Camargo,” FFCLRP/USP (Ribeirão Preto, Brazil). Species- and genus-level classification followed Moure’s Catalogue of Neotropical Bees (Moure et al. 2023), while higher-level classification followed Michener (2007), with updates from Almeida et al. (2025).

2.2.3 Plant-bee interaction network

To build the network topology between bee-plant interactions, we constructed an occurrence interaction matrix M using the visitation data of bees on flowers. The matrix considered each plant i or bee j as a node and the interaction occurrence (m_{ij}) as a link. To describe the structure of the interaction network, we calculated the following network

metrics: (1) Bee niche overlap (R_0), which measures how plant-bee interactions among bee species overlap. Values closer to one indicate bees sharing a greater amount of floral resources. (2) Connectance (C), which represents the proportion of interactions realized in relation to the potential available interactions, considering a scenario in which all bees and plants interact. (3) Nestedness ($NODF$), which quantifies the nestedness based on the overlap and decreasing fill index by quantifying whether specialized species interactions depict subsets of the more generalist ones (Almeida-Neto and Ulrich 2011). $NODF$ varies from 0 to 100, and higher values demonstrate that the network is nested: specialist species interact only with those partners that generalists also engage with, thereby forming a centralized core of generalists linked to a peripheral set of specialists (Bascompte et al. 2003; Almeida-Neto and Ulrich 2011). (4) Modularity (Q'), which quantifies how interactions are structured within compartments, *i.e.*, modules that represent a subset of species that interact more with each other than with species belonging to other subsets. Higher values of Q' (from 0 to 1) indicate the existence of pollinator interaction preferences for a particular subset of plant species. Modularity in the network was calculated through the DIRTLPAbw+algorithm (Beckett 2016). Since the obtained value of Q' might vary somewhat between the algorithm runs, we ran the algorithm 10 times to find the optimal module configuration, which presents the highest Q' score. For this, we set the number of Markov Chain Monte Carlo (MCMC) to 10^9 steps following Maruyama et al. (2015) and Araujo et al. (2018). We calculated all the network metrics with the package ‘*bipartite*’ in R (Dormann 2008; Dormann & Strauss 2014). (5) Robustness to bee species loss (R_r , R_d , and R_t) quantifies the proportion of species within a trophic level that persist in the network after consecutive removal of species from the opposing trophic level, with the assumption that extinction occurs when a species loses all its interconnected partners. R values range from 0 to 1, with higher values indicating greater system stability (Burgos et al. 2007). We conducted cascading extinction simulations focusing on the loss of bee species to assess R values.

As species removal within a community may result from both neutral and niche processes, simulations of species exclusion followed three different rules. Firstly, we initiated

secondary extinctions after random bee species loss (*Rr*), representing species removal by stochastic events (*i.e.*, neutral processes). Secondly, we conducted secondary extinctions by deleting species in order from the least to the most connected species. This procedure simulates cascading extinctions, wherein specialist species, dependent on fewer nodes within a network, are lost first (*Rd*). For instance, in our study, this would represent the loss of oligolectic bees strongly dependent on a particular plant species that has disappeared. Thirdly, we executed secondary extinctions by progressively removing species from the most to the least connected species (*Rt*). This case is particularly rare as the asymmetrical dependence of one species on another enables those with lesser dependency to adjust their interaction frequency following the exclusion of only a subset of species they interact with (Jordano 1987; Bascompte & Jordano 2007; Burgos et al. 2007). Although uncommon in nature (Memmott et al. 2004), this scenario fits our study, as we sampled a pristine ecosystem, in which, perhaps anthropogenic stressors were only beginning to impact generalist bees. For example, the expansion of the Africanized honeybee, *Apis mellifera*, in Brazil since the 1950s (Kerr 1957; Michener 1975; De Jong 1996) could have impacted generalists to the same extent as specialists if this exotic species overlaps in resource use patterns with the most connected bees.

We assessed the significance of each network metric by estimating the *p-value* after comparisons between the obtained values in each network index with the null distribution of each network metric generated by a null model after 10,000 randomizations using the *vaznull* algorithm, which preserves network size, the species richness, and the connectance of the observed interaction network (Vázquez et al. 2007).

2.2.4 Bee topological roles and functional attributes

Further, we tested whether differences in bee species topology were related to bee functional attributes. For this, we first classified bees according to their body size (*i.e.*, small <2cm, large >2cm), life history (*i.e.*, eusocial, solitary, cleptoparasitic), and pattern of floral resource use (*i.e.*, nectar/pollen, nectar/pollen/oil, nectar/pollen/resin,

nectar/pollen/fragrance). We then calculated five species-level network metrics that mirror their topological roles within the interaction network: (1) Nested contribution measures the average contribution of each bee species to network nestedness. It evaluates how a particular bee species (*i.e.*, node) influences network nestedness, accounting for its interactions (Saavedra et al. 2011). For instance, species engaged in specialized interactions within network compartments (*i.e.*, oligolectic and specialist bee species visiting specialist plants) may have a lower contribution to nestedness due to their high interaction specificity, contrasting with a broader array of interacting species that often share interactions with generalist core species (*i.e.*, polylectic bees using a wide range of specialist and generalist plants as resources). (2) Nested rank assesses species generality within ecological networks, determined by species position within the nestedness matrix. Nested rank tends towards 0 if the species interacts with numerous others. (3) Within-module degree (z) indicates the number of interactions that a species establishes within its module relative to other species across the same module in the network modular conformation. (4) Among-module connectivity (c) measures the extent to which a species interacts with species from different modules (Olesen et al. 2007) and, finally, (5) closeness centrality delineates the proximity of a target node to all others within a network (González et al. 2010). Bee species exhibiting higher closeness centrality values also exhibit shorter paths connecting them to other nodes within the network, holding the potential to exert a more significant influence on interacting species through direct and indirect effects.

To assess whether bees' topological roles differentiate according to their functional attributes, we first constructed a distance matrix B_{ij} that combines the score of species-level network metrics j for each bee species i . We then used the Gower dissimilarity coefficient (Gower 1971) to build a distance matrix relating variations in bee species roles (*i.e.*, species-level network scores) and bee functional traits. Finally, we conducted a permutational multivariate analysis of variance (PERMANOVA) using the 'adonis2' function with 10,000 permutations, with the 'vegan' package in R (Dixon 2003). Since some metric outputs are negative values, we performed a linear transformation to ensure all data values are positive

for PERMANOVA. We fitted the PERMANOVA considering the three bee functional attributes as variables explaining their topological roles within the network. Data visualization was achieved by performing a Non-metric Multidimensional Scaling (NDMS) with the *metaMDS* function in 'vegan' package in R.

2.3 RESULTS

2.3.1 Community composition and plant-bee interactions

During the sampling period, we recorded 412 plant-bee unique pairwise interactions, encompassing 115 plant species from 36 families and 127 bee species from 25 tribes (Supplementary Table S2.1, S2.2). Three bee tribes represented 47% of all bee species within the network: Augochlorini (22%, 28 species), Centridini (14%, 18 spp.), and Meliponini (11%, 14 spp.). Among plants, four families represented 51.30% of all plant species within the network: Fabaceae (22%, represented by 25 spp.), Asteraceae (16%, 18 spp.), Bignoniaceae (8%, 9 spp.), and Malpighiaceae (6%, 7 spp.).

Regarding pairwise interactions, the same three bee tribes with higher species richness performed 48% of the interactions with plants: Augochlorini (17%, 71 pairwise interactions), Centridini (15%, 63), Meliponini (15%, 63, 41; Figure 2.1; Figure S2.1). However, among all bee species, the invasive *Apis mellifera*, the only representative of the tribe Apini, established the highest number of interactions (33 pairwise interactions, interacting with 29% of all plant species), followed by *Oxaea flavescens* and *Exomalopsis fulvofasciata* (18 pairwise interactions each, 16% of all plant species) and *Trigona spinipes* (16, 14% of all plants). At the plant side, the most visited species were the Bignoniaceae species *Memora peregrina* and *Tabebuia aurea* (13 pairwise interactions each, representing 10% of all bee species), followed by the Malpighiaceae *Byrsonima coccolobifolia* (11, 9%).

Most bees foraged on flowers for pollen and nectar (94 species, 74.01%), followed by bees, mostly represented by Centridini species, that also included oil in their foraging demands (23 spp., 18.11%), Euglossini bees that included fragrance as an alternative for

pollen and nectar in their visits (6 spp., 4.72%) and, finally, bees that also collected resins (4 spp., 3.14%). Pollen and nectar were the most common resource offered by plants to bees (74 spp., 64.34%), followed by pollen only (22 spp., 19.13%), nectar only (8 spp., 6.95%), pollen along with oil (7 spp., 6.08%), pollen, nectar and scent (2 spp., 1.73%), and finally pollen and floral tissue or pollen, nectar and resin, which were represented by solely one species each. This means that bees could forage on 107 plant species for pollen, 85 species for nectar, seven species (only represented by Malpighiaceae) for oil, two species for fragrance, and lastly, one plant species for floral tissue or resin, respectively (Figure 2.2).

2.3.2 Network structure

The interaction network had a low connectance ($C = 0.028$, Table 2.1) and presented a nested ($NODF = 7.364$, $p < 0.01$, Table 2.1) and modular topology ($Q' = 0.534$, $p < 0.01$, Figure 2, Table 2.1). Nine modules were extracted from the interaction network. One was mainly composed of oil-collecting bees of the tribe Centridini, and floral oil-producing plants belonging to Malpighiaceae, depicting a highly specialized compartment within the interaction network. Niche overlap between bee species was very low ($R_0 = 0.02$), and only marginally different from results obtained in null networks ($p = 0.07$), indicating a high degree of niche partitioning. Finally, the historical plant-bee network exhibited the highest robustness to cascading exclusion from the least to the most connected bee species (Rd). Rd was 1.27 times higher than the network robustness to random bee exclusion (Rr), and 2.11 times higher than the cascading exclusion from the most to the least connected species (Rt , Table 2.1).

2.3.3 Bee topological roles and functional attributes

Regarding topological roles, the three most representative bee tribes had similar mean values across every species-level index measured: Augochlorini: $c\text{-score} = 0.150 \pm 0.212$, $z\text{-score} = 1.040 \pm 0.892$, $closeness = 0.008 \pm 0.001$, $nested\ contribution = 5.276 \pm 0.868$, $nested\ rank = 0.512 \pm 0.292$; Centridini: $c\text{-score} = 0.208 \pm 0.258$, $z\text{-score} = 1.329 \pm 0.825$, $closeness = 0.008 \pm 0.0007$, $nested\ contribution = 6.008 \pm 0.475$, $nested\ rank = 0.345 \pm$

0.224; Meliponini: *c-score* = 0.269 ± 0.297 , *z-score* = 1.527 ± 0.932 , *closeness* = 0.008 ± 0.001 , *nested contribution* = 5.024 ± 2.219 , *nested rank* = 0.472 ± 0.323 .

Additionally, the four species with the highest number of pairwise interactions, also had similar values of every species-level index measured: *A. mellifera*: *c-score* = 0.822, *z-score* = 4.306, *closeness* = 0.012, *nested contribution* = 11.784, *nested rank* = 0.000; *Oxaea flavescens*: *c-score* = 0.414, *z-score* = 3.475, *closeness* = 0.011, *nested contribution* = 9.310, *nested rank* = 0.015; *Exomalopsis fulvofasciata*: *c-score* = 0.732, *z-score* = 3.687, *closeness* = 0.012, *nested contribution* = 8.760, *nested rank* = 0.007; *Trigona spinipes*: *c-score* = 0.528, *z-score* = 2.442, *closeness* = 0.011, *nested contribution* = 10.796, *nested rank* = 0.0238.

None of the bee functional attributes explained differences in bee topological roles (PERMANOVA: size, $F = -32.96$, $p = 0.78$; sociality, $F = -32.124$, $p = 0.95$; resource use, $F = -4.41$, $p = 0.64$; Figure 3), as bee species from different tribes occupied similar topological positions in the network ($F = 49.84$, $p = 0.059$, Figure 2.3).

2.4 DISCUSSION

By reconstructing a historical plant-pollinator network from a Cerrado fragment suppressed in the early 1980s, we revealed a complex and diverse community of interacting plants and bees. To our knowledge, this survey of plant-bee interactions is among the first conducted in the Neotropics and remains the only one available for South America, a region that served as the biogeographic cradle for the early diversification of several major bee clades (Almeida et al. 2023). Remarkably, although the dataset was derived from a single 1-hectare site, we documented interactions that are now considered rare or infrequent in contemporary samples, such as those between oil-collecting bees and oil-producing plants, which accounted for a substantial share of the unique pairwise interactions in this half-century-old network. The network exhibited both nestedness and modularity, structural

properties that reflect robustness to the local loss of weak and specialist interactions. Nonetheless, its low connectance and high niche partitioning suggest that the loss of generalist bee species would have disproportionate and cascading effects on network organization, which may help explain why present-day, heavily impacted fragments tend to support less stable mutualistic networks (Astegiano et al. 2015; Ren et al. 2022; but see Grass et al. 2018 for contrasting evidence).

2.4.1 Community composition and plant-bee interactions

Most studies of bee fauna in Brazil conducted after 1990 report a dominance of polylectic assemblages in fragmented habitats (reviewed by Ferreira et al. 2015). In many of these studies, assemblages were largely composed of a few generalist stingless bee species, primarily from common Neotropical tribes such as Meliponini and Augochlorini (Raiol et al. 2021). In contrast, our historical dataset revealed that a substantial proportion of established plant-bee interactions involved oligolectic species, particularly oil-collecting bees of the tribe Centridini. The prevalence of these specialized bees may be linked to the relatively even distribution of plant species providing distinct floral resources, which strongly influences network assembly, structure, and species roles (Fründ et al. 2010). This suggests that the diversity of floral resources available to bees is directly correlated with the diversity of resources exploited and, consequently, with bee diversity itself (Armbruster et al. 2017; Ballarin et al. 2022).

The interaction among specialized species, such as those between oil-collecting bees and oil-producing plants and between fragrant plants and orchid bees, is very cohesive due to a high interaction intimacy among interacting species (*sensu* Guimarães Jr. et al. 2007). This high interaction intimacy may arise from the high phylogenetic and functional relatedness that both levels of the interaction partners present (Bezerra et al. 2009; Davis et al. 2014; Ospina-Torres et al. 2015). However, it is remarkable that in recent plant-bee interaction samplings across Cerrado fragments and other ecosystems worldwide (Clavel et al. 2011), such specialized relationships are being replaced by generalist species (Lurgi et al.

2012; Hachuy-Filho et al. 2020). This suggests that anthropogenic actions can disrupt even robust and phylogenetically structured interactions among specialized and rare species (Aizen et al. 2012).

Another factor that may contribute to the disruption of specialized interactions is the presence of highly competitive invasive species (Valido et al. 2019). In our study, the invasive bee species, the honeybee, established interactions with 29% of all plant species. This is worrisome and shows how populations of *A. mellifera* were successfully established in natural Cerrado habitats right after their introduction in the Cerrado ecosystem in 1957 (Kerr 1957; Michener 1975; De Jong 1996). Although *A. mellifera* had the highest number of interactions, its species dominance was shared with other native bee species, such as *Oxaea flavescens*, *Exomalopsis fulvofasciata*, and *Trigona spinipes*, and the interactions were evenly distributed across most core bee species. Interestingly, some studies show that *A. mellifera* impacts on native bee populations are much more severe in highly impacted fragment communities, where floral resources are scarce and homogenous (Hachuy-Filho et al. 2020; Ballarin et al. 2022). Hence, we can expect that in communities with high floral resource availability and diversity, like the one in our study, the impacts of the introduced species should be mitigated primarily by niche partitioning with native species (Hachuy-Filho et al. 2020; Ballarin et al. 2022). However, this could represent an initial cue of the onset of the impact caused by the invasion of bees on the native plant-bee network. The balanced contribution of both native and invasive species in the past may now manifest as an overrepresentation of invasive species and the displacement of less competitive bee species. Indeed, our study demonstrates that this half-century-old network exhibited lower resilience to the exclusion of native core species that share plant species with the invasive species *A. mellifera*.

Despite the relatively low impact of *A. mellifera* on the native bee community and also on the structure of the plant-bee interaction network at the moment of our study sampling, since 1970, the extensive fragmentation of Cerrado habitats in the São Paulo state

dramatically reorganized plant communities (Strassburg et al. 2016; Strassburg et al. 2017), with the reduction of floral resource diversity and availability as a side effect (Hachuy-Filho et al. 2020). The reduction of floral resources intensifies competition among co-occurring bee species, which may cascade into competitive exclusion (Thomson & Page, 2020). Because of the high competitiveness of *A. mellifera*, it is likely that in a scenario of reduced resource diversity and availability, the introduced species may expand its niche breadth by enhancing resource usage, thereby disrupting the interactions among native plant and bee species (see Hachuy-Filho et al. 2020). In fact, in a study conducted in a recent Cerrado fragment presenting a lower plant species richness, *A. mellifera* showed a substantially wider resource usage, establishing interactions with 50% of available flowering plant species in the community (Andena et al. 2012), an increase scaling up to 2-fold from what was observed in this 50-year-old plant-bee network. Additionally, in this study, Andena et al. (2012) found significantly lower bee species richness in relation to our Cerrado hectare, which may indicate that consequences of the increased resource usage by *A. mellifera* may already have driven the exclusion of less competitive native specialized bees. Hence, the long-term impacts of *A. mellifera* on native bee communities can be much more pronounced than what we evidenced here, especially considering the synergetic effects of anthropogenic actions such as habitat loss (Weiner et al. 2014), the use of pesticides (Woodcock et al. 2017) and the spread of pathogens (Yang & Cox-Foster 2007) that has intensified in the last decades.

2.4.2 Network structure

The loss of species in highly fragmented habitats increases network connectance due to the loss of specialist species (Hagen et al. 2012). The low-connected plant-bee network we present here demonstrates that, in contrast, this historical inventory harbored interactions with a higher degree of specificity and heterogeneity (Jauker et al. 2019). Furthermore, many interacting species exhibited a high number of non-overlapping links, suggesting the prevalence of opportunistic associations or high-specificity relationships that enable specialists and weaker competitors to avoid overexploited resources (Valdovinos et al. 2016; Ponisio et al. 2017), such as abundant plant species that offer large amounts of nectar, the

most common resource provided by flowering plants to pollinators (Ballarin et al. 2024).

We suggest that the plant-bee interaction network in the Cerrado hectare was likely to be stable due to its network structural properties, namely, nestedness and modularity. The nested structure of the plant-bee network reveals that the interactions made between bees and plants were highly asymmetrical, and the network presented a core group of generalist bee species that interacted with a high number of plant species (Jordano, 1987, Bascompte et al. 2003; Pawar 2014). This kind of structure brings stability to the interacting community, as the trophic overlap among core and peripheral bee species should lead to functional redundancy, a main driver of ecological stability (Memmott et al. 2004; Burgos et al. 2007; Okuyama & Holland 2008; Biggs et al. 2020). Network modularity is also important for community stability, as the loss of pairwise interactions caused by species loss often drives indirect effects on interacting species that share interaction partners with the lost species (Pires et al. 2020). As species from different compartments within the network generally share weak or no connections (Olesen et al. 2007), enhancing connectivity within modules is expected to buffer against the removal of species from other compartments (Stouffer & Bascompte 2011). Conversely, the establishment of highly generalist invasive species, like *A. mellifera*, can strengthen the connectivity among modules, which can merge module units, leading to long-term impacts on the entire network structure (Olesen et al. 2002).

2.4.3 Bee topological roles and functional attributes

Despite the community's high bee richness, our study reveals a balanced distribution of species' topological roles within the interaction network. Moreover, species roles do not differentiate according to species functional attributes, which can indicate that bee species harboring differing traits played equivalent roles across the network. Topological redundancy within ecological communities is widely recognized as a key factor supporting the temporal stability of ecosystem functions. The extent to which the loss of a single species affects overall ecosystem structure depends on whether other species within the community can perform similar ecological roles, thereby buffering the system against functional disruption

(Jordán, 2009). In our case, despite the variation in body sizes among bee species, the collection of a wide range of resources, and diversity of life histories (including social behaviors and brood parasitism), each functional attribute was displayed across large and rich functional groups. This suggests that, despite the high degree of interaction specificity and structural heterogeneity in our network, community stability was maintained through a high level of functional complementarity among species in their pairwise interactions and through topological redundancy in their contributions to the overall network structure.

2.5 CONCLUSION

This study sheds light on the well-structured plant-bee interactions that once characterized a preserved Cerrado fragment in the 1970s. By reconstructing this historical network, we revealed: *i*) a core of generalist bee species, primarily natives; *ii*) a smaller set of peripheral specialists exploiting a wide range of floral resources; *iii*) a highly heterogeneous and compartmentalized organization of interactions; *iv*) a high degree of interaction specificity; and *v*) a weak association between bee functional traits and their topological roles. Together, these findings indicate that the stability of past networks depended on both functional complementarity and topological redundancy. Crucially, they underscore that the erosion of these structures through habitat loss, invasive species, and other anthropogenic pressures can severely undermine ecosystem resilience. Our results, therefore, underscore the urgent need to conserve both generalist and specialist bees to sustain stable plant–pollinator networks. Furthermore, historical datasets offer irreplaceable baselines for guiding restoration initiatives and mitigating the long-term impacts of global change, thereby directly supporting pollinator conservation in the Cerrado.

2.6 REFERENCES

- Aizen MA, Sabatino M & Tylianakis JM (2012) Specialization and rarity predict nonrandom loss of interactions from mutualist networks. *Science* 335: 1486-1489.
- Almeida EAB, Bossert S, Danforth BN, et al. (2023) The evolutionary history of bees in time and space. *Curr Biol* 33: 3409-3422.
- Almeida EAB, López-Urbe MM, Packer L, Porto DS, Camacho GP (2025) Bee systematics and conservation. *Sociobiol* 72: e11416.
- Almeida-Neto M & Ulrich W (2011) A straightforward computational approach for measuring nestedness using quantitative matrices. *Environ Modell Software* 26: 173-178.
- Araujo AC, Martín González AM, Sandel B, et al. (2018) Spatial distance and climate determine modularity in a cross-biomes plant–hummingbird interaction network in Brazil. *J Biogeogr* 45: 1846-1858.
- Andena SR, Santos EF & Noll FB (2012) Taxonomic diversity, niche width and similarity in the use of plant resources by bees (Hymenoptera: Anthophila) in a cerrado area. *J Nat Hist* 46: 1663-1687.
- Armbruster WS (2017) The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation. *Funct ecol* 31: 88-100.
- Astegiano J, Massol F, Vidal M M, et al. (2015) The robustness of plant-pollinator assemblages: linking plant interaction patterns and sensitivity to pollinator loss. *PloS*

one 10: e0117243.

Ballarin CS, Hachuy-Filho L, Doria MJW et al. (2022) Intra-seasonal and daily variations in nectar availability affect bee assemblage in a monodominant afforested Brazilian Cerrado. *Austral Ecol* 47: 1315-1328.

Ballarin CS, Fontúrbel FE, Rech AR et al. (2024) How many animal-pollinated angiosperms are nectar-producing? *New Phytol* 243: 2008-2020.

Bascompte J, Jordano P, Melián CJ, et al. (2003) The nested assembly of plant–animal mutualistic networks. *PNAS* 100: 9383-9387.

Bascompte, J, & Jordano, P (2007) Plant-animal mutualistic networks: the architecture of biodiversity. *Annu Rev Ecol Evol Syst* 38: 567-593.

Bascompte J, & Scheffer M (2023) The resilience of plant–pollinator networks. *Annu Rev Entomol* 68: 363-380.

Beckett SJ (2016) Improved community detection in weighted bipartite networks. *R Soc Open Sci* 3: 140536.

Bezerra EL, Machado IC, & Mello MA (2009) Pollination networks of oil-flowers: a tiny world within the smallest of all worlds. *J Anim Ecol* 78: 1096-1101.

Biggs CR, Yeager LA, Bolser DG, et al. (2020) Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. *Ecosphere* 11: e03184.

Burgos E, Ceva H, Perazzo RP, et al. (2007) Why nestedness in mutualistic networks? *J Theor Biol* 249: 307-313.

- Burkle LA, Marlin JC & Knight TM (2013) Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339: 1611-1615.
- Carvalho FM, Júnior PDM & Ferreira LG. (2009) The Cerrado into-pieces: Habitat fragmentation as a function of landscape use in the savannas of central Brazil. *Biol Conserv* 142: 1392-1403.
- Clavel J, Julliard R & Devictor V (2011) Worldwide decline of specialist species: toward a global functional homogenization? *Front Ecol Environ* 9: 222-228.
- Davis CC, Schaefer H, Xi Z, et al. (2014) Long-term morphological stasis maintained by a plant–pollinator mutualism. *PNAS* 111: 5914-5919.
- De Jong D (1996) Africanized honey bees in Brazil, forty years of adaptation and success. *Bee World* 77: 67-70
- Dixon P (2003) VEGAN, a package of R functions for community ecology. *J Veg Sci* 14: 927-930.
- Dormann CF, Gruber B, & Fründ J (2008) Introducing the bipartite package: analysing ecological networks. *Interaction* 1: 8-11.
- Dormann CF & Strauss R (2014) A method for detecting modules in quantitative bipartite networks. *Methods Ecol Evol* 5: 90-98.
- Elle E, Elwell SL, & Gielens GA (2012) The use of pollination networks in conservation. *Botany* 90: 525-534.

- Ferreira PA, Boscolo D, Carvalheiro LG, et al. (2015) Responses of bees to habitat loss in fragmented landscapes of Brazilian Atlantic Rainforest. *Landscape Ecol* 30 2067-2078.
- Franco J R, Dal Pai E, Calça MVC, et al. (2023) Atualização da normal climatológica e classificação climática de köppen para o município de botucatu-sp. *Irriga* 28: 77-92
- Fründ J, Linsenmair KE, & Blüthgen N. (2010) Pollinator diversity and specialization in relation to flower diversity. *Oikos* 119: 1581-1590.
- González AMM, Dalsgaard B, & Olesen JM (2010) Centrality measures and the importance of generalist species in pollination networks. *Ecol Complexity* 7: 36-43.
- Goulson D, Nicholls E, Botías C, et al. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347: 1255957
- Gottsberger G & Silberbauer-Gottsberger I (2006a) Life in the Cerrado. Vol. I. Pollination and Seed Dispersal. *Reta Ulm*.
- Gottsberger G & Silberbauer-Gottsberger I (2006b) Life in the Cerrado. Vol. II. Origin, Structure, Dynamics and Plant Use. *Reta Ulm*.
- Gottsberger G & Silberbauer-Gottsberger I (2018) How are pollination and seed dispersal modes in Cerrado related to stratification? Trends in a cerrado sensu stricto woodland in southeastern Brazil, and a comparison with Neotropical forests. *Acta Botanica Brasilica* 32: 434-445.
- Gower JC (1971) A general coefficient of similarity and some of its properties. *Biometrics* 857-871.

- Grass I, Jauker B, Steffan-Dewenter I, et al. (2018) Past and potential future effects of habitat fragmentation on structure and stability of plant–pollinator and host–parasitoid networks. *Nat Ecol Evol* 2: 1408-1417.
- Guimaraes Jr PR, Rico-Gray V, Oliveira PS, et al. (2007) Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. *Curr Biol* 17: 1797-1803.
- Hachuy-Filho L, Ballarin CS, & Amorim FW (2020) Changes in plant community structure and decrease in floral resource availability lead to a high temporal β -diversity of plant–bee interactions. *Arthropod-Plant Interact* 14: 571-583.
- Hagen M, Kissling WD, Rasmussen C, et al. (2012) Biodiversity, species interactions and ecological networks in a fragmented world. *Adv Ecol Res* 46: 89-210.
- Jauker F, Jauker B, Grass I, et al. (2019) Partitioning wild bee and hoverfly contributions to plant–pollinator network structure in fragmented habitats. *Ecology* 100: e02569.
- Jordán F (2009) Keystone species and food webs. *Philos Trans R Soc London, Ser B* 1524: 1733-1741.
- Jordano P (1987) Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries, and coevolution. *Am Nat* 129: 657-677.
- Kerr WE (1957) Introdução de abelhas africanas no Brasil. *Brasil Apicola* 3: 211-213.
- Klein AM, Vaissiere BE, Cane JH et al. (2007) Importance of pollinators in changing landscapes for world crops. *Proc R Soc B* 274: 303-313.

- Klein AM, Boreux V, Fornoff F, et al. (2018) Relevance of wild and managed bees for human well-being. *Curr Opin Insect Sci* 26: 82-88.
- Lurgi M, López BC & Montoya JM (2012) Novel communities from climate change. *Phil Trans R Soc B* 367: 2913-2922.
- Maruyama PK, Vizentin-Bugoni J, Dalsgaard B, et al. (2015) Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. *Oecologia* 178: 783-793.
- Mathiasson ME, & Rehan SM (2020) Wild bee declines linked to plant-pollinator network changes and plant species introductions. *Insec Conserv Diversity* 13: 595-605.
- Maia KP, Vaughan IP, & Memmott J (2019a) Plant species roles in pollination networks: an experimental approach. *Oikos* 128: 1446-1457.
- Maia KP, Rasmussen C, Olesen JM, & Guimarães Jr PR (2019b) Does the sociality of pollinators shape the organisation of pollination networks? *Oikos* 128: 741-752.
- Memmott J, Waser NM, & Price MV (2004). Tolerance of pollination networks to species extinctions. *Proc R Soc London, Ser B* 271: 2605-2611.
- Michener CD (1975) The Brazilian bee problem. *Annu Rev Entomol* 20: 399-416.
- Michener CD (2007) The bees of the world, 2nd Ed. *The Johns Hopkins University Press*, Baltimore.
- Moure JS, Urban D, & Melo GAR (2023) Catalogue of Bees (Hymenoptera, Apoidea) in the

Neotropical Region.

Myers N, Mittermeier RA, Mittermeier CG, et al. (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.

Okuyama T & Holland JN (2008) Network structural properties mediate the stability of mutualistic communities. *Ecol Lett* 11: 208-216.

Olesen JM, Eskildsen LI, & Venkatasamy S (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Divers Distrib* 8: 181-192.

Olesen JM, Bascompte J, Dupont YL, et al. (2007) The modularity of pollination networks. *PNAS* 104: 19891-19896.

Oliveira PE, & Gibbs PE (2000) Reproductive biology of woody plants in a cerrado community of Central Brazil. *Flora* 195: 311-329.

Oliveira PS, & Marquis RJ (2002) The cerrados of Brazil: ecology and natural history of a neotropical savanna. *Columbia University Press*, New York.

Ollerton J, Erenler H, Edwards M, et al. (2014) Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. *Science* 346: 1360-1362.

Ollerton J (2017) Pollinator diversity: distribution, ecological function, and conservation. *Annu Rev Ecol Evol Syst* 48: 353-376.

Ospina-Torres R, Montoya-Pfeiffer PM, Solarte V & Otero JT (2015) Interaction networks and the use of floral resources by male orchid bees (Hymenoptera: Apidae:

- Euglossini) in a primary rain forest of the Chocó Region (Colombia). *Revista de biologia tropical* 63: 647-658.
- Ostwald MM, Gonzalez VH, Chang C, et al (2024) Toward a Functional Trait Approach to Bee Ecology. *Ecol Evol* 14: e70465.
- Pawar S (2014) Why are plant-pollinator networks nested? *Science* 345: 383-383.
- Peralta G, Resasco J, Worthy S, et al. (2024) Pollinator intraspecific body size variation and sociality influence their interactions with plants. *Funct Ecol* 38: 875-882.
- Pires MM, O'Donnell JL, Burkle LA, et al. (2020) The indirect paths to cascading effects of extinctions in mutualistic networks. *Ecology* 101: e03080.
- Ponisio LC, Gaiarsa MP, & Kremen C (2017) Opportunistic attachment assembles plant–pollinator networks. *Ecol Lett* 20: 1261-1272.
- Potts SG, Biesmeijer JC, Kremen C, et al. (2010a) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25: 345-353.
- Potts SG, Roberts SP, Dean R, et al. (2010b) Declines of managed honeybees and beekeepers in Europe. *J Apic Res* 49: 15-22.
- Potts SG, Imperatriz-Fonseca V, Ngo HT, et al (2016) Safeguarding pollinators and their values to human well-being. *Nature* 540: 220-229.
- Raiol RL, Gastauer M, Campbell AJ, et al. (2021) Specialist Bee Species Are Larger and Less Phylogenetically Distinct Than Generalists in Tropical Plant–Bee Interaction Networks. *Front Ecol Evol* 9: 699649.

- Ramos-Jiliberto R, Moisset de Espanés P, & Vázquez DP (2020) Pollinator declines and the stability of plant–pollinator networks. *Ecosphere* 11: e03069.
- Raven PH, Gereau RE, Phillipson PB, et al. (2020) The distribution of biodiversity richness in the tropics. *Sci Adv* 6: eabc6228.
- Ren P, Si X, & Ding P (2022) Stable species and interactions in plant–pollinator networks deviate from core position in fragmented habitats. *Ecography* 2022: e06102.
- Sánchez-Bayo F & Wyckhuys KA (2019) Worldwide decline of the entomofauna: A review of its drivers. *Biol Conserv* 232: 8-27.
- Saavedra S, Stouffer DB, Uzzi B, et al. (2011) Strong contributors to network persistence are the most vulnerable to extinction. *Nature* 478: 233-235.
- Silberbauer-Gottsberger I, & Eiten G (1987) hectare of cerrado. I. General aspects of the trees and thick-stemmed shrubs. *Phyton: Annales rei botanicae* 27: 55-91.
- Silberbauer-Gottsberger I, & Gottsberger G (1988) A polinização de plantas do cerrado. *Revista Brasileira de Biologia* 48:651-663
- Stouffer DB & Bascompte J (2011) Compartmentalization increases food-web persistence. *PNAS* 108: 3648-3652.
- Stout JC & Morales CL (2009) Ecological impacts of invasive alien species on bees. *Apidologie* 40: 388-409.
- Strassburg BB, Latawiec A, & Balmford A (2016) Urgent action on Cerrado extinctions.

Nature 540: 199-199.

Strassburg BB, Brooks T, Feltran-Barbieri R, et al. (2017) Moment of truth for the Cerrado hotspot. *Nat Ecol Evol* 1: 1-3.

Thomson DM & Page ML (2020) The importance of competition between insect pollinators in the Anthropocene. *Curr Opin Insect Sci* 38: 55-62.

Valido A, Rodríguez-Rodríguez MC, & Jordano P (2019) Honeybees disrupt the structure and functionality of plant-pollinator networks. *Sci Rep* 9: 4711.

Valdovinos FS, Brosi BJ, Briggs H M, et al. (2016) Niche partitioning due to adaptive foraging reverses effects of nestedness and connectance on pollination network stability. *Ecol lett* 19: 1277-1286.

Van Klink R, Bowler DE, Gongalsky KB, et al. (2020) Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science* 368: 417-420.

Vázquez DP, Melián CJ, Williams NM, et al. (2007) Species abundance and asymmetric interaction strength in ecological networks. *Oikos* 116: 1120-1127.

Weiner CN, Werner M, Linsenmair KE, et al. (2014) Land-use impacts on plant–pollinator networks: interaction strength and specialization predict pollinator declines. *Ecology* 95: 466-474.

Winfree R, Aguilar R, Vázquez DP, et al. (2009) A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90: 2068-2076.

Woodcock BA, Bullock JM, Shore RF, et al. (2017) Country-specific effects of neonicotinoid

pesticides on honey bees and wild bees. *Science* 356: 1393-1395.

Yang X, & Cox-Foster D (2007) Effects of parasitization by *Varroa destructor* on survivorship and physiological traits of *Apis mellifera* in correlation with viral incidence and microbial challenge. *Parasitology* 134: 405-412.

Zattara EE, & Aizen MA (2021) Worldwide occurrence records suggest a global decline in bee species richness. *One Earth* 4: 114-123.

2.7 TABLES

Table 2.1. Plant-bee interaction network indexes. * represents significant values ($p < 0.05$) based on a null distribution using the Vaznull null model.

Community composition	
Bee richness	127
Plant richness	115
Network metrics	Observed values
Total Number of Links	412
Connectance (C)	0.028
Nestedness ($NODF$)	7.364 *
Modularity (Q')	0.534 *
Robustness (R_r)	0.704
Robustness (R_d)	0.893
Robustness (R_t)	0.423
Bee niche's overlap (R_θ)	0.02

2.8 FIGURES

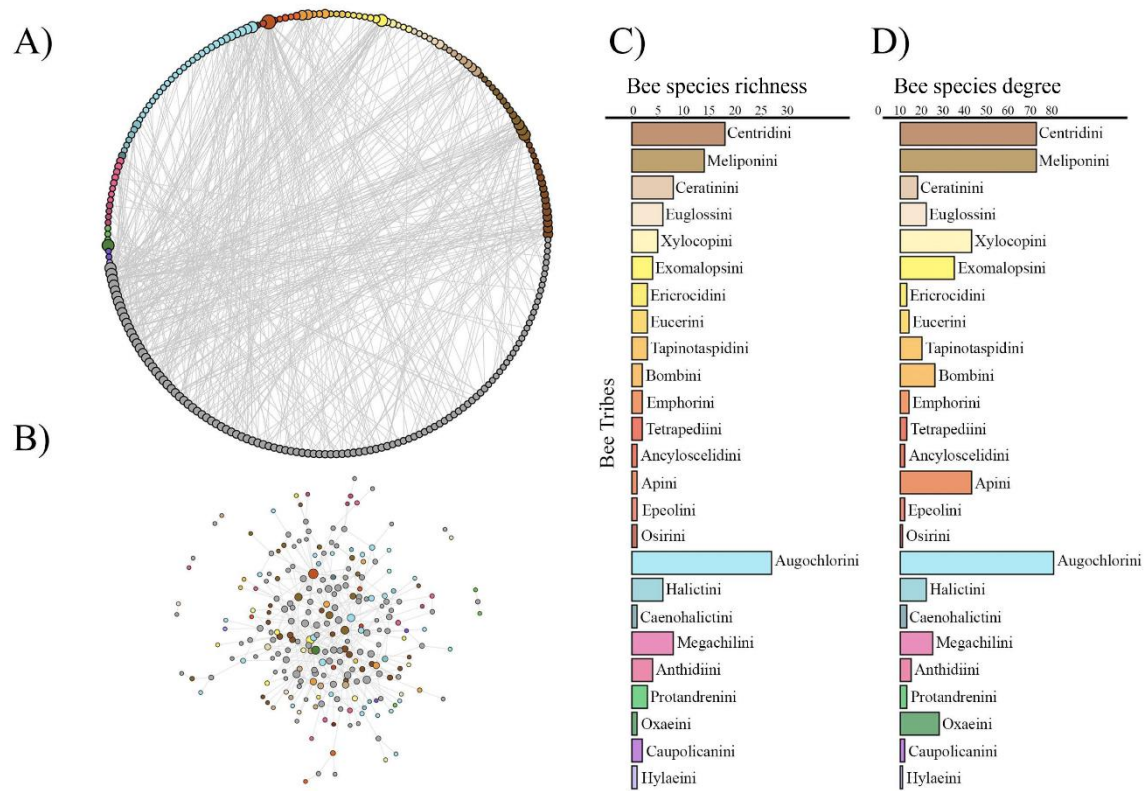
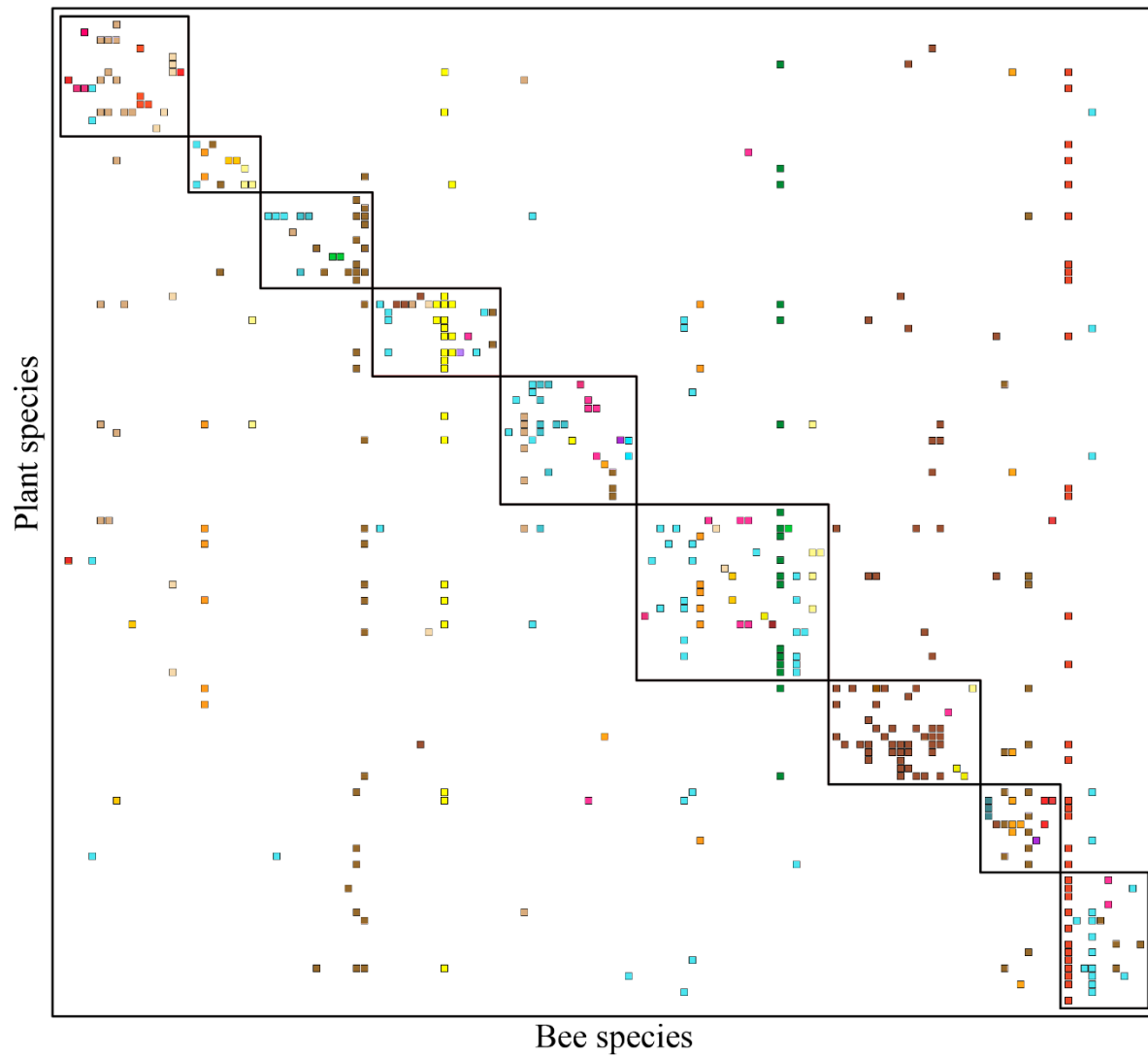
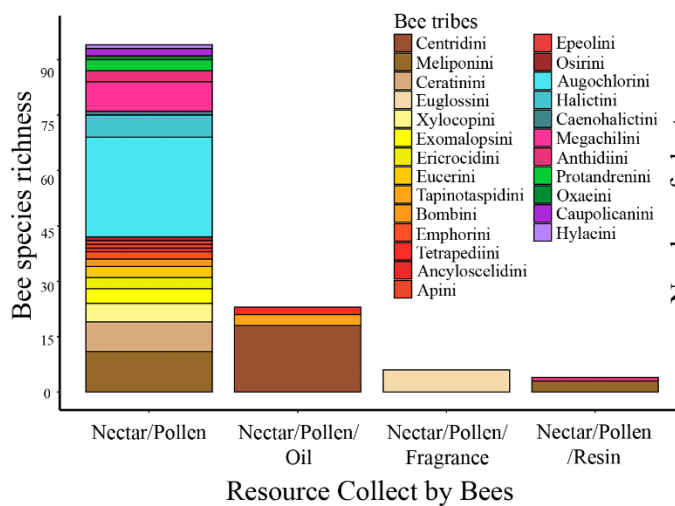


Figure 2.1. Plant-bee interaction network and bee species composition recorded 50 years ago in the studied square hectare of Cerrado. The circular network (A) demonstrates how bee species are linked with plants, focusing on species degree, *i.e.* the number of links each species performs, while the connected network (B) focuses on depicting how species are indirectly linked through direct pairwise interactions and, thus, how much they are integrated into the network. Bee species richness (C) and bee species' degree (D) are also included. Colors in circles (A–B) and bars (C–D) represent different bee tribes. Size in circles (A–B) represents the log-transformed species degree for each bee and plant species.

A) Bee-plant interaction network (modular structure)



B)



C)

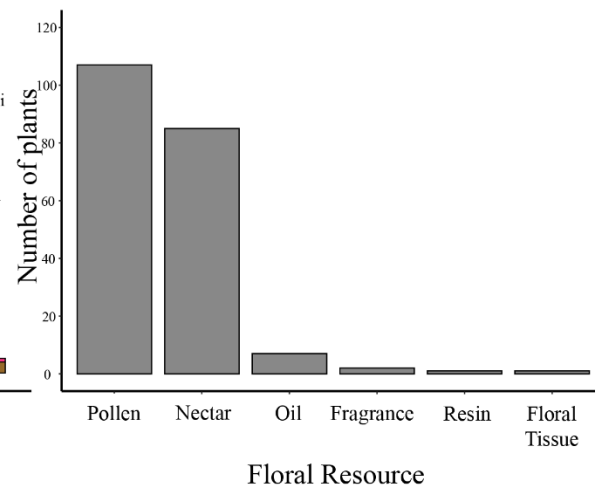


Figure 2.2 The modular structure of the plant-bee interaction network and resources offered by plants to bees in the 50-year-old studied hectare of Cerrado. The modular topology of the web (A) shows how interactions within the interaction network form compartments among groups of bees that visit a particular group of plants in search of floral resources. The number of bee species that collect each resource (B) and the number of plant species that provide each type of floral resource (C) are also shown. Colors (A–B) indicate different bee tribes.

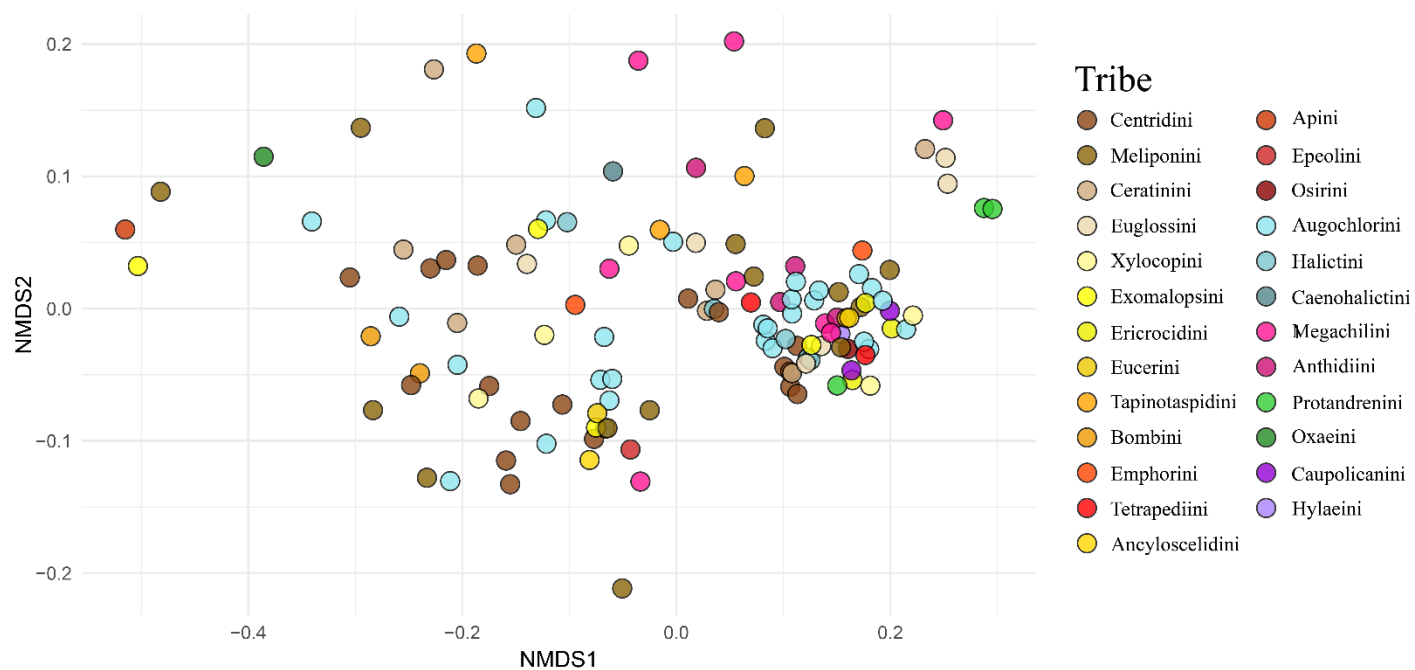


Figure 2.3. Distance among bee species' topological roles. Non-metric multidimensional scaling (NMDS) ordination depicts the position of each bee species (colored points) according to its topological role, with colors representing different bee tribes.

Table S2.1. Bee species collected between 1971 and 1975 at Fazenda Treze de Maio, São Paulo state, Brazil. Floral resource collected by each bee species as well as their body size are also included.

Family	Tribe	Species	Floral resource	Body size
Andrenidae	Oxaeini	<i>Oxaea flavescens</i> Klug, 1807	Nectar/Pollen	Large
	Protandrenini	<i>Parapsaenythia paspali</i> (Schrottky, 1909)	Nectar/Pollen	Small
		<i>Psaenythia</i> Gerstaecker, A. 1868 sp. 1	Nectar/Pollen	Small
		<i>Psaenythia</i> sp. 2	Nectar/Pollen	Small
Apidae	Ancyloscelidini	<i>Ancyloscelis apiformis</i> (Fabricius, 1793)	Nectar/Pollen	Small
	Apini	<i>Apis mellifera</i> Linnaeus, 1758	Nectar/Pollen	Large
	Bombini	<i>Bombus (Fervidobombus) pauloensis</i> Friese, 1913	Nectar/Pollen	Large
		<i>Bombus (Fervidobombus) morio</i> (Swederus, 1787)	Nectar/Pollen	Large
	Centridini	<i>Centris (Melanocentris) discolor</i> Smith, 1874	Nectar/Pollen/Oil	Large
		<i>Centris (Melanocentris) dorsata</i> Lepeletier, 1841	Nectar/Pollen/Oil	Large
		<i>Centris (Trachina) fuscata</i> Lepeletier, 1841	Nectar/Pollen/Oil	Large
		<i>Centris (Aphemisia) mocsaryi</i> Friese, 1899	Nectar/Pollen/Oil	Large
		<i>Centris (Ptilotopus) moerens</i> (Perty, 1833)	Nectar/Pollen/Oil	Large
Apidae	Centridini	<i>Centris (Centris) nitens</i> Lepeletier, 1841	Nectar/Pollen/Oil	Large
		<i>Centris (Melanocentris) obsolete</i> Burmeister, 1876	Nectar/Pollen/Oil	Large
		<i>Centris (Ptilotopus) scopipes</i> Friese, 1899	Nectar/Pollen/Oil	Large
		<i>Centris (Hemisiella) tarsata</i> (Smith, 1874)	Nectar/Pollen/Oil	Large
		<i>Centris (Centris) varia</i> (Erichson, 1848)	Nectar/Pollen/Oil	Large
		<i>Centris (Melanocentris) violácea</i> Lepeletier, 184	Nectar/Pollen/Oil	Large

Family	Tribe	Species	Floral resource	Body size
		<i>Epicharis (Xanthepicharis) bicolor</i> Smith, 1854	Nectar/Pollen/Oil	Large
		<i>Epicharis (Epicharitides) cockerelli</i> Friese, 1900	Nectar/Pollen/Oil	Large
		<i>Epicharis (Anepicharis) dejeanii</i> Lepeletier, 1841	Nectar/Pollen/Oil	Large
		<i>Epicharis (Hoplepicharis) fasciata</i> Lepeletier & Serville,	Nectar/Pollen/Oil	Large
		<i>Epicharis (Epicharis) flava</i> Friese, 1900	Nectar/Pollen/Oil	Large
		<i>Epicharis (Triepicharis) analis</i> Friese, 1899	Nectar/Pollen/Oil	Large
		<i>Epicharis</i> Klug, 1807 sp. 1	Nectar/Pollen/Oil	Large
Apidae	Centridini			
	Caupolicanini	<i>Ptiloglossa hemileuca</i> Moure, J.S. (1944)	Nectar/Pollen	Large
		<i>Ptiloglossa</i> Smith, 1853 sp. 1	Nectar/Pollen	Large
Apidae	Emphorini	<i>Diadasia</i> Patton, 1879 sp. 1	Nectar/Pollen	Small
		<i>Diadasia</i> sp. 2	Nectar/Pollen	Small
	Ericrocidini	<i>Mesocheira bicolor</i> (Fabricius, 1804)	Nectar/Pollen	Small
		<i>Mesoplia rufipes</i> (Perty, 1833)	Nectar/Pollen	Small
		<i>Mesoplia</i> Lepeletier, 1841 sp. 1	Nectar/Pollen	Small
	Eucerini	<i>Florilegus (Floriraptor) melectoides</i> (Smith, 1879)	Nectar/Pollen	Small
		<i>Melissodes</i> Latreille, 1829 sp. 1	Nectar/Pollen	Large
		<i>Melissodes</i> sp. 2	Nectar/Pollen	Large
	Euglossini	<i>Euglossa (Euglossa) cordata</i> (Linnaeus, 1758)	Nectar/Pollen/Fragrance	Large
	Euglossini	<i>Euglossa (Euglossa) townsendi</i> Cockerell, 1904	Nectar/Pollen/Fragrance	Large
Apidae	Euglossini	<i>Euglossa (Euglossa) melanotricha</i> Moure 1967	Nectar/Pollen/Fragrance	Large

Family	Tribe	Species	Floral resource	Body size
Apidae	Exomalopsini	<i>Euglossa (Euglossa) pleosticta</i> Dressler, 1982	Nectar/Pollen/Fragrance	Large
		<i>Eulaema (Apeulaema) nigrita</i> (Lepeletier, 1841)	Nectar/Pollen/Fragrance	Large
		<i>Eufriesea violascens</i> (Mocsáry, 1898)	Nectar/Pollen/Fragrance	Large
		<i>Exomalopsis (Exomalopsis) auropilosa</i> Spinola, M. (1853)	Nectar/Pollen	Small
		<i>Exomalopsis (Exomalopsis) fulvipennis</i> Schrottky, 1910	Nectar/Pollen	Small
		<i>Exomalopsis (Exomalopsis) fulvofasciata</i> Smith, F. (1879)	Nectar/Pollen	Small
		<i>Exomalopsis</i> Spinola, 1853 sp. 1	Nectar/Pollen	Small
	Meliponini	<i>Geotrigona</i> Moure, 1943 sp. 1	Nectar/Pollen	Small
		<i>Leurotrigona muelleri</i> (Friese, 1900)	Nectar/Pollen	Small
		<i>Melipona (Eomelipona) marginata</i> Lepeletier, 1836	Nectar/Pollen	Small
	Meliponini	<i>Melipona (Melipona) quadrifasciata</i> Lepeletier, 1836	Nectar/Pollen	Small
		<i>Melipona (Melipona) quadrifasciata anthidioides</i>	Nectar/Pollen	Small
		<i>Scaptotrigona postica</i> (Latreille, 1807)	Nectar/Pollen	Small
		<i>Nannotrigona testaceicornis</i> (Lepeletier, 1836)	Nectar/Pollen	Small
		<i>Paratrigona lineata</i> (Lepeletier, 1836)	Nectar/Pollen/Resin	Small
		<i>Plebeia droryana</i> (Friese, 1900)	Nectar/Pollen	Small
		<i>Scaptotrigona depilis</i> (Moure, 1942)	Nectar/Pollen	Small
		<i>Tetragona clavipes</i> (Fabricius, 1804)	Nectar/Pollen/Resin	Small
		<i>Tetragonisca angustula</i> (Latreille, 1825)	Nectar/Pollen/Resin	Small
		<i>Trigona hyalinata</i> (Lepeletier, 1836)	Nectar/Pollen	Small

Family	Tribe	Species	Floral resource	Body size
Apidae	Epeolini	<i>Trigona spinipes</i> Fabricius, 1793	Nectar/Pollen	Small
		<i>Thalestria spinosa</i> (Fabricius, 1804)	Nectar/Pollen	Large
		<i>Osiris</i> Smith, 1854 sp. 1	Nectar/Pollen	Small
	Tapinotaspidini	<i>Monoeca</i> Lepeletier & Serville, 1828 sp. 1	Nectar/Pollen/Oil	Small
	Tapinotaspidini	<i>Paratetrapedia</i> Moure, 1941 sp. 1	Nectar/Pollen/Oil	Small
	Tetrapediini	<i>Paratetrapedia</i> sp. 2	Nectar/Pollen/Oil	Small
		<i>Tetrapedia diversipes</i> Klug, 1810	Nectar/Pollen/Oil	Small
		<i>Tetrapedia</i> Klug, 1810 sp. 1	Nectar/Pollen/Oil	Small
	Ceratinini	<i>Ceratina</i> (<i>Crewella</i>) <i>asuncionis</i> Strand, 1910	Nectar/Pollen	Small
		<i>Ceratina</i> (<i>Crewella</i>) <i>gossypii</i> Schrottky, 1907	Nectar/Pollen	Small
		<i>Ceratina</i> (<i>Crewella</i>) <i>maculifrons</i> Smith, 1854	Nectar/Pollen	Small
		<i>Ceratina</i> (<i>Crewella</i>) <i>paraguayensis</i> Schrottky, 1907	Nectar/Pollen	Small
		<i>Ceratina</i> Latreille, 1802 sp. 1	Nectar/Pollen	Small
		<i>Ceratina</i> sp. 2	Nectar/Pollen	Small
		<i>Ceratina</i> (<i>Ceratinula</i>) <i>oxalidis</i> Schrottky, 1907	Nectar/Pollen	Small
		<i>Ceratina</i> (<i>Ceratinula</i>) Moure, J. S. (1941) sp. 1	Nectar/Pollen	Small
	Xylocopini	<i>Xylocopa</i> (<i>Megaxylocopa</i>) <i>frontalis</i> (Olivier, 1789)	Nectar/Pollen	Large
		<i>Xylocopa</i> (<i>Neoxylocopa</i>) <i>hirsutissima</i> Maidl, F. (1912)	Nectar/Pollen	Large
Apidae	Xylocopini	<i>Xylocopa</i> (<i>Schonnherria</i>) <i>macrops</i> Lepeletier, 1841	Nectar/Pollen	Large
		<i>Xylocopa</i> (<i>Neoxylocopa</i>) <i>transitoria</i> Pérez, 1901	Nectar/Pollen	Large

Family	Tribe	Species	Floral resource	Body size
		<i>Xylocopa (Megaxylocopa) fimbriata</i> Fabricius, 1804	Nectar/Pollen	Large
Colletidae	Hylaeini	<i>Hylaeus</i> Fabricius, 1793 sp. 1	Nectar/Pollen	Small
Halictidae	Augochlorini	<i>Augochlora (Augochlora) esox</i> (Vachal, 1911)	Nectar/Pollen	Small
		<i>Augochlora</i> Smith, 1853 sp. 1	Nectar/Pollen	Small
		<i>Augochlora</i> sp. 2	Nectar/Pollen	Small
		<i>Augochlora</i> sp. 3	Nectar/Pollen	Small
		<i>Augochlora</i> sp. 4	Nectar/Pollen	Small
		<i>Augochlorella urania</i> (Smith, 1853)	Nectar/Pollen	Small
		<i>Augochloropsis smithiana</i> (Cockerell, 1900)	Nectar/Pollen	Small
		<i>Augochloropsis electra</i> (Smith, 1853)	Nectar/Pollen	Small
		<i>Augochloropsis aurifluens</i> (Vachal, 1903)	Nectar/Pollen	Small
Halictidae	Augochlorini	<i>Augochloropsis brachycephala</i> Moure, 1943	Nectar/Pollen	Small
		<i>Augochloropsis melanochaeta</i> Moure, 1950	Nectar/Pollen	Small
		<i>Augochloropsis cleopatra</i> (Schrottky, 1902)	Nectar/Pollen	Small
		<i>Augochloropsis cupreola</i> (Cockerell, 1900)	Nectar/Pollen	Small
		<i>Augochloropsis</i> Cockerell, 1897 sp. 1	Nectar/Pollen	Small
		<i>Augochloropsis</i> sp. 2	Nectar/Pollen	Small
		<i>Augochloropsis</i> sp. 3	Nectar/Pollen	Small
		<i>Augochloropsis</i> sp. 4	Nectar/Pollen	Small
		<i>Augochloropsis</i> sp. 5	Nectar/Pollen	Small

Family	Tribe	Species	Floral resource	Body size
		<i>Ceratalictus clonius</i> (Brèthes, 1909)	Nectar/Pollen	Small
		<i>Megalocta</i> Smith, 1853 sp. 1	Nectar/Pollen	Large
		<i>Pseudaugochlora graminea</i> (Fabricius, 1804)	Nectar/Pollen	Small
		<i>Pseudaugochlora nigerrima</i>	Nectar/Pollen	Small

Table S2.2 Plant species collected between 1971 and 1975 at Fazenda Treze de Maio in a Brazilian Cerrado, São Paulo state, Brazil.

Family	Species
Acanthaceae	<i>Ruellia geminiflora</i> Kunth
Annonaceae	<i>Annona cornifolia</i> A.St.-Hil.
Apocynaceae	<i>Hancornia speciosa</i> Gomes
Araliaceae	<i>Schefflera vinosa</i> (Cham. & Schltdl.) Frodin & Fiaschi
Arecaceae	<i>Acrocomia hassleri</i> (Barb.Rodr.) W.J.Hahn
	<i>Butia paraguayensis</i> (Barb.Rodr.) L.H.Bailey
	<i>Syagrus</i> Mart. sp. 1
	<i>Syagrus</i> sp. 2
Asteraceae	<i>Baccharis dracunculifolia</i> DC.
	<i>Baccharis selloi</i> Baker
	<i>Bidens segetum</i> Mart. ex Colla
	<i>Calea mediterranea</i> (Vell.) Pruski
	<i>Chromolaena barbacensis</i> (Hieron.) R.M.King & H.Rob.
	<i>Chromolaena campestris</i> (DC.) R.M.King & H.Rob.
	<i>Gochnatia barrosoae</i> Cabrera
	<i>Lessingianthus bardanoides</i> (Less.) H.Rob.
	<i>Lessingianthus grandiflorus</i> (Less.) H.Rob.
	<i>Mikania micrantha</i> (Hieron.) B.L.Rob.
	<i>Moquiniastrium polymorphum</i> (Less.) G.Sancho
	<i>Porophyllum lanceolatum</i> DC.
	<i>Stomatanthes warmingii</i> (Baker) H.Rob.
Asteraceae	<i>Trichogonia attenuata</i> G.M.Barroso
	<i>Vernonanthura ferruginea</i> (Less.) H.Rob.

Table S2.2
Continued.

	<i>Vernonanthura lindbergii</i> (Baker) H.Rob.
	<i>Wedelia floribunda</i> (Gardner) B.L.Turner
Bignoniaceae	<i>Adenocalymma peregrinum</i> (Miers) L.G.Lohmann
	<i>Amphilophium elongatum</i> (Vahl) L.G.Lohmann
	<i>Anemopaegma arvense</i> (Vell.) Stellfeld ex J.F.Souza
	<i>Cuspidaria pulchra</i> (Cham.) L.G.Lohmann
	<i>Fridericia platyphylla</i> (Cham.) L.G.Lohmann
	<i>Handroanthus ochraceus</i> (Cham.) Mattos
	<i>Jacaranda decurrens</i> Cham.
	<i>Jacaranda rufa</i> Silva Manso
	<i>Tabebuia aurea</i> Benth. & Hook.f. ex S.Moore
Bixaceae	<i>Cochlospermum regium</i> (Schrunk) Pilg.
Bromeliaceae	<i>Ananas comosus</i> var. <i>microstachys</i> L.B.Sm.
Calophyllaceae	<i>Kielmeyera variabilis</i> Mart. & Zucc.
Caryocaraceae	<i>Caryocar brasiliense</i> Cambess.
Celastraceae	<i>Tontelea micrantha</i> (Mart. ex Schult.) A.C.Sm.
Chrysobalanaceae	<i>Licania humilis</i> Cham. & Schltdl.
Commelinaceae	<i>Commelina erecta</i> L.
Connaraceae	<i>Connarus suberosus</i> Planch.
Convolvulaceae	<i>Jacquemontia blanchetii</i> Moric.
	<i>Distimake digitatus</i> (Spreng.) A.R.Simões & Staples
Dilleniaceae	<i>Davilla elliptica</i> A.St.-Hil.
Erythroxylaceae	<i>Erythroxylum pelleterianum</i> A.St.-Hil.
	<i>Erythroxylum suberosum</i> A.St.-Hil.

Table S2.2
Continued.

	<i>Erythroxylum tortuosum</i> Mart.
Euphorbiaceae	<i>Manihot violacea</i> Pohl
Fabaceae	<i>Bauhinia holophylla</i> (Bong.) Steud.
	<i>Bowdichia virgilioides</i> Kunth
	<i>Chamaecrista campestris</i> H.S.Irwin & Barneby
	<i>Chamaecrista cathartica</i> (Mart.) H.S.Irwin & Barneby
	<i>Chamaecrista desvauxii</i> (Collad.) Killip
	<i>Chamaecrista desvauxii</i> var. <i>latistipula</i> (Benth.) G.P.Lewis
	<i>Chamaecrista desvauxii</i> var. <i>parvifoliola</i>
	<i>Chamaecrista flexuosa</i> (L.) Greene
	<i>Chamaecrista labouriaee</i> (H.S.Irwin & Barneby) H.S.Irwin & Barneby
	<i>Crotalaria micans</i> Link
	<i>Crotalaria paulina</i> Schrank
	<i>Dimorphandra mollis</i> Benth.
	<i>Leptolobium elegans</i> Vogel
	<i>Machaerium acutifolium</i> Vogel
	<i>Mimosa falcipinna</i> Benth.
	<i>Plathymenia reticulata</i> Benth.
	<i>Rhynchosia pyramidalis</i> (Lam.) Urb.
	<i>Rhynchospora exaltata</i> Kunth
	<i>Senna rugosa</i> (G.Don) H.S.Irwin & Barneby
	<i>Stryphnodendron adstringens</i> (Mart.) Coville
Fabaceae	<i>Stylosanthes gracilis</i> Kunth
	<i>Stylosanthes guianensis</i> (Aubl.) Sw.

Table S2.2
Continued.

	<i>Tachigali aurea</i> Tul.
	<i>Tephrosia sessiliflora</i> Hassl.
	<i>Vigna longifolia</i> (Benth.) Verdc.
Lamiaceae	<i>Eriope crassipes</i> Benth.
	<i>Medusantha crinita</i> (Benth.) Harley & J.F.B.Pastore
Malpighiaceae	<i>Banisteriopsis argyrophylla</i> (A.Juss.) B.Gates
	<i>Banisteriopsis latifolia</i> (A.Juss.) B.Gates
	<i>Byrsonima coccolobifolia</i> Kunth
	<i>Byrsonima intermedia</i> A.Juss.
	<i>Byrsonima verbascifolia</i> Rich. ex Juss.
	<i>Diplopterys pubipetala</i> (A.Juss.) W.R.Anderson & C.Davis
	<i>Tetrapteryx ramiflora</i> A.Juss.
Malvaceae	<i>Eriotheca gracilipes</i> (K.Schum.) A.Robyns
	<i>Luehea grandiflora</i> Mart.
	<i>Pavonia biflora</i> Fryxell
	<i>Sida glaziovii</i> K.Schum.
	<i>Sida</i> L. sp. 1
	<i>Waltheria indica</i> L.
Melastomataceae	<i>Miconia ligustroides</i> Naudin
Myrtaceae	<i>Campomanesia pubescens</i> O.Berg
	<i>Eugenia aurata</i> O.Berg
	<i>Eugenia pyriformis</i> Cambess.
Myrtaceae	<i>Eugenia</i> L.
	<i>Myrcia bella</i> Cambess.

Table S2.2
Continued.

	<i>Myrcia torta</i> DC.
Nyctaginaceae	<i>Guapira graciliflora</i> (Mart. ex J.A.Schmidt) Lundell
Ochnaceae	<i>Ouratea spectabilis</i> Engl.
Polygalaceae	<i>Bredemeyera floribunda</i> Willd.
	<i>Securidaca divaricata</i> Nees & Mart.
Proteaceae	<i>Roupala montana</i> Aubl.
Rubiaceae	<i>Cordia sessilis</i> Kuntze
	<i>Spermacoce capitata</i> Ruiz & Pav.
	<i>Palicourea rigida</i> Kunth
	<i>Richardia grandiflora</i> (Cham. & Schltdl.) Steud.
Salicaceae	<i>Casearia sylvestris</i> Sw.
Sapindaceae	<i>Serjania reticulata</i> Cambess.
Solanaceae	<i>Solanum lycocarpum</i> A.St.-Hil.
Styracaceae	<i>Styrax ferrugineus</i> Nees & Mart.
Verbenaceae	<i>Lantana camara</i> L.
	<i>Lippia lupulina</i> Cham.
	<i>Lippia origanoides</i> Kunth
	<i>Stachytarpheta simplex</i> Hayek
Vochysiaceae	<i>Vochysia cinnamomea</i> Pohl

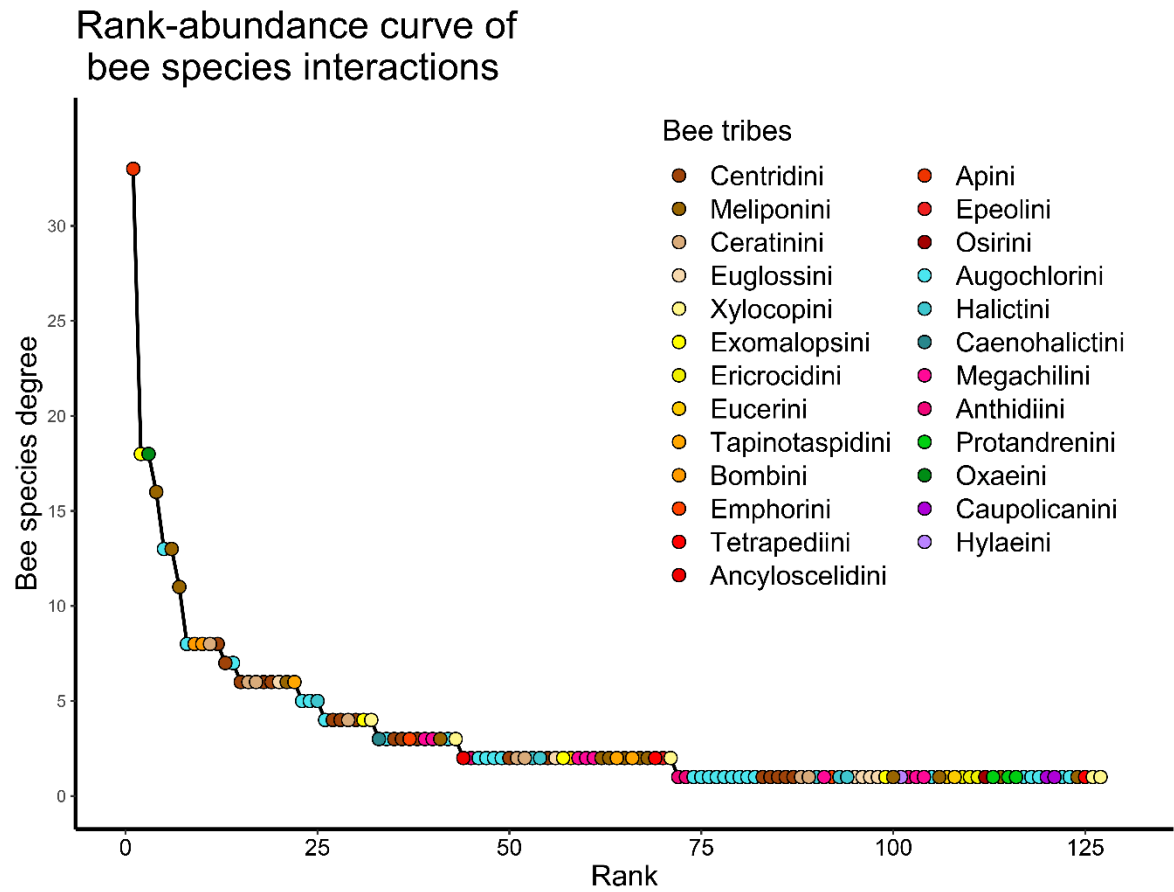


Figure S2.1. Rank-abundance curve showing bee species degree *i.e.*, the number of pairwise interactions performed by each bee with plants. Colors depict each bee tribe.

3 CHAPTER II*

**Global convergence and fine-scale shifts in plant–bee network structure across the
honeybee’s native and non-native ranges**

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Global convergence and fine-scale shifts in plant–bee network structure across the honeybee’s native and non-native ranges

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Abstract

Pollinator communities are increasingly influenced by human-mediated species introductions, yet the global impacts of invasive pollinators on network structure remain poorly understood. We performed a systematic review that compiled 80 quantitative plant–bee interaction networks encompassing the native and non-native ranges of the western honeybee (*Apis mellifera*) and analyzed network organization at three hierarchical levels: the overall network architecture (macro-scale), participation in motif classes (meso-scale), and species’ structural roles (micro-scale). Macro-scale metrics (including connectance, nestedness, modularity, and robustness) did not differ between ranges, indicating broad structural similarity across the species’ global distribution. However, finer-scale analyses revealed significant range-dependent shifts. In non-native regions, *A. mellifera* exhibited a lower within-module degree, suggesting reduced integration within network compartments, and participated more frequently in fan motifs but less in core–peripheral motifs, implying altered dominance and resource-use patterns. These findings show that *A. mellifera* maintains a largely conserved structural role worldwide, but that subtle shifts in local connectivity and sub-structural organization occur outside its native range. Overall, our results reveal that while invasive generalists can promote structural convergence in mutualistic networks at macro scales, cross-scale analyses uncover hidden variations that may influence the long-term stability and resilience of pollination systems under global change.

Keywords: *Apis*–bee interaction *mellifera*; biological invasions; ecological homogenization; network convergence; plant networks; pollination network resilience

3.1 INTRODUCTION

Pollinator communities and the ecosystem services they provide are increasingly shaped by anthropogenic pressures (Potts et al. 2016; Vanbergen 2013). One of the most pervasive outcomes of the Anthropocene is the large-scale movement of species across biogeographic boundaries through human activities (Dicks et al. 2016; Valido et al. 2019). Introduced species can alter community composition, disrupt species interactions, and modify ecosystem functioning, including processes fundamental to angiosperm reproduction such as pollination (Peller & Altermatt 2024). Because pollination underpins both plant reproduction and community stability (Ollerton 2017), understanding how introduced pollinators integrate into native interaction networks is key to predicting their long-term ecological consequences.

Among introduced pollinators, the western honeybee (*Apis mellifera*, Hymenoptera: Apidae) is exceptional for its ecological breadth and global reach (Moritz & Neumann 2005). Native to Africa, Europe, and parts of western Asia (Dogantzis et al. 2021; Ji 2021), it has been introduced on all continents except Antarctica for apiculture and crop pollination (Dicks et al. 2016; Carpenter & Harpur 2021). Traits such as eusociality, generalist foraging, and nesting flexibility facilitate its establishment across a wide range of ecological contexts (Weislo et al. 2017; Magrach et al. 2017; Visick & Ratnieks 2023). Local-scale studies have shown that *A. mellifera* can influence native bee communities by competing for floral resources, altering visitation frequencies, and changing relative abundances of native species, though the magnitude and direction of these effects are often context-dependent (Roubik & Wolda 2001; Paine 2004; Valido et al. 2019; Hachuy-Filho et al. 2020; Ballarin et al. 2022; Sponsler et al. 2023). Honeybee super-generalist behaviour allows it to interact with a broad diversity of flowering plants, often reshaping the distribution of interactions among native pollinators.

Such species-level changes can cascade through entire mutualistic networks, affecting community-wide patterns of interaction. By interacting with many plant species, *A.*

mellifera may increase connectance and nestedness while reducing modularity and specialization, patterns that can enhance functional redundancy but ultimately reduce the overall network specialization (Aizen et al. 2008; Traveset et al. 2013; Stouffer et al. 2014). However, the structural effects of a super-generalist may differ across macro-, meso-, and micro-scales, affecting the overall network architecture, network motifs (*i.e.*, the building blocks of ecological networks), and species roles in distinct ways (Guimerà & Amaral 2005; Milo et al. 2002; Olesen et al. 2007; Simmons et al. 2019a). These scale-dependent effects may remain undetected when only macro-scale properties are considered, potentially leading to an underestimation of invasion impacts.

Here, we performed a systematic review and collected a global dataset of 80 plant–bee interaction networks to test whether network structure at multiple organizational scales differs across *A. mellifera*’s native and non-native ranges. We quantified network properties at three scales: (i) macro-scale (the overall network architecture), (ii) meso-scale (participation in distinct motif classes), and (iii) micro-scale (species-level roles). This approach enables us to assess how the introduction of a single, globally distributed pollinator influences the structure of plant-bee interaction networks worldwide, and how biological invasions may reshape the architecture of ecological interactions across scales.

3.2 MATERIAL AND METHODS

3.2.1 Plant-bee interaction network dataset

Following the PRISMA protocol (Moher et al. 2015), we compiled a comprehensive dataset of plant–bee interaction networks including *Apis mellifera*. We searched the Web of Science database from 1976 to 2024 and complemented this search with data from Google Scholar, Dryad, Web of Life, and articles cited in systematic reviews. To ensure a broad and inclusive search, we accounted for both American and British spelling variations by using logical operators (AND/OR). The search string “(networ AND pollina*) OR floral-visit* OR plant-bee*” captured all relevant variations of the keywords “network,” “pollinator,” “floral-visitor,” and “plant-bee.” The initial search produced 1,513 records, from which duplicates

were removed following the PRISMA 2020 guidelines (Moher et al. 2015; Figure S3.1). We then applied a series of inclusion criteria to select suitable networks for analysis. Eligible networks had to (i) include *A. mellifera* among pollinators; (ii) represent only interactions between plants and bees; (iii) be quantitative (weighted); (iv) be sampled using the phytocentric approach, *i.e.*, recording legitimate pollinator visits on flowers; and (v) represent entire communities rather than experimental or focal-species datasets. Networks collected less than 2 km apart were considered part of the same community and were excluded to avoid spatial pseudoreplication. We also removed all pairwise interactions involving animal taxa outside Anthophila. After these filtering steps, our dataset included 80 plant–bee networks, 39 from *A. mellifera*’s native range and 41 from its non-native range (Figure 3.1, S3.1). To evaluate potential sampling biases, we estimated sampling completeness as the proportion of observed to estimated interaction richness using the Chao1 estimator (Chao 1984). Sampling completeness did not differ between native and non-native ranges ($\chi^2 < 0.01$, $p = 0.96$; $R^2 < 0.01$; Figure S3.2).

3.2.2 Network properties across organizational scales

To investigate how *A. mellifera*’s range influenced network structure, we quantified properties across three organizational scales. At the macro-scale, we compared overall network architecture between ranges by calculating several metrics related to structure, specialization, and robustness. We estimated connectance (C), the proportion of realized versus potential interactions; bee niche overlap (R_o), representing the degree of overlap in plant use among bee species; and network-wide specialization ($H2'$), quantifying interaction exclusivity (Blüthgen et al. 2006). Weighted nestedness ($wNODF$) was calculated to describe asymmetrical dependency between specialists and generalists (Bascompte et al. 2003; Almeida-Neto & Ulrich 2011), while weighted modularity (Q') was used to measure the compartmentalization of networks into internally cohesive modules (Beckett 2016). Both nestedness and modularity were Δ -transformed to represent deviations from null expectations (Fründ et al. 2016; Dalsgaard et al. 2017), and null distributions were generated using 10,000 randomizations of the *vaznull* algorithm, which preserves network size, species richness, and connectance (Vázquez et al. 2009). Finally, we calculated network robustness (Rd) as the proportion of bee species persisting after sequential removal of plants, simulating cascading

extinctions from least to most connected species (Burgos et al. 2007).

At the meso-scale, we examined the composition of interaction motifs, *i.e.*, small, recurring sub-networks that represent distinct topological arrangements of species interactions (Milo et al. 2004; Simmons et al. 2019a). Following Simmons et al. (2021), we classified motifs into four categories according to their connectivity patterns and path lengths: (1) core–peripheral motifs, composed of interconnected generalists linked to one or more specialists; (2) complete motifs, where all species interact with each other; (3) asymmetric complete motifs, similar to complete ones but with an attached specialist; and (4) fan motifs, characterized by multiple specialists sharing a single generalist or a single pollinator visiting multiple plants. We quantified motif frequencies using the R package *bmotif* (Simmons et al. 2019b) and measured the proportion of each motif type in which *A. mellifera* and other bee species participated across networks in honeybee’s native and non-native ranges. This approach allowed us to assess potential shifts in *A. mellifera*’s structural role and its effect on other bee species at the meso-scale structural level.

At the micro-scale, we characterized species-level structural roles using indices that describe how *A. mellifera* and other bees contribute to the network’s internal architecture in native and non-native contexts. The within-module degree (z) measures how strongly a species is connected within its own module, indicating its local integration; the among-module connectivity (c) assesses how evenly a species distributes its interactions among modules, identifying potential connectors; and the nestedness contribution quantifies each species’ influence on the network’s nested organization (Guimerà & Amaral 2005; Olesen et al. 2007; Saavedra et al. 2011).

3.2.3 Statistical Analysis

To test whether network properties at multiple scales differed between *A. mellifera*’s native and non-native ranges, we fitted generalized linear mixed models (GLMMs) using *glmmTMB* in R, validated with the *DHARMA* package. For each organizational scale, we used the calculated metrics as response variables and *A. mellifera* range (native vs. non-native) as the fixed effect, with biome included as a random factor to account for environmental

variability. For the meso-scale analysis, we also tested the interaction between range and motif class to evaluate whether differences in motif participation varied across motif types. Models for proportional data (C , R_o , R_d , motif proportions, nestedness contribution) were fitted with beta regressions, whereas Gaussian error distributions were used for continuous variables. This multi-scale modelling framework allowed us to assess whether *A. mellifera*'s global distribution is associated with consistent patterns of network organization across ecological contexts (*i.e.*, native vs. non-native range).

3.3 RESULTS

Our literature search identified 41 studies that met the inclusion criteria, resulting in a dataset of 80 quantitative plant–bee interaction networks encompassing all continents except Antarctica (Figure 3.1). Among these, 51.25% ($n = 41$) were from *Apis mellifera*'s non-native range and 48.75% ($n = 39$) from its native range. Across all networks, we recorded 1181 plant species and 1047 bee species in the non-native range, and 572 plant species and 496 bee species in the native range. On average, *A. mellifera* accounted for 26.67% of floral visits in its native range and 25.8% in its non-native range.

At the macro-scale, we found no significant differences in any of the overall network metrics across *A. mellifera*'s native and non-native distributions. Connectance (C ; $\chi^2 = 0.21$, $p = 0.65$, $R^2_{\text{adj}} < 0.01$, Figure S3.3a), bee niche overlap (R_o ; $\chi^2 = 0.03$, $p = 0.87$, $R^2_{\text{adj}} < 0.01$, Figure 3.2a), and network-wide specialization ($H2'$; $\chi^2 = 1.20$, $p = 0.27$, $R^2_{\text{adj}} = 0.32$, Figure 3.2b) did not vary significantly between ranges. Likewise, no differences were detected in weighted nestedness ($\Delta\text{-wNODF}$; $\chi^2 = 0.38$, $p = 0.54$, $R^2_{\text{adj}} = 0.09$, Figure 3.2c), weighted modularity ($\Delta\text{-Q}'$; $\chi^2 = 0.38$, $p = 0.54$, $R^2_{\text{adj}} < 0.01$, Figure 3.2d), or robustness to bee loss (R_d ; $\chi^2 = 0.20$, $p = 0.65$, $R^2_{\text{adj}} = 0.60$, Figure S3.3b).

At the meso-scale, the overall proportion of motif classes differed significantly across all networks ($\chi^2 = 208.22$, $p < 0.01$, Figure 3.2e), but there was no main effect of range ($\chi^2 = 1.64$, $p = 0.20$, $R^2_{\text{adj}} = 0.57$; Figure 3.2e). When considering only motifs that involved *A.*

mellifera, however, the interaction between range and motif class was significant ($\chi^2 = 19.92$, $p < 0.01$, $R^2 = 0.88$; Figure 3.2f). In its native range, *A. mellifera* participated more frequently in core-peripheral motifs (mean $\pm$ SE: 0.675 ± 0.023) than in non-native networks (0.569 ± 0.025 , Figure 3.2f). Conversely, in non-native networks, the species was proportionally more involved in fan motifs (0.316 ± 0.023) than in native ones (0.219 ± 0.020). No significant differences were detected in the frequency of complete motifs (0.041 ± 0.006 , $p = 0.55$) or asymmetric complete motifs (0.091 ± 0.012 , $p = 0.48$, Figure 3.2f). These results indicate that *A. mellifera* tends to occupy different structural positions within interaction sub-networks depending on whether it occurs inside or outside its native range.

At the micro-scale, when considering all bee species within the networks, we found no significant differences in nestedness contribution ($\chi^2 = 0.60$, $p = 0.44$, $R^2_{\text{adj}} = 0.03$, Figure S3.4a), within-module degree ($\chi^2 < 0.01$, $p = 0.99$, $R^2_{\text{adj}} < 0.01$, Figure 3.2g), or among-module connectivity ($\chi^2 = 3.27$, $p = 0.07$, $R^2_{\text{adj}} = 0.99$, Figure 3.2h) between the native and non-native ranges of *A. mellifera*. Similarly, when analyzing only the honeybee's role within networks, neither nestedness contribution ($\chi^2 = 2.21$, $p = 0.10$, $R^2_{\text{adj}} = 0.14$, Figure S3.4b) nor among-module connectivity ($\chi^2 = 1.75$, $p = 0.18$, $R^2_{\text{adj}} = 0.08$; Figure 3.2i) differed significantly between ranges. However, *A. mellifera*'s within-module degree differed between regions ($\chi^2 = 8.70$, $p < 0.01$, $R^2_{\text{adj}} = 0.38$, Figure 3.2j), with higher values in native networks (mean $\pm$ SE: 1.55 ± 0.35) compared to non-native ones (0.71 ± 0.30 ; Figure 3.2j). These results indicate that honeybees in non-native regions tend to be less integrated within network modules. Residual diagnostics for all models are presented in Figures S3.5–S3.9. Collectively, these results demonstrate that while overall network architecture remains consistent across *A. mellifera*'s global range, subtle but informative differences arise in the species' local connectivity and motif participation, revealing scale-dependent shifts in its structural role across ecological contexts.

3.4 DISCUSSION

By examining networks spanning most of the global distribution of *Apis mellifera*,

we found that plant–bee network structures are broadly similar across its native and non-native ranges when assessed at the macro-scale, *i.e.*, the level most commonly used to diagnose differences among communities or disturbance regimes. However, we also identified subtle but meaningful shifts at finer organizational levels. At the micro-scale, *A. mellifera* exhibited a lower within-module degree in non-native networks, indicating reduced integration within modules. More importantly, at the meso-scale, the species showed a distinct participation in motif classes, being more frequently associated with fan motifs and less with core–peripheral motifs in non-native regions. This means that outside its native range, the honeybee tends to form more asymmetric connections in which a single pollinator interacts with many plants, suggesting a more dominant pattern of resource use.

Collectively, these results suggest that *A. mellifera* plays an overall similar structural role across its global range, yet fine-scale metrics reveal range-dependent differences that are not reflected in broad network architecture. These findings emphasize that cross-scale analyses are crucial for detecting the subtle, context-specific effects of invasive species that may remain hidden when only broad and macro-scale network properties are considered.

The apparent global consistency in *A. mellifera*'s structural role may reflect long-term ecological integration into non-native communities, although alternative explanations such as convergence in network metrics cannot be excluded. Over time, pollination networks may reorganize around a dominant invader, producing structures that converge toward those found in its native range (Cadotte et al. 2018; Bascompte et al. 2023). Such apparent equilibria could obscure the transient effects of competition or displacement that likely occurred during early invasion stages but are rarely captured without pre-invasion baselines (Paini 2004). Our findings demonstrate that exploring less commonly studied scales, such as interaction motifs and modular integration, reveal patterns that would remain undetected by macro-scale metrics alone. These finer-scale approaches provide a more sensitive framework for identifying subtle interaction shifts and potential early signals of structural reorganization following biological invasions.

Reduced within-module degree in non-native networks may indicate that *A. mellifera*

contributes less to the internal cohesion of interaction compartments outside its native range. This pattern could arise if native bees within the same module broaden their foraging spectrum to reduce competition with honeybees, thereby weakening module integrity (Valido et al. 2019). Likewise, the increased frequency of fan motifs in non-native regions, where multiple specialists interact indirectly through a single generalist, suggests changes in resource-use patterns and dominance dynamics. Core–peripheral motifs, more common in native ranges, represent dense clusters of interactions among generalists and specialists, whereas fan motifs capture systems in which a dominant species monopolizes interactions with many partners (Simmons et al. 2021). Hence, the observed shift toward fan motifs may reflect a transition toward resource monopolization by *A. mellifera*, or a compensatory rearrangement of native pollinator behaviour to maintain network connectivity. Although our dataset does not include temporal data to directly assess competitive outcomes, these differences align with previous evidence that honeybee introductions can reshape the topology of pollination networks (Magrach et al. 2017; Dattilo et al. 2022; Cruz et al. 2022).

Despite these local changes, macro-scale properties such as connectance, nestedness, modularity, and robustness remained remarkably stable across the honeybee’s native and introduced ranges. This apparent invariance may reflect a process of structural convergence, in which community-level architecture stabilizes around a recurring pattern despite underlying variation in local species composition. Several mechanisms may contribute to this outcome, such as functional similarity among generalist pollinators across regions and long-term rewiring of interactions that restores network stability following disturbance (Bascompte & Stouffer 2009; Rodríguez-Rodríguez et al. 2017; Ballarin et al. 2024). Importantly, these forms of convergence are not necessarily indicative of system stability. Even when network-level metrics remain unchanged, shifts in how interactions are distributed among species can have profound implications for the long-term resilience and redundancy of pollination systems.

3.5 CONCLUSION

Whether the convergence observed here represents a stable configuration or a transient phase of adjustment remains uncertain. If stable, it may indicate that *A. mellifera* has become functionally integrated into many ecosystems, generating a new equilibrium where its dominance defines network architecture. In this scenario, pollination networks may exhibit a form of ecological homogenization, where structural similarity arises across biogeographic boundaries through the spread of a single generalist species. Conversely, if transient, these networks could still be undergoing reorganization, and their apparent resilience may mask delayed disruptions that manifest only after further losses of native species or resources. The resilience of pollination networks under ongoing global change will therefore depend on whether native species can adapt to, coexist with, or functionally replace *A. mellifera* within these restructured systems. Integrating long-term datasets and historical baselines will be essential to distinguish between stable equilibria and transitional phases and to forecast the persistence of pollination services in an increasingly homogenized world (Dáttilo et al. 2022; Worthy et al. 2023).

3.6 REFERENCES

- Aizen MA, Morales CL, & Morales JM (2008) Invasive mutualists erode native pollination webs. *PLoS biology* 6: e31.
- Almeida-Neto M & Ulrich W (2011) A straightforward computational approach for measuring nestedness using quantitative matrices. *Environmental Modelling & Software* 26: 173-178
- Araujo AC, Martín González AM, Sandel B, et al. (2018) Spatial distance and climate determine modularity in a cross-biomes plant–hummingbird interaction network in Brazil. *Journal of Biogeography* 45: 1846-1858
- Ballarin CS, Vizentin-Bugoni J, Hachuy-Filho L & Amorim, FW (2024) Imprints of indirect interactions on a resource-mediated ant–plant network across different levels of network organization. *Oecologia* 204: 661-673.
- Bascompte J, Jordano P, Melián CJ, et al. (2003) The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences* 100: 9383-9387.
- Bascompte J & Scheffer M (2023) The resilience of plant–pollinator networks. *Annual Review of Entomology* 68: 363-380.
- Bascompte J & Stouffer DB (2009) The assembly and disassembly of ecological networks. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364: 1781-1787.
- Beckett SJ (2016) Improved community detection in weighted bipartite networks. *Royal Society open science* 3: 140536.

- Blüthgen N, Menzel F, & Blüthgen N (2006) Measuring specialization in species interaction networks. *BMC ecology* 6: 9.
- Burgos E, Ceva H, Perazzo RP, et al. (2007) Why nestedness in mutualistic networks? *Journal of theoretical biology* 249: 307-313.
- Carpenter MH, & Harpur BA (2021) Genetic past, present, and future of the honey bee (*Apis mellifera*) in the United States of America. *Apidologie* 52: 63-79.
- Chao, A. (1984) Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of statistics* 265-270.
- Cruz CP, Luna P, Guevara R et al. (2022) Climate and human influence shape the interactive role of the honeybee in pollination networks beyond its native distributional range. *Basic and Applied Ecology* 63: 186-195.
- Cadotte MW, Campbell SE, Li SP, et al. (2018) Preadaptation and naturalization of nonnative species: Darwin's two fundamental insights into species invasion. *Annual Review of Plant Biology* 69: 661-684.
- Dalsgaard B, Schleuning M, Maruyama PK, et al (2017) Opposed latitudinal patterns of network-derived and dietary specialization in avian plant–frugivore interaction systems. *Ecography* 40: 1395-1401.
- Dattilo W, Cruz CP, Luna P, Ratoni B et al. (2022) The impact of the honeybee *Apis mellifera* on the organization of pollination networks is positively related with its interactive role throughout its geographic range. *Diversity* 14: 917.
- Dicks LV, Viana B, Bommarco R. et al. (2016) Ten policies for pollinators. *Science* 354: 975-976.

- Dogantzis KA, Tiwari T, Conflitti IM, et al. (2021) Thrice out of Asia and the adaptive radiation of the western honey bee. *Science advances* 7: eabj2151.
- Dohzono I, & Yokoyama J (2010) Impacts of alien bees on native plant-pollinator relationships: A review with special emphasis on plant reproduction. *Applied Entomology and Zoology* 45: 37-47.
- Dáttilo W, Cruz CP, Luna P, et al (2022) The impact of the honeybee *Apis mellifera* on the Organization of Pollination Networks is positively related with its interactive role throughout its geographic range. *Diversity* 14: 917.
- Fründ J, McCann KS & Williams NM (2016) Sampling bias is a challenge for quantifying specialization and network structure: lessons from a quantitative niche model. *Oikos* 125: 502-513.
- Goulson D (2003) Effects of introduced bees on native ecosystems. *Annual Review of Ecology, Evolution, and Systematics* 34: 1-26.
- Goulson D, Hanley ME, Darvill B, et al. (2005) Causes of rarity in bumblebees. *Biological conservation* 122: 1-8.
- Guimera R & Nunes Amaral LA (2005) Functional cartography of complex metabolic networks. *nature* 433: 895-900.
- Hachuy-Filho L, Ballarin CS, & Amorim FW (2020) Changes in plant community structure and decrease in floral resource availability lead to a high temporal β -diversity of plant-bee interactions. *Arthropod-Plant Interactions* 14: 571-583.
- Ji Y (2021) The geographical origin, refugia, and diversification of honey bees (*Apis* spp.) based on biogeography and niche modeling. *Apidologie*, 52: 367-377.

- Lanuza JB, Allen-Perkins A & Bartomeus I (2023) The non-random assembly of network motifs in plant–pollinator networks. *Journal of Animal Ecology* 92: 760-773.
- Magrach A, González-Varo JP, Boiffier M, et al (2017) Honeybee spillover reshuffles pollinator diets and affects plant reproductive success. *Nature Ecology & Evolution* 1: 1299-1307.
- Maruyama PK, Vizentin-Bugoni J, Dalsgaard B, et al. (2015) Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. *Oecologia* 178: 783-793.
- Moher D, Shamseer L, Clarke M, et al (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic reviews* 4: 1-9.
- Moritz RF, Härtel S, & Neumann P (2005) Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. *Ecoscience* 12: 289-301.
- Milo R, Shen-Orr S, Itzkovitz S, et al. (2002) Network motifs: simple building blocks of complex networks. *Science* 298: 824-827
- Milo R, Itzkovitz S, Kashtan N, et al (2004) Superfamilies of evolved and designed networks. *Science* 303: 1538-1542.
- Myers N, Mittermeier RA, Mittermeier CG et al. (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.
- Olesen JM, Bascompte J, Dupont YL et al. (2007) The modularity of pollination networks. *Proceedings of the national academy of sciences* 104: 19891-19896.
- Ollerton J (2017) Pollinator diversity: distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics* 48: 353-376.

- Ollerton J & Cranmer L (2002) Latitudinal trends in plant-pollinator interactions: are tropical plants more specialised? *Oikos* 98: 340-350.
- Paini DR (2004) Impact of the introduced honey bee (*Apis mellifera*)(Hymenoptera: Apidae) on native bees: a review. *Austral ecology* 29: 399-407.
- Peller T, & Altermatt F (2024) Invasive species drive cross-ecosystem effects worldwide. *Nature Ecology & Evolution* 1-11.
- Potts SG, Imperatriz-Fonseca V, Ngo HT, et al (2016) Safeguarding pollinators and their values to human well-being. *Nature* 540: 220-229.
- Rodríguez-Rodríguez MC, Jordano P & Valido A (2017) Functional consequences of plant-animal interactions along the mutualism-antagonism gradient. *Ecology* 98:1266-1276.
- Roubik DW, & Wolda H (2001) Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. *Population Ecology* 43: 53-62.
- Saavedra S, Stouffer DB, Uzzi, B, et al. (2011) Strong contributors to network persistence are the most vulnerable to extinction. *Nature* 478: 233-235.
- Simmons BI, Cirtwill AR, Baker NJ, et al (2019a) Motifs in bipartite ecological networks: uncovering indirect interactions. *Oikos* 128: 154-170.
- Simmons BI, Sweering MJ, Schillinger M, et al. (2019b) bmotif: A package for motif analyses of bipartite networks. *Methods in Ecology and Evolution* 10: 695-701.
- Simmons BI, Beckerman AP, Hansen K, et al. (2021) Niche and neutral processes leave distinct structural imprints on indirect interactions in mutualistic networks.

Functional Ecology 35: 753-763.

Sponsler D, Iverson A & Steffan-Dewenter I (2023) Pollinator competition and the structure of floral resources. *Ecography* 2023: e06651.

Stouffer DB, Cirtwill AR & Bascompte J (2014) How exotic plants integrate into pollination networks. *Journal of Ecology* 102:1442-1450.

Traveset A, Heleno R, Chamorro S, et al. (2013) Invaders of pollination networks in the Galápagos Islands: emergence of novel communities. *Proceedings of the Royal Society B: Biological Sciences* 280: 20123040.

Valido A, Rodríguez-Rodríguez MC, & Jordano P (2019) Honeybees disrupt the structure and functionality of plant-pollinator networks. *Scientific reports* 9: 4711.

Vanbergen AJ, & Insect Pollinators Initiative (2013) Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment* 11: 251-259.

Vázquez DP, Chacoff NP, Cagnolo L. (2009). Evaluating multiple determinants of the structure of plant–animal mutualistic networks. *Ecology* 90: 2039-2046.

Visick OD & Ratnieks FL (2023) Density of wild honey bee, *Apis mellifera*, colonies worldwide. *Ecology and Evolution* 13: e10609.

Wcislo W, Fewell JH, Rubenstein DR, et al. (2017) Sociality in bees. *Comparative social evolution* 50-83.

Worthy SH, Acorn JH, & Frost CM (2023) Honey bees (*Apis mellifera*) modify plant-pollinator network structure, but do not alter wild species' interactions. *Plos one* 18: e0287332.

3.7 FIGURES

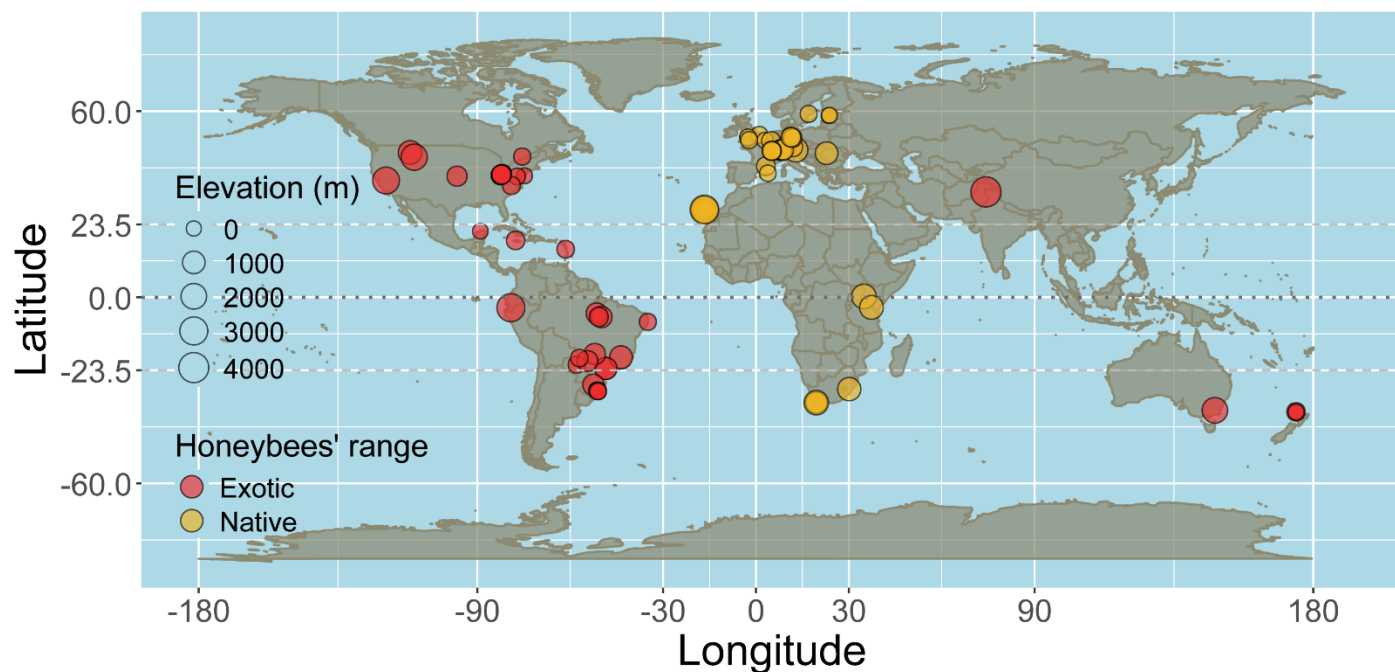


Figure 3.1. Global distribution of plant–bee interaction networks used in this study. Geographical locations of the 80 plant–bee networks compiled for analysis, shown across *Apis mellifera*’s native (yellow, $n = 39$) and non-native (red, $n = 41$) ranges. Symbol size indicates the elevation (m) of each sampling site. The dataset spans all continents except Antarctica and covers a wide range of biogeographic and environmental conditions.

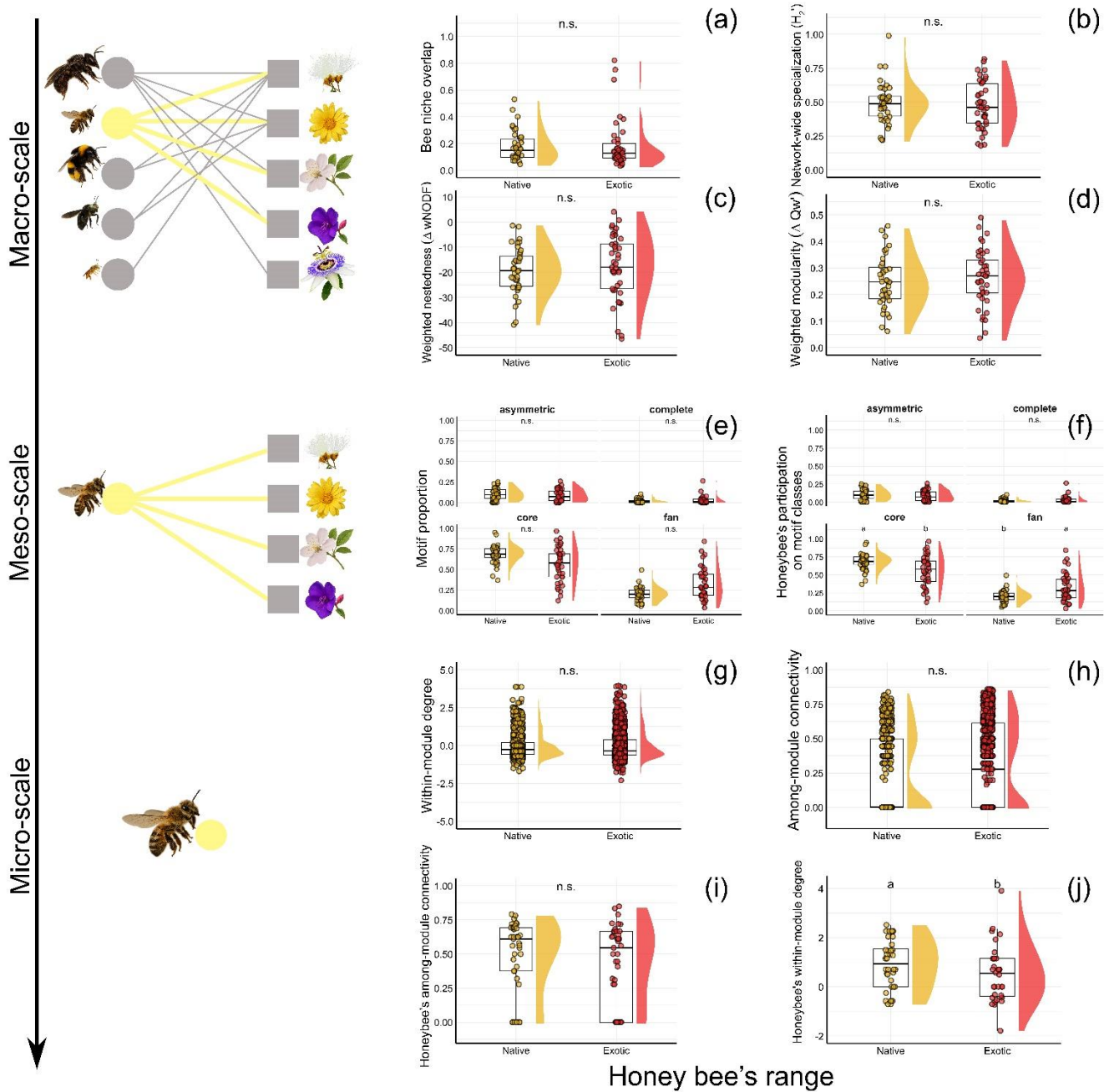


Figure 3.2. Cross-scale comparison of plant–bee network structure across the native and non-native ranges of *Apis mellifera*. Illustration of the three organizational scales analyzed in this study (left) and comparison of network metrics between the honeybee's native (yellow) and non-native (red) ranges (right). Macro-scale panels (a–d) show whole-network properties: (a) bee niche overlap (R_0), (b) network-wide specialization (H_2'), (c)

weighted nestedness ($\Delta\text{-}wNODF$), and (d) weighted modularity ($\Delta\text{-}Qw'$). Meso-scale panels (e–f) show the proportion of motifs in each class for all bees (e) and specifically for *A. mellifera* (f), including asymmetric, complete, core–peripheral, and fan motifs. Micro-scale panels (g–j) show species-level structural roles: (g) within-module degree (z) for all bees, (h) among-module connectivity (c) for all bees, (i) among-module connectivity, and (j) within-module degree for *A. mellifera* only. Boxes and points represent observed values across networks; violins show density distributions. “n.s.” indicates no significant difference between ranges; different letters denote significant pairwise differences within motif classes or micro-scale indices.

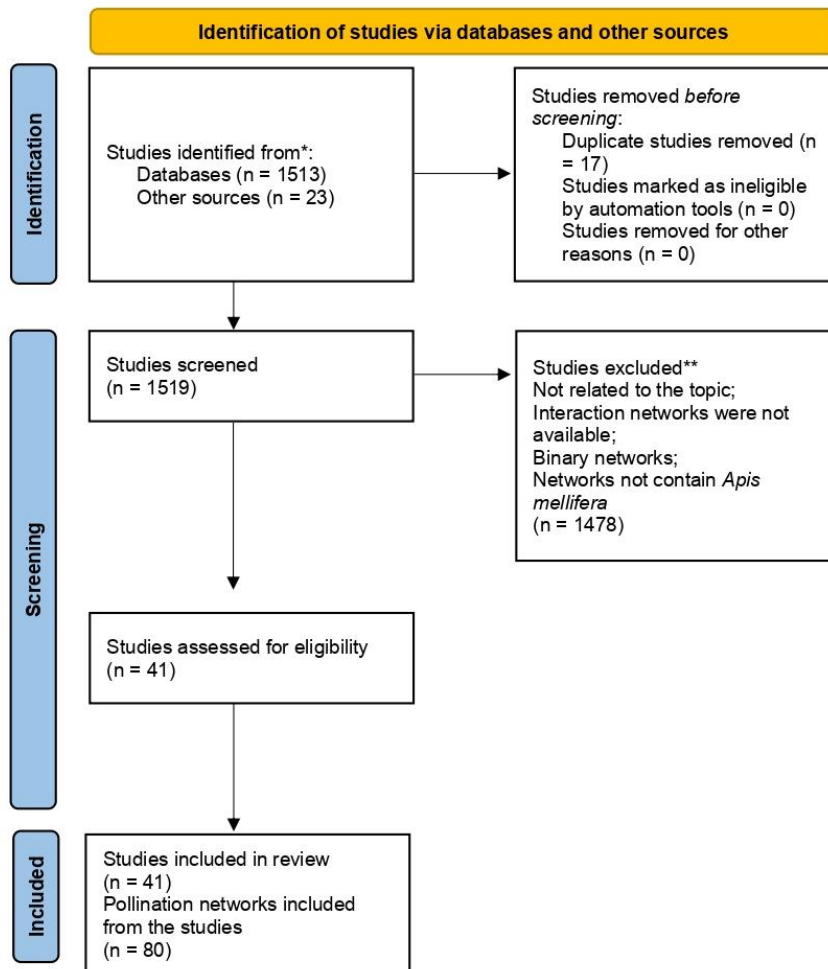


Figure S3.1. PRISMA 2020 flow diagram for study selection. Flow diagram showing the process of identifying and selecting studies that reported interaction networks including *Apis mellifera* worldwide. The screening process involved removing duplicates and excluding studies that did not report interaction networks, used binary networks, or did not include *A. mellifera*. A total of 41 studies were included in the review, encompassing 80 pollination networks containing *A. mellifera* within both its native and non-native ranges.

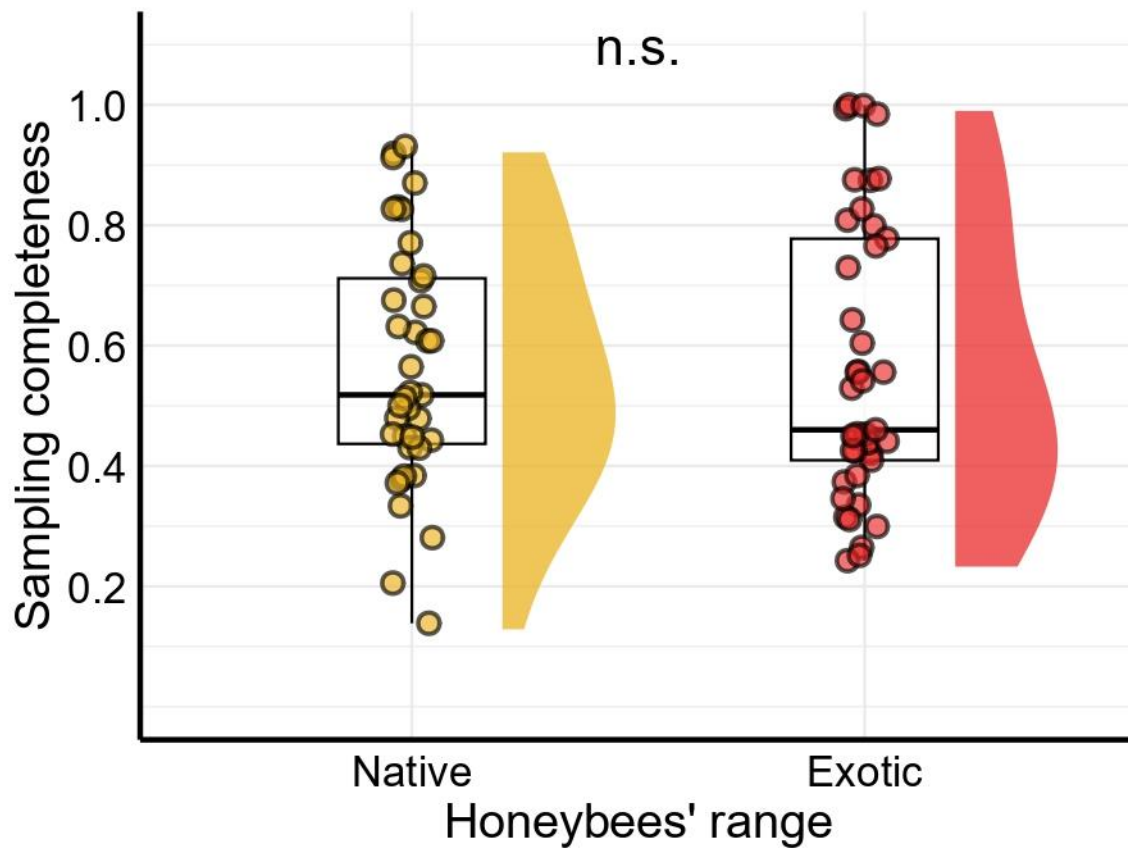


Figure S3.2. Comparison of sampling completeness between plant–bee networks from *Apis mellifera*'s native and non-native ranges. Raw data points (native, yellow; non-native, red) are shown alongside boxplots and density distributions. Sampling completeness was estimated as the proportion of observed interactions relative to the expected total interactions in each network. A post-hoc Tukey test indicated no significant difference between ranges (n.s., not significant).

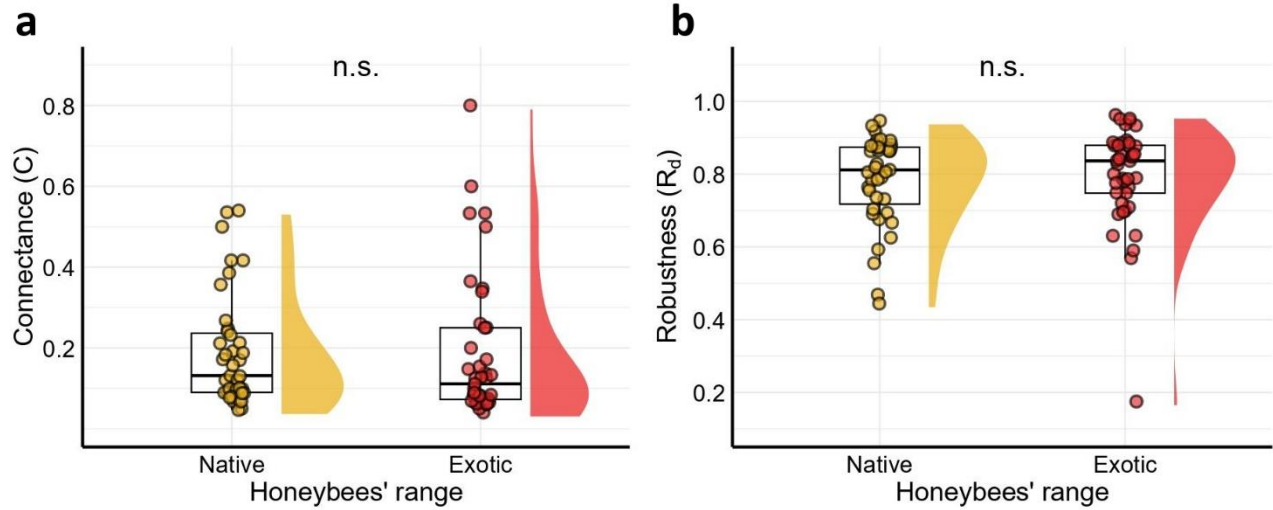


Figure S3.3. Macro-scale network-level metrics for interaction networks including *Apis mellifera* across its native and non-native ranges. Connectance (C ; a) and robustness (R_d ; b) to species loss (R_d) did not differ significantly between honeybees' native (yellow) and non-native (red) ranges (Tukey's post-hoc tests, n.s. = not significant). Each point represents a pollination network, with violins showing the distribution and boxplots indicating median and interquartile ranges.

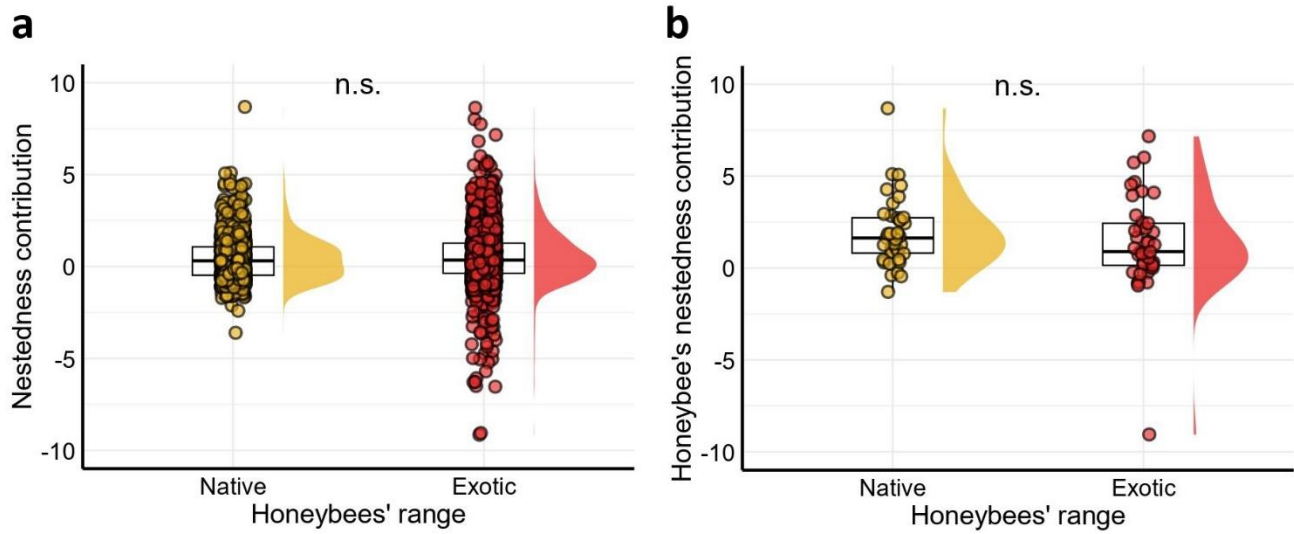


Figure S3.4. Micro-scale species-level network metris depicting *Apis mellifera*'s contribution to network nestedness across honeybees' native and non-native ranges.

Average nestedness contribution of all bee pollinator species within each network (a). Nestedness contribution of *Apis mellifera* only (b). No significant differences were detected between honeybee's native (yellow) and non-native (red) ranges (Tukey's post-hoc tests, n.s. = not significant). Violin plots show the distribution of values, with boxplots indicating median and interquartile ranges.

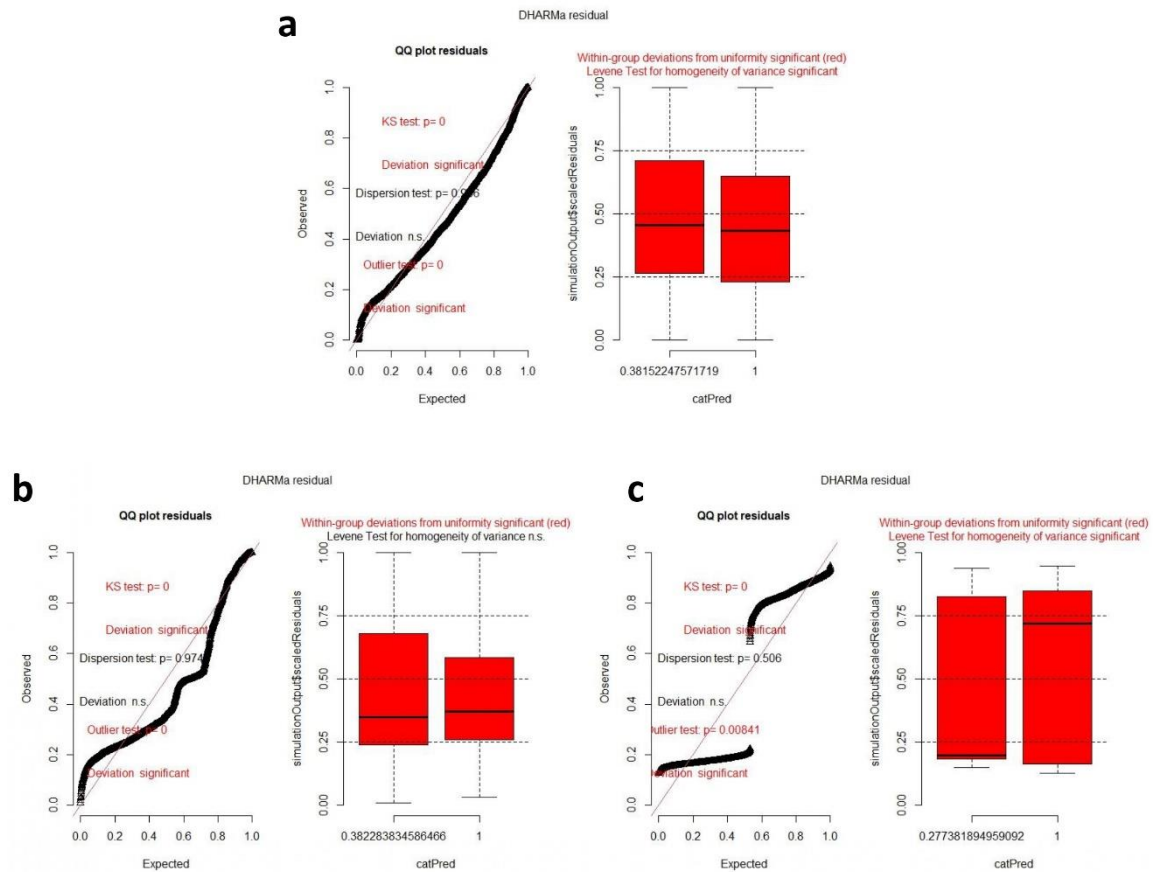


Figure S3.5. Residual diagnostics of micro-scale network metrics measured for all bee species within the network. Residual analysis of linear models for micro-scale network metrics (nestedness contribution - a, within-module degree - b, and among-module connectivity - c) for all bee species present in plant–bee networks containing *Apis mellifera*, located within the native and non-native range of the species. Panels show (i) QQ-plots assessing residual normality and (ii) residual distributions evaluating homoscedasticity.

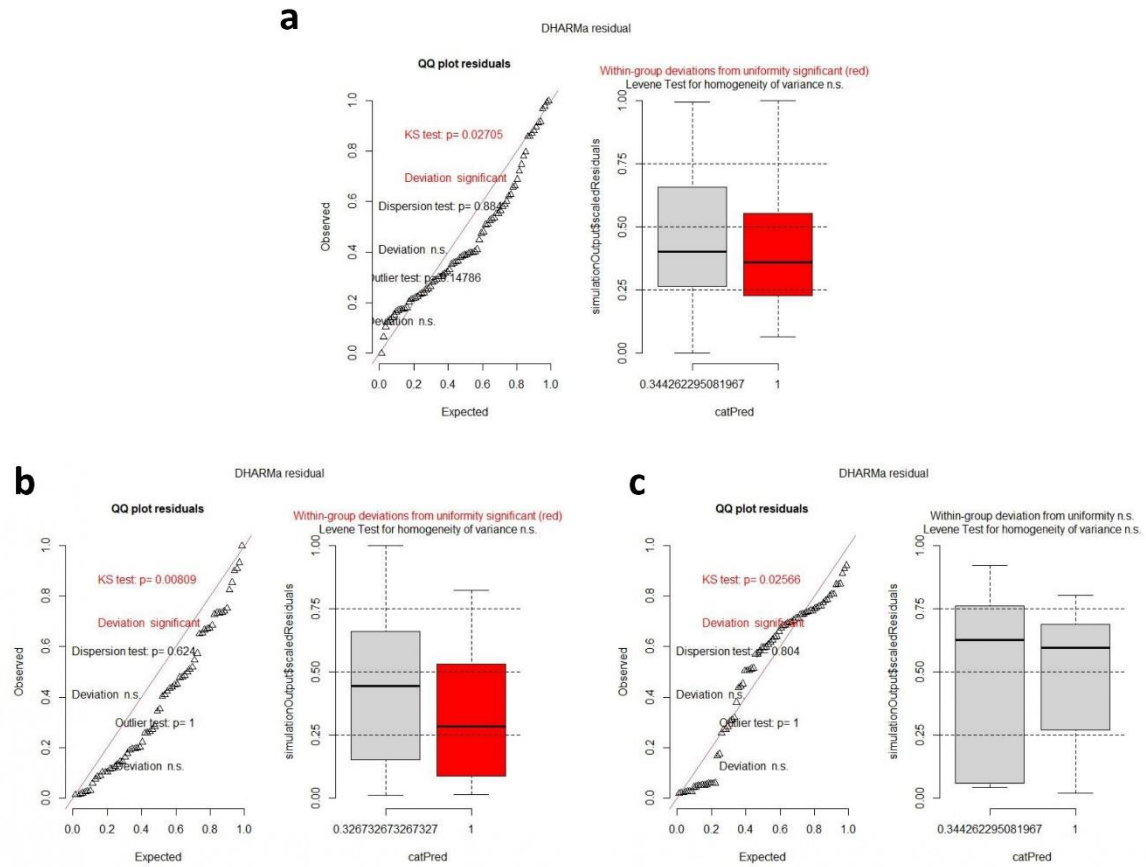


Figure S3.6. Residual diagnostics of micro-scale network metrics for *Apis mellifera*. Residual analysis of linear models for micro-scale network metrics (nestedness contribution - a, within-module degree - b, and among-module connectivity - c) considering only *Apis mellifera* in plant–bee networks, located within the native and non-native range of the species. Panels show (i) QQ-plots assessing residual normality and (ii) residual distributions evaluating homoscedasticity.

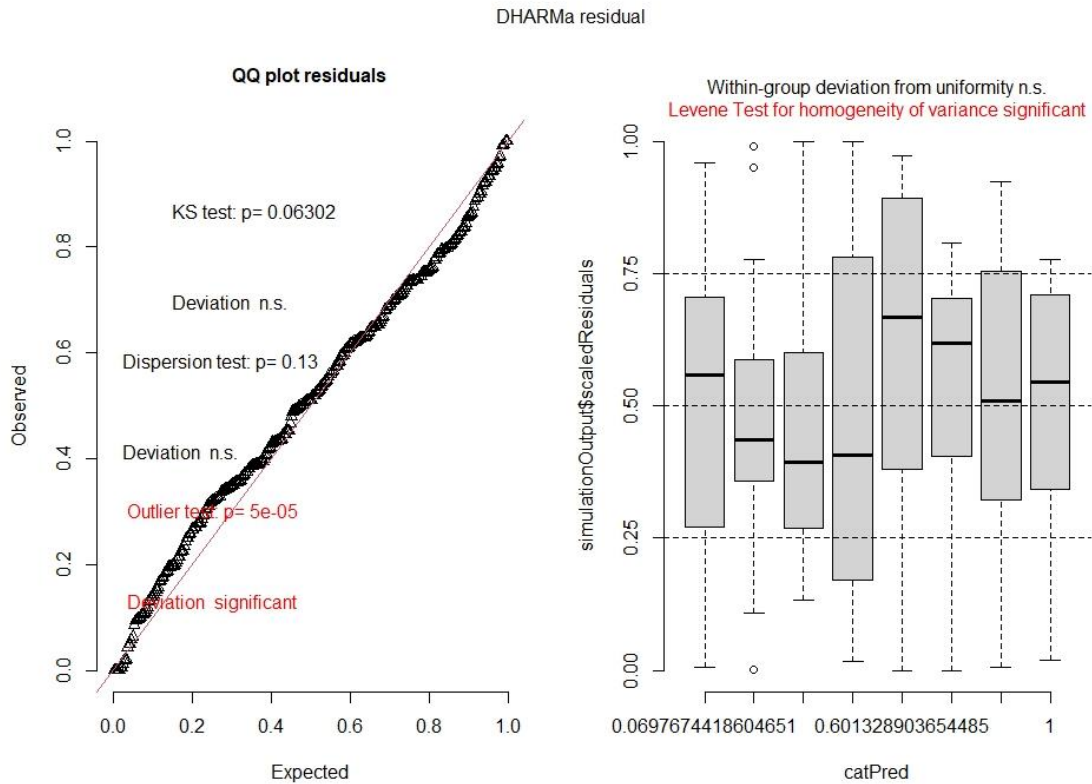


Figure S3.7. Residual diagnostics of meso-scale network metrics. Residual analysis of linear models for meso-scale network metrics (participation in different motif classes) for all bee species in plant–bee networks. Panels show (i) QQ-plots assessing residual normality and (ii) residual distributions evaluating homoscedasticity.

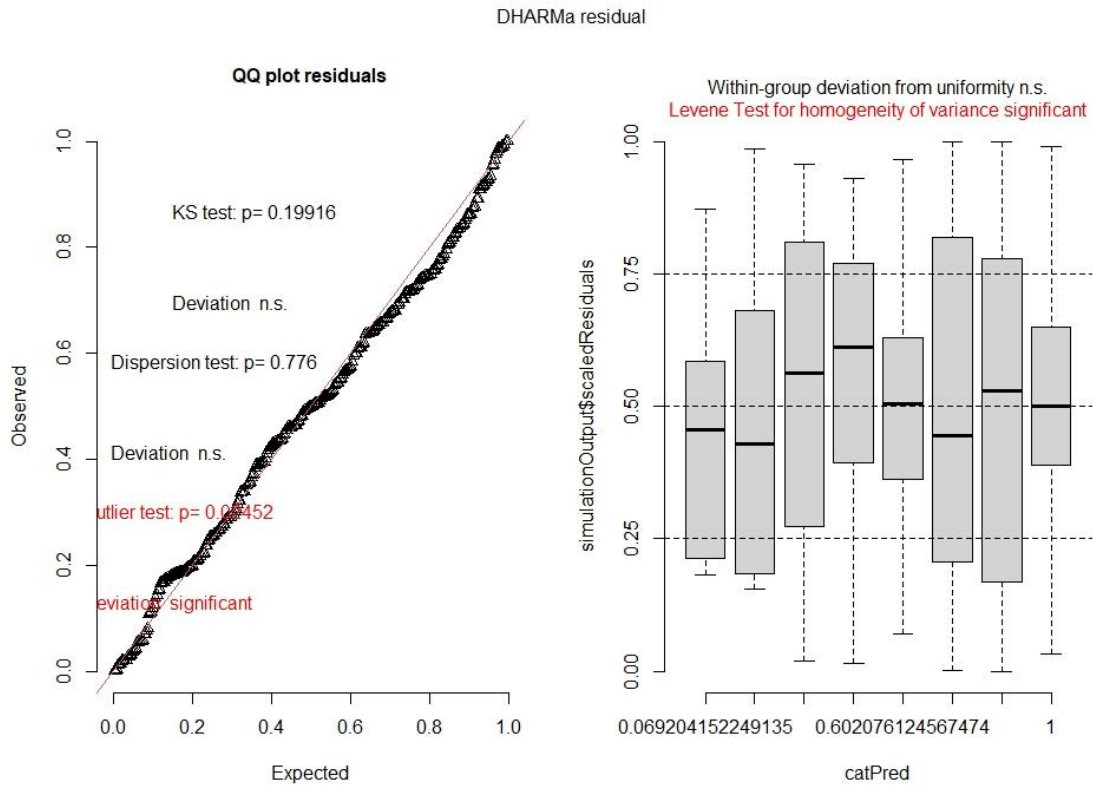


Figure S3.8. Residual diagnostics of meso-scale network metrics for *Apis mellifera*. Residual analysis of linear models for meso-scale network metrics (participation in motif classes) considering only motifs in which *Apis mellifera* participates in plant–bee networks. Panels show (i) QQ-plots assessing residual normality and (ii) residual distributions evaluating homoscedasticity.

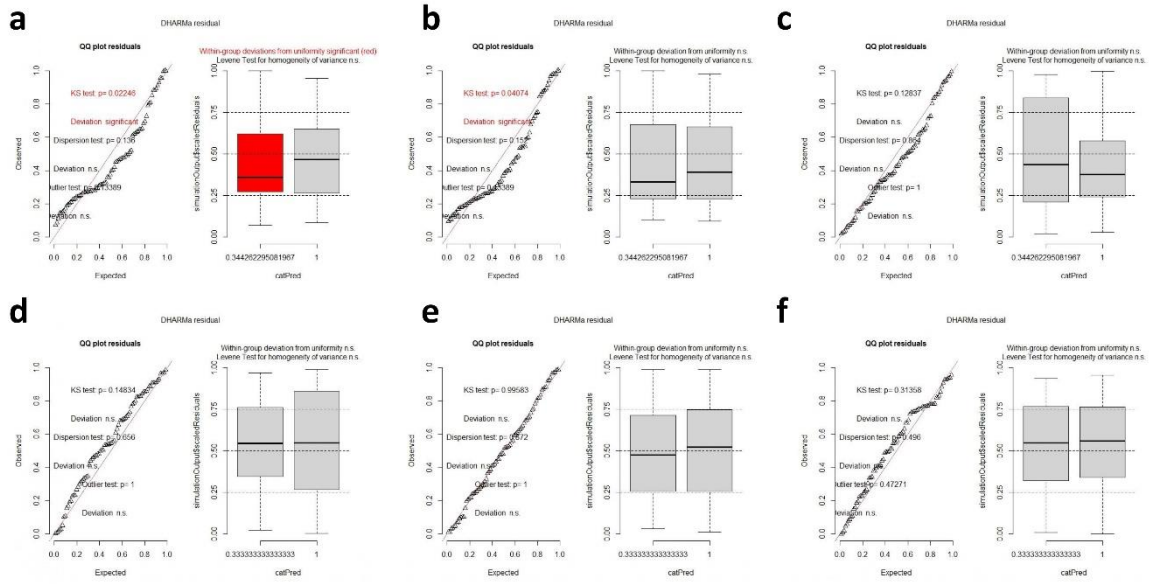


Figure S3.9. Residual diagnostics of macro-scale network metrics. Residual analysis of linear models for macro-scale network metrics (a: bee niche overlap - R_0 , b: connectance - C , c: network-wide specialization - H_2' , d: weighted nestedness - $\Delta\text{-wNODF}$, e: weighted modularity $\Delta\text{-}Q'$, f: robustness to bee species loss - R_d) in plant-bee networks. Panels show (i) QQ-plots assessing residual normality and (ii) residual distributions evaluating homoscedasticity.

4 CHAPTER III

**Rapid community shifts in native bees following the introduction of managed
honeybee hives in the Cerrado**

Rapid community shifts in native bees following the introduction of managed honeybee hives in the Cerrado

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Abstract

Managed honeybee (*Apis mellifera*) colonies are frequently introduced into natural habitats to support apiculture, yet their effects on native pollinator communities remain poorly understood in tropical ecosystems. Here, we assessed short-term changes in native bee diversity and community structure in a Cerrado fragment (southeastern Brazil) before and after the introduction of *A. mellifera* hives. Bee assemblages were surveyed for one year using active floral sampling and pan traps, and diversity was evaluated through rarefaction and extrapolation analyses based on Hill numbers. Community composition was assessed using non-metric multidimensional scaling (NMDS) and PERMANOVA. Species richness remained stable between periods, whereas Shannon diversity declined markedly from 6.29 before to 4.36 after hive introduction, indicating reduced diversity. NMDS and PERMANOVA revealed subtle but significant compositional shifts in the bee community. Site diversity was unaffected by proximity to hive boxes, suggesting fragment-wide effects of honeybee foraging. Our findings demonstrate that honeybee introductions can rapidly alter the structure of native bee communities in the Cerrado, primarily through changes in community diversity rather than species loss, with potential implications for pollination dynamics in human-modified landscapes.

Keywords: Honeybees, Native Bees, Pollinator Competition

4.1 INTRODUCTION

Animal pollination is a key driver of angiosperm reproduction, contributing to the reproductive success of more than 75% of flowering plants worldwide, including numerous crops of economic importance (Aizen et al 2019; Klein et al. 2007). Pollination services provide billions of dollars in global agricultural value, positioning pollinators as essential components of food security and ecosystem functioning (Potts et al. 2016a,b). Despite their importance, multiple lines of evidence indicate global declines in wild pollinator populations and associated deficits in crop pollination (Dicks et al. 2021). In contrast, the use of managed pollinators, particularly the western honeybee (*Apis mellifera*), has expanded sharply in recent decades (Osterman et al. 2021).

Brazil ranks among the world's largest agricultural producers, with an estimated pollination service value from bees of approximately USD 12–14 billion annually (Giannini et al. 2015). *A. mellifera* is the primary managed pollinator in the country and is routinely employed in mass-flowering crops such as orange (*Citrus sinensis*; Carvalho et al. 2019), cotton (*Gossypium hirsutum*), and coffee (*Coffea* spp.; Pires et al. 2014; Hipólito et al. 2018; Basualdo et al. 2022). After crop flowering, beekeepers often relocate hives to nearby natural fragments, where colonies can recover using native floral resources. While these practices can momentarily enhance pollination of some wild plants, *A. mellifera* may also compete with native pollinators for nectar and pollen, especially in resource-limited environments, and its visits are often less effective for native plant reproduction (Garibaldi et al. 2021).

Competitive interactions between honeybees and native pollinators can occur through resource depletion and behavioral dominance. The honeybee's recruitment system allows rapid exploitation of abundant floral resources, potentially excluding less competitive bee species from shared foraging sites (Hung et al. 2019; Page & Williams 2023). Such effects may be particularly strong in small or degraded fragments, where resource diversity and floral density are reduced (Theodorou et al. 2020). However, direct

assessments of how sudden hive introductions alter native bee diversity within tropical ecosystems remain scarce.

In São Paulo state, southeastern Brazil, managed *A. mellifera* colonies are commonly placed in Cerrado fragments adjacent to orange orchards following crop flowering. These introductions create a natural experiment for evaluating short-term community-level responses to honeybee presence. Here, we surveyed the bee assemblage of a Cerrado fragment before and after hive introduction, including both sites near and away from the introduced hives, to test how honeybee presence influences native bee diversity and community composition.

We hypothesized that the introduction of *A. mellifera* would reduce native bee diversity by increasing dominance and reducing diversity, while total species richness would remain stable. Specifically, we predicted that (1) Shannon and Simpson diversity would decrease after honeybee introduction, reflecting shifts in relative abundance patterns; (2) species richness would show minimal change, indicating persistence of most taxa; and (3) overall community composition would differ between periods, with a reorganization of species assemblages. We further expected that these effects would be intensified in sites immediately adjacent to hive boxes.

4.2 MATERIAL AND METHODS

4.2.1 Study site

The study was conducted in the natural reserve Fazenda Treze de Maio (FTM), located in Botucatu, São Paulo state, southeastern Brazil (22°43'24.89"S, 48°26'04.87"W; 550 m a.s.l.). The climate in the region is transitional between humid subtropical (Cwa) and tropical savanna (Aw) according to the Köppen classification (Kottek et al. 2006), with a mean annual temperature of 21 °C and annual precipitation of approximately 1500 mm. The region exhibits two well-defined seasons: a cool and dry winter (March–September) and a warm, humid summer (October–April). The reserve covers approximately 160 ha and

is characterized by a mosaic of Cerrado physiognomies, including Cerrado *sensu stricto*, dominated by shrubs and small trees, and Cerradão, with a denser tree canopy reaching up to 12 m (Oliveira & Marquis 2002; Gottsberger & Silberbauer-Gottsberger 2006; Figure S4.1).

Managed *Apis mellifera* colonies are recurrently introduced at FTM following the flowering of adjacent orange (*Citrus sinensis*) orchards. During these periods, colonies remain for several months and forage extensively within the fragment, providing an ideal setting to evaluate short-term community responses of native bees to honeybee introductions.

4.2.2 Bee sampling

Bee sampling was conducted monthly from January 2023 to January 2024. The fragment was divided into six sites with similar area and vegetation structure. Along these sites, we established ten sampling stations spaced 200 m apart (Figure S4.1). Each year, following the orange flowering period (September), beekeepers introduce *A. mellifera* hives into three of the six sites, while the remaining three sites remain hive-free and served as reference areas.

Bees were collected using two complementary methods to maximize species detection:

1. *Active sampling* – Bees visiting flowers were actively collected using entomological nets during one full day each month. Specimens were euthanized using 10% ammonia solution and preserved for identification.

2. *Passive sampling* – Sets of three colored bowls (white, fluorescent yellow, and fluorescent blue; 500 ml each) were deployed as pan traps (Campbell et al. 2007). Twenty trap sets were placed per hectare, spaced by at least 10 m, and operated weekly.

All specimens were pinned, labeled, and identified to the lowest possible taxonomic

level using available keys (Silveira et al. 2002) and confirmed by specialists. Each record was georeferenced and assigned to its corresponding trap and site.

4.2.3 Data organization

Each individual bee record was associated with metadata including species identity, sampling location, and sampling period (before or after hive introduction). We treated sampling locations as the sampling unit, and insertion period (“BEFORE” or “AFTER”) and hive presence (“YES” or “NO”) as explanatory factors.

To construct the species-by-sampling abundance matrix used in analyses, individual records were aggregated by counting the number of individuals of each species per sampling unit per period. The final dataset contained all bee species detected before and after hive introduction, with non-detections treated as zero abundance.

4.2.4 Diversity analysis

Rarefaction analyses

We evaluated changes in bee diversity before and after the introduction of *A. mellifera* using rarefaction and extrapolation curves based on Hill numbers ($q = 0, 1, 2$), corresponding to species richness, Shannon diversity, and Simpson diversity, respectively. Analyses were performed with the *iNEXT* package (Hsieh et al. 2016) in R.

To ensure fair comparisons between periods, we standardized sampling effort by extrapolating both assemblages to a common sample size equal to the larger of the two. Diversity estimates were bootstrapped with 200 replications to calculate 95% confidence intervals. We considered non-overlapping confidence intervals as evidence of significant differences between periods.

Site-proximity effect and BACI analysis

To evaluate the local effects of *Apis mellifera* hive introductions, we implemented

a Before–After, Control–Impact (BACI) framework at the site level. Sites were classified according to future hive status: PREY for sites that received hive boxes and PREN for control sites. Insertion period (BEFORE vs AFTER) and future hive assignment (PREY vs PREN) were treated as fixed factors.

For each site, we calculated species richness and Shannon diversity. Each metric was analyzed using generalized linear models (GLMs) including the interaction between insertion period and future hive assignment to test for site-specific effects of hive proximity. Species richness was modeled with a Poisson GLM, and overdispersion was checked; if present, a negative binomial GLM was applied. Shannon diversity was modeled with a Gaussian GLM. All analyses were conducted in R (version 4.4.0) using the *stats*, *vegan*, and *MASS* packages.

Community composition analyses

To assess changes in bee community composition, we calculated Bray–Curtis dissimilarities on the abundance matrix and performed non-metric multidimensional scaling (NMDS) with two dimensions ($k = 2$) using the metaMDS function in *vegan* package. We visually inspected ordination plots to evaluate separation between assemblages before and after hive introduction.

We statistically tested the effect of insertion period on community composition using a PERMANOVA (*adonis2*, 999 permutations) based on the Bray–Curtis distance matrix. The resulting F , R^2 , and p -values were used to quantify the strength and significance of compositional differences.

4.3 RESULTS

4.3.1 Bee sampling

During the sampling period (January 2023–January 2024), we recorded a total of 49

bee species belonging to five families and eleven tribes (Supplementary Table S4.1). The family Apidae represented the highest species richness, encompassing the majority of tribes detected (including Centridini, Ceratinini, Emphorini, Eucerini, Euglossini, Exomalopsini, Meliponini, Isepeolini, and Tapinotaspidini).

4.3.2 Rarefaction and diversity

Rarefaction-extrapolation curves revealed changes in bee diversity before and after the introduction of *Apis mellifera*. Species richness ($q = 0$) showed a slight decrease after the introduction, although confidence intervals of the curves still overlapped, indicating no statistically strong difference in total species number between the periods (Figure 4.1A).

In contrast, Shannon diversity ($q = 1$), which accounts for both species richness and abundance, exhibited a decline after the introduction of *A. mellifera* (Figure 4.1B). Extrapolated Shannon diversity decreased from 6.29 (95% CI: 5.21–7.37) before to 4.36 [3.49–5.24] after, representing a ~31% reduction (Figure 4.1B). Importantly, the confidence intervals no longer overlapped, suggesting a significant reduction in bee species diversity. Simpson diversity ($q = 2$), reflecting dominance patterns, showed a minor decline after the introduction, but the confidence intervals still overlapped (Figure 4.1C).

4.3.3 Hive proximity on diversity (BACI analysis)

The BACI analysis showed no statistically significant effects of hive installation on any diversity metric (Figure 4.2). Species richness did not differ significantly between periods (insertion: $F = 1.54$, $p = 0.232$, Figure 4.2) or between sites destined to receive hives versus control sites (future_hive: $F = 0.32$, $p = 0.579$, Figure 4.2), and the interaction term was also not significant (insertion $\times$ future_hive: $F = 0.11$, $p = 0.747$, Figure 4.2). Shannon diversity similarly showed no significant effects of time ($F = 0.26$, $p = 0.615$, Figure 4.2), hive assignment ($F = 0.64$, $p = 0.436$, Figure 4.2), or their interaction ($F = 0.07$, $p = 0.794$, Figure 4.2). Finally, Simpson diversity, reflecting dominance patterns, was not affected by insertion ($F = 0.065$, $p = 0.801$, Figure 4.2), future hive assignment ($F = 1.14$, $p = 0.302$, Figure 4.2), or the interaction between these factors ($F = 0.067$, $p = 0.799$, Figure 4.2).

Overall, these results indicate that although Shannon diversity declined across the fragment in the rarefaction analysis, this decline was not specifically associated with sites that received hive boxes. In other words, sites that later received hives (PREY) did not exhibit a different trajectory in diversity compared to control sites (PREN).

4.3.4 Community composition

Non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities revealed a subtle separation between assemblages sampled before and after hive introduction ($stress = 0.232$). Although the ordination axes showed partial overlap, before samples generally clustered apart from after samples (Figure 4.3).

PERMANOVA confirmed that insertion period had a significant effect on bee community composition ($R^2 = 0.023$, $F = 2.27$, $p = 0.025$, Figure 4.3). The proportion of variation explained was modest, indicating a mild but consistent compositional shift following *A. mellifera* introduction.

4.4 DISCUSSION

Our results demonstrate that the short-term introduction of *Apis mellifera* hives in a Cerrado fragment led to measurable alterations in the structure of the native bee community. Although overall species richness remained stable, the community experienced a pronounced reduction in diversity and subtle but significant changes in species composition. Also, the BACI analysis confirmed that this decline was not influenced by proximity to hive boxes suggesting that honeybee effects were diffuse across the fragment rather than spatially localized. These patterns indicate that *A. mellifera* primarily affected the relative abundance distribution of the bee assemblage rather than driving local extinctions or species loss.

The observed decline in Shannon diversity implies that honeybee foraging activity increased its dominance within the community, likely by concentrating foraging pressure

on shared floral resources (Pasquaretta et al. 2017). Honeybees are efficient exploiters of floral rewards due to their recruitment communication and mass-foraging behavior (Hung et al. 2019), which enables them to monopolize abundant nectar and pollen sources. This behavioral dominance can reduce resource availability for native species, particularly solitary or less competitive bees (Page & Williams 2023). While most native taxa persisted after hive introduction, their relative abundances were suppressed across the fragment, rather than being concentrated at sites near hives, possibly as a result of resource depletion or displacement from preferred foraging sites.

Changes in community composition detected through NMDS and reinforce the interpretation of subtle but consistent shifts in species assemblages following *A. mellifera* introduction. Although the explained variation was modest, such small yet significant differences are typical of ecological community responses to short-term disturbances (Campbell et al. 2015). Consistent with the BACI results, the absence of detectable spatial effects at the site level suggests that honeybee influence was not confined to sites immediately adjacent to hive boxes. Instead, foraging activity likely extended throughout the fragment, producing a diffuse community-wide effect. This spatial pattern is consistent with the high mobility and broad foraging range of honeybee workers, which often exceeds several hundred meters (Beekman & Ratnieks 2000).

The stability of species richness despite declines in diversity indicates resilience of the native community to short-term honeybee presence, at least in terms of species persistence. However, reductions in diversity can have long-term implications for community function and stability. More dominant taxa may increasingly control resources, potentially reducing species' ecological redundancy and diminishing community responses to environmental change (Winfrey et al. 2014; Fontúrbel et al. 2021). In the Cerrado, where many native bees depend on seasonally scarce floral resources, recurring hive introductions could amplify competitive pressures over time, leading to cumulative shifts in community structure and function.

4.5 CONCLUSION

While our study captures only a single annual cycle, it provides empirical evidence that honeybee introductions can rapidly alter native bee community structure in a tropical savanna environment. Future studies should extend monitoring across multiple years and include direct measures of resource availability and floral visitation to clarify whether declines in diversity arise from competitive displacement or differential resource use. Assessing long-term persistence and functional redundancy of native pollinators will be essential to understanding the broader ecosystem consequences of managed honeybee use in natural habitats.

4.6 REFERENCES

- Aizen MA, Aguiar S, Biesmeijer JC (2019) Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. *Global change biology* 25: 3516-3527.
- Basualdo M, Cavigliasso P, de Avila Jr RS, et al. (2022) Current status and economic value of insect-pollinated dependent crops in Latin America. *Ecological Economics* 196: 107395.
- Beekman M, & Ratnieks FLW (2000) Long-range foraging by the honey-bee, *Apis mellifera* L. *Functional Ecology* 14: 490-496.
- Campbell C, Yang S, Albert R, et al. (2015) Plant–pollinator community network response to species invasion depends on both invader and community characteristics. *Oikos* 124: 406-413.
- Carvalho SAD, Girardi EA, Mourão Filho FDAA, et al. (2019) Advances in citrus propagation in Brazil. *Revista Brasileira de Fruticultura* 41: e-422.
- Dicks LV, Breeze TD, Ngo HT, et al. (2021) A global-scale expert assessment of drivers and risks associated with pollinator decline. *Nature Ecology & Evolution* 5: 1453-1461.
- Dixon P (2003) VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14: 927-930.
- Fontúrbel FE, Nespolo RF, Amico GC et al (2021) Climate change can disrupt ecological interactions in mysterious ways: Using ecological generalists to forecast community-wide effects. *Climate Change Ecology* 2: 100044.

- Franco J R, Dal Pai E, Calça MVC, et al. (2023) ATUALIZAÇÃO DA NORMAL CLIMATOLÓGICA E CLASSIFICAÇÃO CLIMÁTICA DE KÖPPEN PARA O MUNICÍPIO DE BOTUCATU-SP. *Irriga* 28: 77-92.
- Garibaldi LA, Pérez-Méndez N, Cordeiro GD, et al (2021) Negative impacts of dominance on bee communities: Does the influence of invasive honey bees differ from native bees? *Ecology* 102: e03526.
- Giannini TC, Cordeiro GD, Freitas BM, et al (2015) The dependence of crops for pollinators and the economic value of pollination in Brazil. *Journal of economic entomology* 108: 849-857.
- Hipólito J, Boscolo D, & Viana BF (2018) Landscape and crop management strategies to conserve pollination services and increase yields in tropical coffee farms. *Agriculture, Ecosystems & Environment* 256: 218-225.
- Hsieh TC, Ma KH, Chao A (2016) iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution* 7:1451-1456.
- Hung KLJ, Kingston JM, Lee A, et al. (2019) Non-native honey bees disproportionately dominate the most abundant floral resources in a biodiversity hotspot. *Proceedings of the Royal Society B* 286: 20182901.
- Klein AM, Vaissière BE, Cane JH, et al (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the royal society B: biological sciences*, 274: 303-313.
- Kottek M, Grieser J, Beck C, et al. (2006) World map of the Köppen-Geiger climate classification updated.

- Oliveira PS, & Marquis RJ (2002) The cerrados of Brazil: ecology and natural history of a neotropical savanna. *Columbia University Press*, New York.
- Osterman J, Aizen MA, Biesmeijer JC et al (2021) Global trends in the number and diversity of managed pollinator species. *Agriculture, Ecosystems & Environment* 322: 107653.
- Page ML & Williams NM (2023) Honey bee introductions displace native bees and decrease pollination of a native wildflower. *Ecology* 104: e3939.
- Pires VCP, Silveira FA, Sujii ER., et al. (2014) Importance of bee pollination for cotton production in conventional and organic farms in Brazil. *Embrapa*.
- Potts SG, Ng HT, Biesmeijer JC, et al. (2016A) The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production.
- Potts SG, Imperatriz-Fonseca V, Ngo HT, et al. (2016B) Safeguarding pollinators and their values to human well-being. *Nature* 540: 220-229.
- Pasquaretta C, Jeanson R, Andalo C, et al (2017) Analysing plant-pollinator interactions with spatial movement networks. *Ecological Entomology* 42: 4-17.
- Silveira FA, Melo GA, & Almeida EA (2002) Abelhas brasileiras: sistemática e identificação. *Guilherme Carnevale Carmona*.
- Gottsberger G & Silberbauer-Gottsberger I (2006b) Life in the Cerrado. Vol. I. Origin, Structure, Dynamics and Plant Use. *Reta, Ulm*.
- Winfree R, Williams NM, Dushoff J, et al. (2014) Species abundance, not diet breadth,

drives the persistence of the most linked pollinators as plant-pollinator networks disassemble. *The American Naturalist* 183: 600-611.

Theodorou P, Herbst SC, Kahnt B, et al. (2020) Urban fragmentation leads to lower floral diversity, with knock-on impacts on bee biodiversity. *Scientific Reports* 10: 21756.

4.7 FIGURES

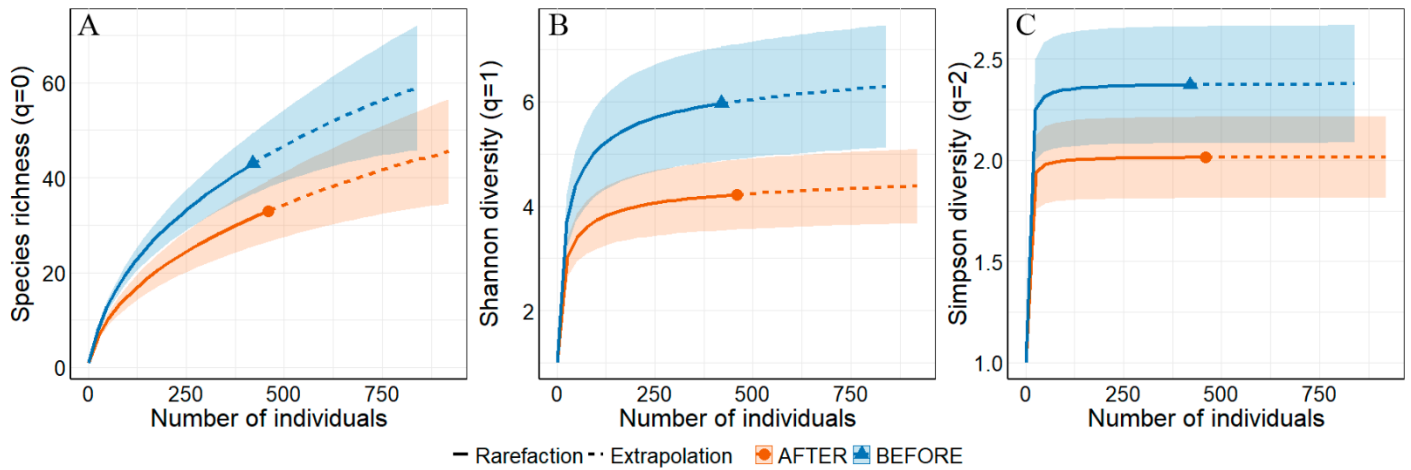


Figure 4.1 Rarefaction–extrapolation curves of bee diversity before (blue) and after (orange) the introduction of *Apis mellifera*. (A) Species richness ($q = 0$), (B) Shannon diversity ($q = 1$), and (C) Simpson diversity ($q = 2$) estimated across increasing numbers of individuals. Solid lines represent rarefaction, dashed lines represent extrapolation, and shaded areas indicate 95% confidence intervals. Species richness showed only a slight decline after the introduction of *A. mellifera*, with overlapping confidence intervals indicating no strong statistical difference between periods. In contrast, Shannon diversity exhibited a marked reduction after honeybee introduction, with no overlap in confidence intervals and a ~31% decrease in extrapolated diversity (from 6.29 to 4.36), indicating a significant loss of community diversity. Simpson diversity also declined after honeybee arrival, reflecting reduced dominance-adjusted diversity. Together, these patterns reveal a shift toward a less even and more homogenized bee community following the establishment of *A. mellifera*.

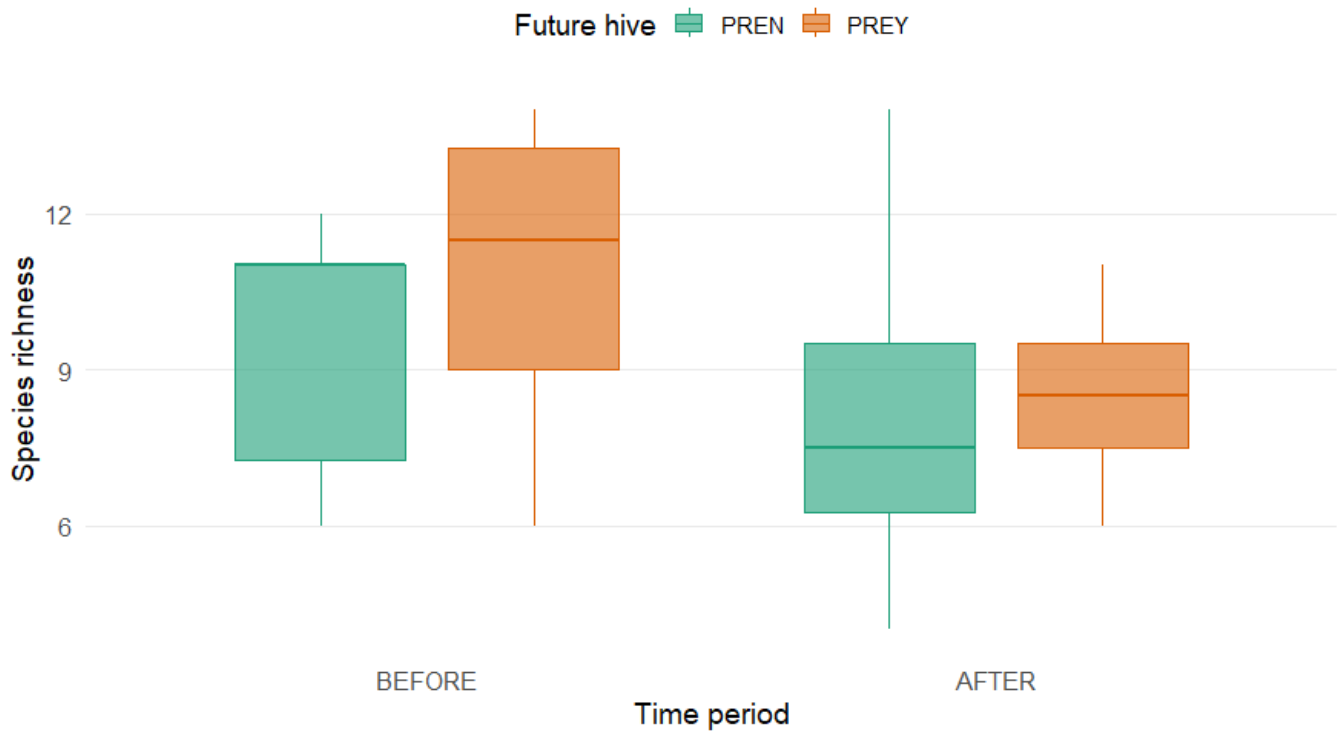


Figure 4.2 Bee species richness before and after hive installation in control (PREN) and future-hive (PREY) sites. Boxplots show the distribution of species richness across sampling sites in the two periods. Green boxes represent PREN sites (controls), and orange boxes represent PREY sites (sites assigned to receive hives). Although rarefaction analyses revealed a decline in diversity at the community level, the BACI analysis detected no significant effects of hive installation on species richness. Neither time (insertion), site type (future_hive), nor their interaction influenced richness (all $p > 0.23$), indicating that PREY and PREN sites exhibited similar temporal trajectories and that diversity changes were not specifically associated with hive placement.

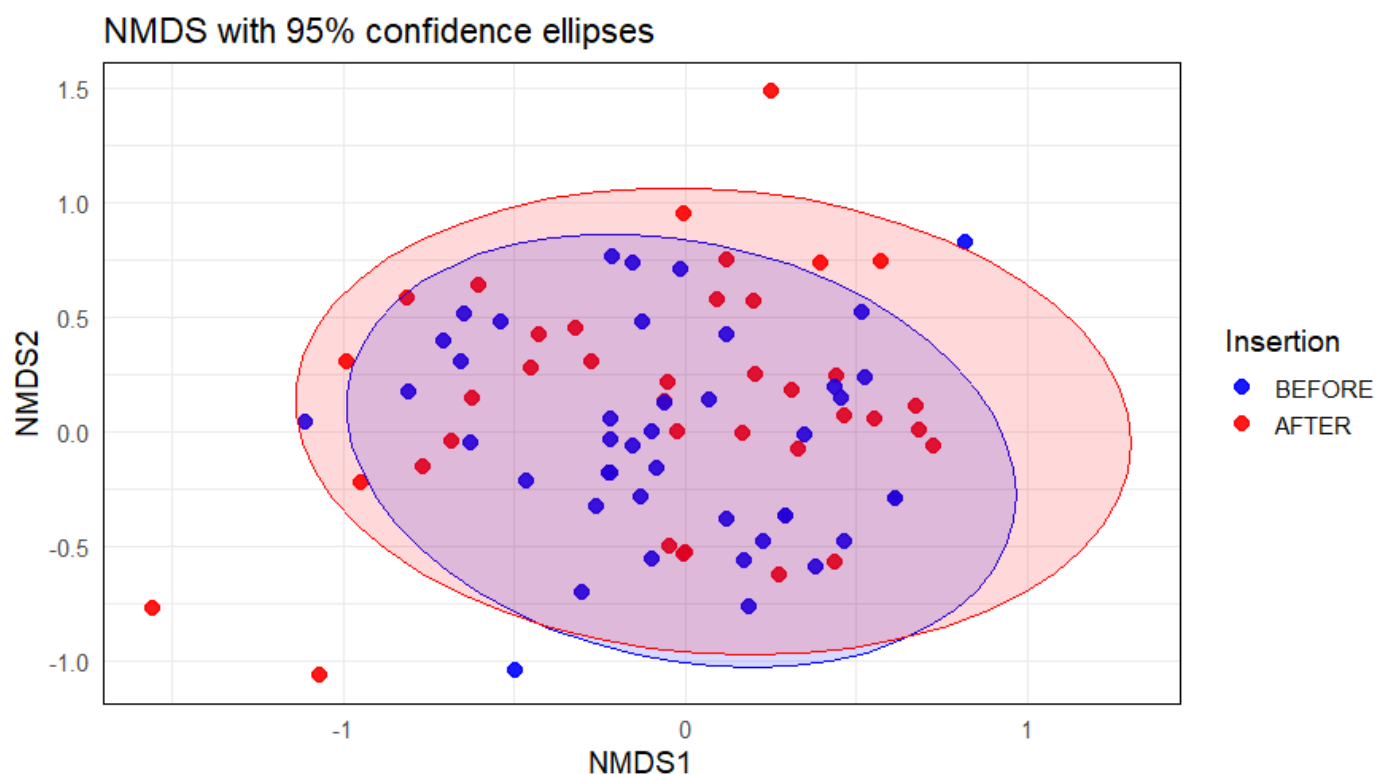


Figure 4.3 Non-metric multidimensional scaling (NMDS) of bee community composition before and after honeybee hive introduction. NMDS ordination based on Bray–Curtis dissimilarities ($stress = 0.232$) showing the distribution of bee assemblages sampled before (blue points) and after (red points) the introduction of *Apis mellifera* colonies. Shaded ellipses represent 95% confidence regions for each sampling period. Although the two groups partially overlap, after-hive communities show a slight shift in multivariate space. This pattern is supported by PERMANOVA, which detected a significant but modest effect of insertion period on community composition ($R^2 = 0.023$, $F = 2.27$, $p = 0.025$), indicating a mild but consistent compositional change following hive installation.



Figure S4.1. Map of the study area showing the spatial distribution of bee sampling stations within the Cerrado fragment. Blue points represent control stations located in sites that did not receive *Apis mellifera* hives, while red points indicate stations situated in sites where honeybee hives were introduced annually after the orange flowering period.

Table S4.1. Bee species collected between January 2023 - 2024 at Fazenda Treze de Maio.

Family	Tribe	Species
Andrenidae	Oxaeini	<i>Oxaea flavescens</i> (Klug, 1807)
Apidae	Apini	<i>Apis mellifera</i> (Linnaeus, 1758)
	Centridini	<i>Centris (Trachina) fuscata</i> (Lepeletier, 1841)
		<i>Centris</i> sp.
		<i>Centris (Hemisiella) tarsata</i> (Smith, 1874)
		<i>Epicharis (Epicharis) flava</i> (Fries, 1900)
		<i>Epicharis (Epicharitides) iheringi</i> (Fries, 1899)
	Ceratinini	<i>Ceratina (Calloceratina) chloris</i> (Fabricius, 1804)
		<i>Ceratina</i> sp.1
		<i>Ceratina</i> sp.2
		<i>Ceratina</i> sp.3
	Emphorini	<i>Alepidosceles imitatrix</i> (Schroetky, 1909)
		<i>Melitoma segmentaria</i> (Fabricius, 1804)
	Eucerini	<i>Florilegus cf. festibus</i> (Smith, 1854)
		<i>Thygater (Thygater) analis</i> (Lepeletier, 1841)
	Euglossini	<i>Euglossa</i> sp.1
		<i>Euglossa</i> sp.2
		<i>Eulaema (Apeulaema) nigrita</i> (Lepeletier, 1841)
	Exomalopsini	<i>Exomalopsis (Exomalopsis) analis</i> (Spinola, 1853)

Family	Tribe	Species
Apidae	Exomalopsini	<i>Exomalopsis (Exomalopsis) analis</i> (Spinola, 1853)
		<i>Exomalopsis (Exomalopsis) auropilosa</i> (Spinola, 1853)
		<i>Exomalopsis (Exomalopsis) fulvofasciata</i> (Smith, 1879)
	Meliponini	<i>Trigona spinipes</i> (Fabricius, 1793)
	Isepeolini	<i>Isepeolus viperinus</i> (Holmberg, 1886)
	Tapinotaspidini	<i>Lophopedia pygmaea</i> (Schrottky 1902)
Colletidae	Caupolicanini	<i>Ptiloglossa pretiosa</i> (Friesse, 1898)
Halictidae	Augochlorini	<i>Augochlora (Augochlora) caerulior</i> (Cockerell, 1900)
		<i>Augochlora (Augochlora) esox</i> (Vachal, 1911)
		<i>Augochlora (Augochlora) francisca</i> (Schrottky, 1902)
		<i>Augochlora (Augochlora) perimelas</i> (Cockerell, 1900)
		<i>Augochlora (Augochlora) scabrata</i> (Lepeco & Gonçalves, 2020)
		<i>Augochlora (Augochlora) seitzii</i> (Cockerell, 1929)
		<i>Augochlora (Augochlora) sp.1</i>
		<i>Augochlora (Augochlora) sp.2</i>
		<i>Augochlora (Augochlora) tantilla</i> (Moure, 1943)
		<i>Augochlora (Oxystoglossella) aurinasis</i> (Vachal, 1911)
		<i>Augochlora (Oxystoglossella) morrae</i> (Strand, 1910)
		<i>Augochlorella cf. ephyra</i> (Schrottky, 1910)
		<i>Augochlorella tredecim</i> (Vachal, 1911)

Family	Tribe	Species
Halictidae	Augochlorini	<i>Augochloropsis brachycephala</i> (Moure, 1943)
		<i>Augochloropsis cupreola</i> (Cockerell, 1900)
		<i>Augochloropsis electra</i> (Smith, 1853)
		<i>Augochloropsis smithiana</i> (Cockerell, 1900)
		<i>Augochloropsis sp.1</i>
		<i>Augochloropsis sp.2</i>
	Halictini	<i>Dialictus sp.1</i>
		<i>Dialictus sp.2</i>
Megachilidae	Anthidiini	<i>Hypanthidium divaricatum</i> (Smith, 1854)
	Megachilini	<i>Megachile (Acentron) cf. tupinaquina</i> (Schrottky, 1913)

5 FINAL CONSIDERATIONS AND CONCLUSION

5.1 GENERAL DISCUSSION

This thesis examined the ecological role of *Apis mellifera* across temporal, spatial, and mechanistic dimensions, integrating historical reconstruction, global comparative analysis, and field experimentation to understand how the species influences plant–bee interaction networks. Taken together, the results demonstrate that honeybee impacts are both context-dependent and scale-dependent. Rather than producing uniform or easily detectable changes, *A. mellifera* influences ecological networks in subtle yet pervasive ways that become evident only when interactions are examined simultaneously across multiple organizational levels (Valido et al 2019; Worthy et al. 2023).

From a temporal perspective (Chapter 1), the historical network reconstructed from a Cerrado fragment in the early 1970s revealed a system characterized by pronounced niche partitioning, high specialization, and strong modularity. These attributes reflect a community shaped by diverse floral resources and a rich native bee assemblage. Notably, *A. mellifera* was present but marginal during this period, occupying a relatively peripheral structural role. This contrasts sharply with contemporary Cerrado landscapes, where environmental homogenization and land-use change have shifted resource availability and potentially amplified competitive interactions with honeybees (Hidasi et al. 2019; Graf et al. 2020; Jacquemin et al. 2020). The historical baseline provided by Chapter 1 thus contextualizes present-day patterns by demonstrating how Cerrado plant–pollinator communities were structured prior to intense anthropogenic pressure. This insight is especially valuable because the limited historical reconstructions of pollination networks in Brazilian biomes have so far been restricted to regions outside the Cerrado, such as the Atlantic Forest (Pereira et al. 2022).

The global analysis (Chapter 2) revealed that the ecological role of honeybees varies substantially between their native and non-native ranges, but these differences are often undetectable at the scale of traditional network metrics (Petanidou et al. 2008). While macro-scale properties such as nestedness and modularity showed limited variation across

regions, finer-scale analyses demonstrated significant differences in motif structure and species roles (Fisogni et al. 2022). In particular, *A. mellifera* tends to adopt more central, structurally influential roles outside its native range, suggesting a disproportionate effect on local interaction dynamics (Cruz et al. 2022; Dattilo et al. 2022). These results highlight the importance of multi-scale analyses for detecting the nuanced ways in which generalist invaders influence community structure (Crichton et al. 2019). They also suggest that honeybee-driven homogenization may operate less through dramatic changes in overall network architecture and more through shifts in the distribution of interaction strengths and functional roles (Paini 2004).

The experimental findings (Chapter 3) further illuminate the mechanisms underlying these global and historical patterns. The short-term introduction of honeybee colonies into a Cerrado fragment triggered detectable changes in bee species composition and diversity (Garibaldi et al. 2021). Honeybees may rapidly concentrated their foraging on abundant floral resources, which in turn can displace native bees from preferred plant species and induced shifts in their foraging strategies (Pasquaretta et al. 2017). These behavioral responses occur even over a short timescale, supporting the hypothesis that honeybees can modify plant–pollinator interactions locally, even when macro-scale structural metrics remain stable (Iwasaki & Hogendoorn 2022). The experimental results thereby provide mechanistic support for the scale-dependent patterns observed globally and help explain the long-term structural changes inferred from historical data (Page & Williams 2023b).

Across all chapters, a unifying theme emerges: the impact of *Apis mellifera* cannot be understood through a single scale of analysis. When viewed only through coarse or community-wide measures, honeybee effects may appear minimal or inconsistent. Yet when interactions are examined at fine spatial, temporal, or structural scales, patterns of competition, displacement, and network reorganization become increasingly apparent (Page & Williams 2023a). This multi-scale perspective reveals that honeybee effects accumulate not through catastrophic ecological disruption, but through gradual changes in interaction patterns, species roles, and behavioral dynamics (Ackerman 2021). Such cumulative changes are consistent with studies that have monitored bee communities over

longer periods and documented progressive honeybee-driven impacts (e.g., Valido et al. 2019). These findings underscore the importance of adopting holistic frameworks to assess biological invasions, particularly in mutualistic systems where subtle changes can have long-term consequences for ecological stability.

5.2 FUTURE RESEARCH DIRECTIONS

The integrative approach adopted in this thesis opens several promising avenues for future research.

First, the reconstruction of historical networks highlights the need for more long-term datasets in the Neotropics. Digitization of museum specimens, field notebooks, and unpublished surveys may provide new opportunities for reconstructing past ecological communities. Such efforts would enable robust comparisons between historical and modern networks, shedding light on how mutualistic systems respond to decades of land-use change and species introductions.

Second, the global analysis points to the importance of expanding comparative studies that incorporate abundance data, phylogenetic information, and functional traits. Understanding whether honeybee impacts are filtered by phylogenetic relatedness, morphological similarity, or floral specialization would offer deeper insight into the mechanisms shaping community assembly across regions.

Third, the experimental work suggests that behavioral responses play a central role in mediating competition between honeybees and native pollinators. Future research could examine how learning, memory, and foraging efficiency influence competitive dynamics. Experiments manipulating resource distribution, colony density, or temporal patterns of resource availability would further clarify the conditions under which honeybees exert the strongest ecological pressures (Cane & Tepedino 2017).

Fourth, integrating plant reproduction into network-level analyses remains a key frontier. While this thesis focused primarily on interactions among bees and plants, quantifying pollen deposition, seed set, and offspring quality would allow direct assessment of the functional consequences of honeybee dominance for plant fitness and community composition.

Finally, there is a growing need for predictive models that incorporate both network structure and behavioral mechanisms. Agent-based or trait-based simulations, calibrated with empirical data, could forecast how honeybee introductions influence plant–pollinator communities under future scenarios of land-use change, climate warming, and resource fluctuation (Larson et al. 2014).

Together, these directions emphasize the importance of multi-disciplinary, multi-scale approaches that connect behavioral ecology, community ecology, and conservation biology.

5.3 THESIS KEY FINDINGS

This thesis shows that understanding the ecological role of *Apis mellifera* requires an integrative framework that bridges temporal baselines, spatial comparisons, and experimental tests. The historical reconstruction revealed that Cerrado plant–bee communities were once characterized by distinct resource partitioning and high specialization, with honeybees occupying peripheral roles. The global analysis demonstrated that honeybee impacts are context-dependent and become evident primarily at fine structural scales, where the species often assumes more central or influential positions outside its native range. The experimental results confirmed that honeybee introduction leads to rapid changes in native bee foraging behavior and floral resource use, supporting the idea that subtle behavioral shifts can drive broader structural changes in ecological networks.

Overall, these findings indicate that honeybee impacts are subtle, cumulative, and highly dependent on ecological context. Rather than dramatically restructuring entire networks, *A. mellifera* influences communities through changes in species roles, increases in functional homogenization, and shifts in interaction patterns that unfold across time and space. By integrating multiple scales and approaches, this thesis contributes to a more nuanced understanding of biological invasions in mutualistic systems and provides essential insights for the conservation of pollination networks in biodiversity hotspots such as the Cerrado.

5.4 REFERENCES

- Ackerman JD (2021) Island invasions by introduced honey bees: what can be expected for Puerto Rico and the Caribbean? *Frontiers in Ecology and Evolution* 8: 556744.
- Cane JH, & Tepedino VJ (2017) Gauging the effect of honey bee pollen collection on native bee communities. *Conservation Letters* 10: 205-210.
- Crichton A, Francis N, Doherty S, et al. (2019) Low endemic bee diversity and very wide host range in lowland Fiji: support for the pollinator super-generalist hypothesis in island biogeography. *Pacific conservation biology* 25: 135-142.
- Cruz CP, Luna P, Guevara R, Hinojosa-Diaz IA, et al. (2022) Climate and human influence shape the interactive role of the honeybee in pollination networks beyond its native distributional range. *Basic and Applied Ecology* 63: 186-195.
- Dattilo W, Cruz CP, Luna P, et al. (2022) The impact of the honeybee *Apis mellifera* on the organization of pollination networks is positively related with its interactive role throughout its geographic range. *Diversity* 14: 917.
- Fisogni A, Hautekèete N, Piquot Y, et al (2022) Seasonal trajectories of plant-pollinator interaction networks differ following phenological mismatches along an urbanization gradient. *Landscape and Urban Planning* 226: 104512.
- Garibaldi LA, Pérez-Méndez N, Cordeiro GD, et al (2021) Negative impacts of dominance on bee communities: Does the influence of invasive honey bees differ from native bees? *Ecology* 102: 12.
- Graf LV, Zenni RD, & Gonçalves RB (2020) Ecological impact and population status of non-native bees in a Brazilian urban environment. *Revista Brasileira de Entomologia* 64: e20200006.

Hidasi-Neto J, Joner DC, Resende F, et al. (2019) Climate change will drive mammal species loss and biotic homogenization in the Cerrado Biodiversity Hotspot. *Perspectives in Ecology and Conservation* 17: 57-63.

Iwasaki JM, & Hogendoorn K (2022) Mounting evidence that managed and introduced bees have negative impacts on wild bees: an updated review. *Current Research in Insect Science* 2: 100043.

Jacquemin F, Violle C, Munoz F, et al. (2020) Loss of pollinator specialization revealed by historical opportunistic data: Insights from network-based analysis. *PLoS One* 15: e0235890.

Johnson AL, & Ashman TL (2019) Consequences of invasion for pollen transfer and pollination revealed in a tropical island ecosystem. *New Phytologist* 221: 142-154.

Larson DL, Droege S, Rabie PA, Larson JL, et al. (2014) Using a network modularity analysis to inform management of a rare endemic plant in the northern Great Plains, USA. *Journal of Applied Ecology* 51: 1024-1032.

Page ML, & Williams NM (2023a) Honey bee introductions displace native bees and decrease pollination of a native wildflower. *Ecology* 104: e3939.

Page ML, & Williams, NM (2023b) Evidence of exploitative competition between honey bees and native bees in two California landscapes. *Journal of Animal Ecology* 92: 1802-1814.

Paini DR (2004) Impact of the introduced honeybee (*Apis mellifera*)(Hymenoptera: Apidae) on native bees: a review. *Austral ecology* 29: 399-407.

Pasquaretta C, Jeanson R, Andalo C, et al (2017) Analysing plant-pollinator interactions

with spatial movement networks. *Ecological Entomology* 42: 4-17.

Pereira J, Ribeiro MC, Battiston F, & Jordán F (2022) Reconstruction and variability of tropical pollination networks in the Brazilian Atlantic Forest. *Community Ecology* 23: 315-325.

Petanidou T, Kallimanis AS, Tzanopoulos J, et al. (2008) Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology letters* 11: 564-575.

Valido A, Rodríguez-Rodríguez MC, & Jordano P (2019) Honeybees disrupt the structure and functionality of plant-pollinator networks. *Scientific reports* 9: 4711.

Worthy SH, Acorn JH, & Frost CM (2023) Honey bees (*Apis mellifera*) modify plant-pollinator network structure, but do not alter wild species' interactions. *Plos one* 18: e0287332.