Universidade de Brasília

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Ecologia

Scaling up coccinellid ecology: Multiscale drivers of coccinellid habitat use, trophic interactions, and biological control in organic agroecosystems

Escalonando a ecologia de coccinelídeos: Determinantes em múltiplas escalas do uso do habitat, das interações tróficas e do controle biológico em agroecossistemas orgânicos

Ana Claudia da Silva

Orientador: Prof. Dr. Pedro H. B. Togni

Brasília – DF

Dezembro de 2025

Universidade de Brasília

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Ecologia

Scaling up coccinellid ecology: Multiscale drivers of coccinellid habitat use, trophic interactions, and biological control in organic agroecosystems

Escalonando a ecologia de coccinelídeos: Determinantes em múltiplas escalas do uso do habitat, das interações tróficas e do controle biológico em agroecossistemas orgânicos

Ana Claudia da Silva

Tese de Doutorado apresentada ao Programa de Pós-Graduação *stricto sensu* em Ecologia da Universidade de Brasília como parte dos requisitos para obtenção do título de Doutor em Ecologia.

Orientador: Prof. Dr. Pedro H. B. Togni

Brasília – DF

Dezembro de 2025

AGRADECIMENTOS

Expresso meus sinceros agradecimentos àqueles que, de maneira direta ou indireta, tornaram este trabalho possível. À minha família, meu porto seguro e fonte de inspiração, em especial à minha mãe Claudina, ao meu pai Ricardo e ao meu irmão Marcelo, pelo incentivo e força constantes. Aos meus avós, Arminda, Janira e Delfino, por todo o carinho e exemplo de vida. Ao meu amado companheiro, Pedro Santos, pelo suporte emocional incondicional, carinho e infinita paciência demonstrados ao longo de toda esta desafiadora jornada. Ao meu orientador, Prof. Dr. Pedro H. B. Togni, pela inestimável orientação humana, pelo constante empenho e dedicação à minha formação e ao desenvolvimento desta tese. Sua liderança e apoio foram fundamentais em todo o processo. À Dra. Gabriela B. Nardoto e ao Dr. Jason M. Schimidt, por suas contribuições indispensáveis e valiosas que enriqueceram significativamente o conteúdo desta tese. Aos membros da banca examinadora: Dra. Marina R. Frizzas, Dr. Lessando Gontijo, Dr. Arne Janssen e Dra. Cecília Monteiro. Meu reconhecimento pela pronta disponibilidade, dedicação e pelas sugestões criteriosas que, sem dúvida, contribuíram para a melhoria e o aprimoramento final deste trabalho. Aos colegas, estagiários, amigos do Laboratório de Ecologia de Insetos (LECOI) que colaboraram direta ou indiretamente com as diversas etapas e atividades relacionadas a esta pesquisa, e que compartilharam comigo momentos felizes que irei guardar para sempre. Um agradecimento especial a Patrícia Sujii, só gostaria de dizer “Obrigada Patrícia!”, você é incrível. Agradeço também a Nícholas Camargo, que me ajudou diversas vezes com as análises estatísticas. À University of Georgia, onde tive a oportunidade enriquecedora de realizar meu período de Doutorado Sanduíche junto ao Schimidt Lab. Aos docentes do Programa de Pós-Graduação em Ecologia pela excelência e rigor na transmissão do conhecimento, que me propiciou uma base sólida e essencial para minha formação científica. Ao Programa de Pós-Graduação em Ecologia e à Universidade de Brasília (UnB), por me oferecerem o acesso e o suporte para a realização da Pós-Graduação e o desenvolvimento de minha carreira científica. À Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF) e o Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo financiamento que permitiu a realização dessa tese. À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão das bolsas de estudo no Brasil e no exterior, as quais propiciaram o apoio financeiro crucial e essencial durante todo o meu processo de formação.

Summary

RESUMO

A intensificação agrícola leva a perda de biodiversidade global, impactando diretamente serviços de controle biológico essenciais para os agroecossistemas tropicais. Os coccinelídeos afidófagos, um importante grupo de inimigos naturais, são significativamente afetados por fatores que operam em múltiplas escalas espaciais, por exemplo, beneficiando-se de recursos florais locais e de habitats seminaturais na escala da paisagem. Portanto, um principal desafio é desvendar os mecanismos que regem esses processos em diferentes escalas, particularmente no que concerne às interações tróficas e à coexistência de espécies. Esta tese investigou como fatores bióticos e abióticos em diferentes escalas espaciais possibilitam ou restringem a coexistência de coccinelídeos predadores em agroecossistemas orgânicos. Para isso, a tese foi dividida em três capítulos. O Capítulo 1 analisou como os coccinelídeos particionam espacialmente habitat e microhabitat em cultivos orgânicas de brássicas, como a sobreposição de nicho varia ao longo dos estágios de vida e a influência das plantas espontâneas na sua abundância. O Capítulo 2 avaliou como as interações tróficas e as posições tróficas das espécies variam com a disponibilidade de presas, enfatizando o papel das plantas espontâneas. O Capítulo 3 investigou como a composição da paisagem influencia as comunidades de coccinelídeos, suas interações tróficas e o consequente controle biológico de afídeos em diferentes escalas. Os resultados mostraram uma segregação espacial estágio-específica por espécies de coccinelídeos, com competidores superiores dominando culturas ricas em presas durante os estágios móveis, empupando apenas em locais abrigados fora das plantas. Inversamente, competidores inferiores persistiram ao utilizar as plantas espontâneas, o que promoveu a coexistência ao facilitar a segregação espacial. Além disso, a abundância de coccinelídeos correlacionou-se com a densidade de afídeos, mas as plantas não cultivadas sustentaram as comunidades durante a escassez de presas. A densidade de afídeos modulou o tamanho e a sobreposição do nicho isotópico das espécies e a estrutura geral da rede trófica. Os coccinelídeos consomem uma alta diversidade de gêneros de plantas espontâneas, permitindo-lhes mudar de uma dieta à base de plantas para uma dieta à base de animais, dependendo da disponibilidade de presas. A rede planta-afídeo-coccinelídeo refletiu o comportamento generalista dos coccinelídeos em sistemas agrícolas, com plantas não cultivadas apoiando interações chave. A composição da paisagem, em última análise, afetou a densidade de afídeos, a abundância e diversidade de coccinelídeos, e suas interações tritróficas em diferentes escalas, influenciando, por fim, a prestação do serviço de controle biológico.

No geral, a paisagem foi confirmada como um importante preditor da diversidade e abundância de coccinelídeos, mas também determinando a estruturação local das interações a partir do pool regional. Essa estrutura é sustentada pelas plantas espontâneas, que servem como recursos alimentares essenciais, explicando em conjunto a variação trófica e a transferência de energia, embora os resultados sejam modulados por diferenças espécie-específicas nas histórias de vida, características funcionais e comportamento das espécies.

PALAVRAS-CHAVE: Cerrado, controle biológico conservativo, DNA metabarcoding, redes ecológicas, serviços ecossistêmicos, composição da paisagem, partição de nicho, isótopos estáveis

ABSTRACT

Agricultural intensification drives global biodiversity loss, directly impacting essential biological control services in tropical agroecosystems. Within this context, aphidophagous coccinellids, a key group of natural enemies, are significantly affected by drivers operating at multiple spatial scales, for instance, benefiting from local floral resources and landscape-scale semi-natural habitats. Therefore, one main challenge is to unravel the mechanisms driving these patterns across scales, particularly concerning trophic interactions and species coexistence, as they directly influence the structure of natural enemy communities and, consequently, the provision of biological control services. This thesis investigated how biotic and abiotic factors across spatial scales enable or constrain coexistence among predatory coccinellid species in organic agroecosystems. To achieve this, this thesis was divided into three chapters. Chapter 1 analyzes how coccinellid species spatially partition habitat and microhabitat in organic brassica crops, niche overlap across life stages, and the influence of adjacent non-crop plants habitats on coccinellids abundance. Chapter 2 evaluates how trophic interactions and trophic positions of species vary with prey availability, emphasizing the role of non-crop plants species. Chapter 3 investigates how landscape composition influences coccinellid communities, their trophic interactions with plants and aphids, and the resulting aphid biological control across different scales. I found stage-specific spatial segregation among coccinellid species, with competitively superior species dominating crop plants during their mobile life stages (larvae and adults) and pupating more frequently in sheltered sites outside the plants. Conversely, inferior competitors persisted by utilizing non-crop plants, which promoted coexistence through spatial segregation. Furthermore, coccinellid abundance correlated with aphid density, yet non-crop plants sustained communities during prey scarcity. Aphid density modulated species isotopic niche size/overlap and the overall trophic network structure. Moreover, high diversity of non-crop plants supplied coccinellid species with resources (e.g., pollen and nectar), enabling them to shift from a plant-based to an animal-based diet based on prey availability. The plant–aphid–coccinellid network was highly connected, with numerous direct plant–coccinellid interactions, reflecting the generalist feeding behavior of coccinellids and highlighting the role of non-crop plants in supporting key interactions. Landscape composition affected aphid density, coccinellid abundance, diversity, and their tritrophic interactions across scales, influencing biological control service provision. Overall, landscape composition is a critical predictor of diversity and abundance of coccinellids but also influences how species from the regional pool structure their interactions locally. This structure is sustained by non-crop plants, which serve as essential food resources, jointly explaining trophic variation and energy transfer, although outcomes are modulated by species-specific differences in life histories, functional traits, and behavior of species.

KEYWORDS: Cerrado, Conservation biological control, DNA metabarcoding, ecological networks, ecosystem services, landscape composition, niche partitioning, stable isotopes

GENERAL INTRODUCTION

Human activities have modified Earth's terrestrial ecosystems, especially over the past 50 years, with the intensification of agriculture being the main cause of biodiversity loss (Nelson, 2005; Baessler, 2006; Power, 2010). These changes have led to the emergence of “novel ecosystems”, characterized by species composition and abundance adapted to human-modified environments, such as pest insects (Hobbs et al., 2006). However, these ecosystems are not isolated, as biodiversity interacts across natural and anthropogenic habitats (Seastedt et al., 2008; Aguiar et al., 2021), which are connected through species dispersal (Hagen et al., 2012; Togni et al., 2019). Therefore, it is necessary to understand the connections between natural and human-modified environments and their role in providing ecosystem services supported by biodiversity, such as biological control (Kremen & Ostfeld, 2005; Bommarco et al., 2013). To achieve this, an approach that considers factors operating at multiple spatial scales influencing species and their services is required (Togni et al., 2021). These scales may range from the microhabitat level (e.g., spatial partitioning of plant use among species) to the landscape level (e.g., effects of landscape composition on local communities).

In this context, natural biological control and natural enemies are influenced by processes occurring at different spatial scales (Togni et al., 2021). At the local scale, organic farming has been shown to enhance biodiversity and its associated ecosystem services within agricultural landscapes (Gabriel et al., 2010; Tuck et al., 2014). Organic systems alone can increase the diversity and abundance of natural enemies (Garratt et al., 2011; Lichtenberg et al., 2017; Aldebron et al., 2020), resulting in a positive relationship between biological control and natural enemy diversity in most cases (Letourneau et al., 2009; Togni et al., 2019; Rosado et al., 2021). However, such effects are often highly context-dependent (Macfadyen et al., 2011; Maalouly et al., 2013; Gontijo, 2019). Thus, even though habitat management usually focuses on local-scale interventions, targeted habitat manipulation can generate spillovers of natural enemies and their interactions among habitats at the landscape scale (Togni et al., 2021; Allen et al., 2022).

The simplification of landscapes directly impacts the provision of multiple ecosystem services supported by biodiversity (Baude et al., 2019; Ellis et al., 2021; Grez & Zaviezo, 2024). Landscape-level processes, such as dispersal facilitation or limitation and trait filtering, can strongly influence the effectiveness of biological control and the dynamics of natural enemies, as the landscape context shapes the species pool available to colonize local habitats. The presence of non-agricultural habitats within the landscape can directly affect biological control (Albrecht et al., 2020; Yang et al., 2021; Jachowicz & Sigsgaard, 2025), particularly when interspersed with agricultural areas (Le Gal et al., 2020). For instance, the presence of natural areas can positively affect parasitoid species richness while negatively influencing their abundance across different spatial scales (Marins et al., 2024). These findings indicate that the effects of landscape structure on natural enemies depend on both the spatial scale and the specific metrics considered. In particular, the negative effects of landscape simplification on biodiversity-driven ecosystem services are largely mediated by species loss (Dainese et al., 2019), which in turn may directly alter species interactions.

In this context, aphidophagous coccinellids, one of the well-known groups of natural enemies (Gordon, 1985; Michaud, 2012), are also influenced by factors operating across multiple spatial scales. Although not the only natural enemies of aphids, coccinellids are predatory in both larval and adult stages, which makes them highly efficient and the main aphid predators. Due to their diverse feeding habits, coccinellids can benefit from plant diversification practices used in conservation biological control (Andow, 1991; Amaral et al., 2013; Gontijo et al., 2013; Togni et al., 2016; Gontijo et al., 2018; Blassioli-Moraes et al., 2022). Conservation biological control consists of strategies that modify the habitat of natural enemies to favor their survival and improve their performance, thereby enhancing their effectiveness as biological control agents (Barbosa, 1998; Venzon et al., 2021). In this context, conservation biological control depends on a range of ecological and behavioral processes that promote pest suppression through habitat management strategies designed to benefit natural enemies and that can be implemented across different spatial scales (Gontijo et al., 2013; Begg et al., 2017; Togni et al., 2021).

At the local scale, the presence of flowering plants can increase coccinellid abundance and survival (Lixa et al., 2010; Togni et al., 2016; Fonseca et al., 2017; da Silva et al., 2025). In addition, manipulating non-crop plants can affect the movement patterns and spatial distribution of aphidophagous coccinellids (Venzon et al., 2019a), as these predators must cope with the ephemeral nature of their aphid prey and frequently move among habitats (Hodek & Honěk, 1996). Some species may even exploit suboptimal habitats, relying primarily on pollen and nectar from these plants (Amaral et al., 2013). Although floral resources can improve survival of predatory coccinellids, especially when prey is not available, they do not typically support reproduction (e.g., He and Sigsgaard, 2019; Hatt and Osawa, 2019; Xia et al., 2024). Beyond habitat and resource use by coccinellids, interactions operating at smaller spatial scales, such as intraguild predation, can further influence species coexistence (Amarasekare, 2007; Marques et al., 2018). Increased plant diversity can contribute to this pattern by enhancing habitat structural complexity, providing alternative food sources and prey, as well as oviposition and shelter sites (Venzon et al., 2019b).

Greater plant diversity may also facilitate spatial partitioning in microhabitat use, allowing coexistence among intraguild predators and thereby strengthening their overall impact on prey populations (Janssen et al., 20007; da Silva et al., 2022). Nonetheless, simply increasing plant diversity does not guarantee the provision of biological control. Understanding the underlying ecological interactions evolving coccinellid species associated with these plants, such as apparent competition or intraguild predation, is therefore essential (Blassioli-Moraes et al., 2022). This is because species interactions are complex, and predators may respond differently to habitat diversification (Gontijo, 2019). For example, although intercropping systems have been shown to reduce aphid infestation, they can also decrease parasitoid emergence, likely due to increased intraguild predation (Saldanha et al., 2019), which can disrupt aphid biological control. Furthermore, it has been demonstrated that increasing coccinellid abundance alone is not sufficient to enhance biological control because higher species diversity is also required, as this may increase complementary effects among predators and their net effect on pest densities (da Silva et al., 2025). However, this is not always true, as increasing diversity by adding intraguild predators may decrease pest control, since one species can interfere with another's ability to capture and consume prey (e.g., Finke and Denno, 2005; Letourneau et al., 2009), reinforcing the context dependence of biodiversity to biological control services.

At broader spatial scales, the landscape context has also proven to be an important factor influencing coccinellid populations. The amount of semi-natural habitat within the landscape has shown positive effects on coccinellid abundance in studies conducted in temperate regions (Gardiner et al., 2009; Woltz & Landis, 2014; Yang et al., 2019; Iuliano et al., 2024). Landscape structure has also been linked to changes in the composition of coccinellid communities in urban environments (Liere et al., 2019). In tropical regions, however, agricultural landscape simplification and urbanization have been associated with negative effects on the abundance and diversity of lady beetles (Grez et al., 2019; Frizzo et al., 2020; Catzim et al., 2022; Grez & Zaviezo, 2024). Together, these findings highlight that landscape-related factors, such as habitat loss, simplification, agricultural intensification, and urbanization, represent major threats to coccinellid populations worldwide, alongside other drivers such as climate change and biological invasions (Honek et al., 2017; Soares et al., 2022).

Although highly desirable, integrating knowledge of the factors shaping the coexistence of predatory coccinellid species acting complementarily in pest suppression across spatial and temporal scales remains a major challenge. In this regard, the study of ecological interaction networks can be particularly valuable, as it helps identify which interactions occur within communities, assess the robustness of these interactions, and understand how individuals use resources across different habitats (Bascompte, 2009; Tylianakis et al., 2017; Valdovinos, 2019; Assunção et al., 2022). Network metrics such as nestedness, modularity and niche overlap allow researchers to identify which species are more likely to interact and whether subsets of tightly connected species occur within communities (Vázquez et al., 2009; Almeida-Neto & Ulrich, 2011; Dormann & Strauss, 2014). By revealing how species are organized and interact within networks, these metrics indicate whether predators share prey, partition resources, or are segregated into modules, which may influence the strength, redundancy, and stability of aphid suppression.

From this network perspective, landscape features are known to shape species interactions across multiple organism groups (e.g., Hedlund et al., 2004; Tylianakis et al., 2007; Martínez-Núñez et al., 2019; Fortin et al., 2021; Syahidah et al., 2021; Pardee et al., 2023), yet the influence of landscape context on coccinellid interaction networks remains poorly understood. This functional dimension may be crucial for clarifying how diversity patterns modulate the relationship between biodiversity and ecosystem services.

Another valuable approach for understanding species interactions and resource use is the analysis of stable isotopes of carbon and nitrogen. By tracing these isotopic signatures, this method provides important insights in ecological studies, such as feeding preferences (Akamatsu et al., 2004; Adams et al., 2016), trophic position, niche overlap (Hood-Nowotny & Knols, 2007; Hyodo, 2015; Choi et al., 2020; Quinby et al., 2020), feeding strategies (Paetzold et al., 2005; Chari et al., 2018), and dispersal patterns (Medeiros et al., 2017; Madeira et al., 2019). Nitrogen isotopes allow the estimation of the trophic position of organisms, since each trophic level is typically enriched by 3–4‰ of ^{15}N (Post, 2002). Carbon isotopes provide information on the dietary carbon sources of organisms. For instance, consumers feeding on C_3 plants contain distinct $\delta^{13}C$ values from those feeding on C_4 plants, as C_3 plants have lower

$\delta^{13}C$ levels (Post, 2002). Consequently, the diet of an herbivore species that feeds on C_3 or C_4 plants can influence the $\delta^{13}C$ isotopic profile of its predator. This method relies on the principle that the ratio of stable isotopes varies in predictable ways as elements cycle through the food web. When an animal consumes and assimilates food, the isotopic composition of that food becomes reflected in its tissues (Hyodo, 2015).

However, a major limitation of isotopic analysis is its inability to identify the resources consumed by species at a meaningful taxonomic level. This becomes particularly important when investigating predator–prey trophic interactions, where prey is partially or completely digested and morphology-based identification is not possible. In such cases, high-throughput sequencing (HTS) combined with DNA metabarcoding can be particularly advantageous, as it enables the identification of multiple species in mixed samples, including gut contents, regurgitates, and feces (Kamenova et al., 2018; Andriollo et al., 2019). Given these advantages, this methodology has been increasingly adopted in ecological research, with numerous studies applying it to investigate ecological interactions (e.g., Lunde et al., 2022; Miller-ter Kuile et al., 2022; Slusher et al., 2024), describe dietary composition (Deagle et al., 2013; Mollot et al., 2014; Kerley et al., 2018; Grabarczyk et al., 2025), evaluate foraging preferences (Leponiemi et al., 2023), assess biodiversity responses to land-use change (Wood et al., 2017; Schmidt et al., 2018), estimate predation rates (Andow &Paula, 2024), and monitor insect pests in agricultural systems (Morales-Hojas, 2017).

The aim of this thesis was to evaluate how biotic and abiotic factors operating at different spatial scales enable or constrain the coexistence of predatory coccinellids in organic agroecosystems. The coexistence of multiple coccinellid species can enhance biodiversity, which may result in either increased or reduced pest control, depending on the degree of functional complementarity in predation and the strength and frequency of negative interspecific interactions. By addressing the structure and functioning of coccinellid communities from different perspectives (e.g., temporal and spatial), it is possible to gain a better understanding of the processes that allow the stable coexistence of individuals within communities, which may improve the provision of biological control services. Based on this framework, we formulated three general hypotheses:

1. Fine-scale habitat and microhabitat partitioning facilitates the coexistence of coccinellid species across life stages by reducing niche overlap.
2. Variation in prey availability and plant resource use drives shifts in coccinellid trophic interactions and trophic position across the crop cycle.

3. Landscape composition acts as a broad-scale abiotic filter species or individual traits that structures coccinellid communities, shapes trophic interactions, and ultimately influences aphid biological control.

To address these hypotheses, the thesis is divided into three chapters. In the first chapter, we investigated how four species of aphidophagous coccinellids commonly found in organic brassica crops partition habitat and microhabitat spatially, and how niche overlap among species varies throughout their life cycles. We also examined the role of different non-crop plant species in influencing coccinellid abundance. This chapter focused on mechanisms operating at finer spatial scales, which were the microhabitat (spatial use of plants) and habitat (movement of individuals between crops and surrounding non-crop plants) scales. In the second chapter, we evaluated how trophic interactions among coccinellids vary with prey availability and the role of non-crop plants in shaping these interactions. We also investigated the trophic position of each species throughout the brassica crop cycle, during which prey density gradually increases. This chapter focused on mechanisms operating at the habitat scale, aiming to uncover the trophic interactions of the species under different prey regimes. In the third chapter, we investigated how landscape composition influences coccinellid communities, their trophic interactions, and the biological control services they provide across different spatial scales. We also examined how the interactions among aphids, plants, and coccinellids affect aphid biological control in brassica crops. This chapter, therefore, addressed mechanisms operating at broader spatial scales that determine the regional species pool through abiotic filters such as land-cover types.

REFERENCES

Adams, M. O., Seifert, C. L., Lehner, L., Truxa, C., Wanek, W., & Fiedler, K. (2016). Stable isotope signatures reflect dietary diversity in European forest moths. *Frontiers in zoology*, *13*(1), 1-10.

Aguiar, L. M., Bueno-Rocha, I. D., Oliveira, G., Pires, E. S., Vasconcelos, S., Nunes, G. L., ... & Togni, P. H. (2021). Going out for dinner—The consumption of agriculture pests by bats in urban areas. PLoS One, 16(10), e0258066.

Akamatsu, F., Toda, H., & Okino, T. (2004). Food source of riparian spiders analyzed by using stable isotope ratios. *Ecological Research*, *19*(6), 655-662.

Albrecht, M., Kleijn, D., Williams, N. M., Tschumi, M., Blaauw, B. R., Bommarco, R., ... & Sutter, L. (2020). The effectiveness of flower strips and hedgerows on pest control, pollination services and crop yield: a quantitative synthesis. *Ecology letters*, *23*(10), 1488-1498.

Aldebron, C., Jones, M. S., Snyder, W. E., & Blubaugh, C. K. (2020). Soil organic matter links organic farming to enhanced predator evenness. Biological Control, 146, 104278.

Allen, W. J., Bufford, J. L., Barnes, A. D., Barratt, B. I., Deslippe, J. R., Dickie, I. A., ... & Tylianakis, J. M. (2022). A network perspective for sustainable agroecosystems. Trends in Plant Science.

Almeida-Neto, M., & Ulrich, W. (2011). A straightforward computational approach for measuring nestedness using quantitative matrices. Environmental Modelling & Software, 26(2), 173-178.

Amaral, D. S., Venzon, M., Duarte, M. V., Sousa, F. F., Pallini, A., & Harwood, J. D. (2013). Non-crop vegetation associated with chili pepper agroecosystems promote the abundance and survival of aphid predators. Biological Control, 64(3), 338-346.

Amarasekare, P. (2007). Spatial dynamics of communities with intraguild predation: the role of dispersal strategies. *The American Naturalist*, *170*(6), 819-831.

Andow, D. A. (1991). Vegetational diversity arthropod population. Annu. rev. entomol, 36, 561-6.

Andow, D. A., & Paula, D. P. (2024). Estimating relative per capita predation rates from molecular gut content analysis. *Biological Control*, *192*, 105499.

Andriollo, T., Gillet, F., Michaux, J. R., & Ruedi, M. (2019). The menu varies with metabarcoding practices: A case study with the bat Plecotus auritus. *PloS one*, *14*(7), e0219135.

Assunção, R. M., Camargo, N. F., Souza, L. S., Rocha, E. M., Tostes, G. M., Sujii, E. R., ... & Togni, P. H. (2022). Landscape conservation and local interactions with non-crop plants aid in structuring bee assemblages in organic tropical agroecosystems. Journal of Insect Conservation, 26(6), 933-945.

Baessler, C., & Klotz, S. (2006). Effects of changes in agricultural land-use on landscape structure and arable weed vegetation over the last 50 years. Agriculture, ecosystems & environment, 115(1-4), 43-50.

Barbosa, P. A. (Ed.). (1998). *Conservation biological control*. Elsevier.

Bascompte, J. (2009). Disentangling the web of life. Science, 325(5939), 416-419.

Baude, M., Meyer, B. C., & Schindewolf, M. (2019). Land use change in an agricultural landscape causing degradation of soil based ecosystem services. Science of the Total Environment, 659, 1526-1536.

Begg, G. S., Cook, S. M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., ... & Birch, A. N. E. (2017). A functional overview of conservation biological control. Crop Protection, 97, 145-158.

Blassioli-Moraes, M. C., Venzon, M., Silveira, L. C. P., Gontijo, L. M., Togni, P. H. B., Sujii, E. R., ... & Birkett, M. (2022). Companion and Smart Plants: Scientific Background to Promote Conservation Biological Control. Neotropical Entomology, 1-17.

Bommarco, R., Kleijn, D., & Potts, S. G. (2013). Ecological intensification: harnessing ecosystem services for food security. Trends in ecology & evolution, 28(4), 230-238.

Catzim, V. V., Philpott, S. M., Henaut, Y., García-Arellano, A., & Pérez-Lachaud, G. (2022). Local and landscape correlates of coccinellid species richness, abundance, and assemblage change along a rural–urban gradient in Quintana Roo, Mexico. *Biotropica*, *54*(3), 776-788.

Chari, L. D., Moyo, S., & Richoux, N. B. (2018). Trophic ecology of adult male Odonata. I. Dietary niche metrics by foraging guild, species, body size, and location. *Ecological Entomology*, *43*(1), 1-14.

Choi, J. Y., Kim, S. K., Kim, J. C., & Kwon, S. J. (2020). Habitat preferences and trophic position of Brachydiplax chalybea flavovittata Ris, 1911 (Insecta: Odonata) larvae in Youngsan River wetlands of South Korea. Insects, 11(5), 273.

da Silva, A. C., Cahú, R. C., Cogitskei, M. M., Kubota, K. S., Sujii, E. R., & Togni, P. H. (2022). Intercropping collard plants with coriander modulates behavioral interactions among aphidophagous predators by altering microhabitat structure. Biological Control, 105084.

da Silva, A. C., Oliveira, L. C., de Lacerda, J. G., Venzon, M., Frizzas, M. R., Sujii, E. R., & Togni, P. H. (2025). Floral availability outweighs aphid presence in supporting coccinellid abundance across life stages and promotes aphid predation. *Basic and Applied Ecology*.

Dainese, M., Martin, E. A., Aizen, M. A., Albrecht, M., Bartomeus, I., Bommarco, R., ... & Steffan-Dewenter, I. (2019). A global synthesis reveals biodiversity-mediated benefits for crop production. Science advances, 5(10), eaax0121.

Deagle, B. E., Thomas, A. C., Shaffer, A. K., Trites, A. W., & Jarman, S. N. (2013). Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: Which counts count?. *Molecular Ecology Resources*, *13*(4), 620-633.

Dormann, C. F., & Strauss, R. (2014). A method for detecting modules in quantitative bipartite networks. Methods in Ecology and Evolution, 5(1), 90-98.

Ellis, E. C., Gauthier, N., Klein Goldewijk, K., Bliege Bird, R., Boivin, N., Díaz, S., ... & Watson, J. E. (2021). People have shaped most of terrestrial nature for at least 12,000 years. Proceedings of the National Academy of Sciences, 118(17), e2023483118.

Finke, D. L., & Denno, R. F. (2005). Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology letters*, *8*(12), 1299-1306.

Fonseca, M. M., Lima, E., Lemos, F., Venzon, M., & Janssen, A. (2017). Non-crop plant to attract and conserve an aphid predator (Coleoptera: Coccinellidae) in tomato. Biological Control, 115, 129-134.

Fortin, M. J., Dale, M. R., & Brimacombe, C. (2021). Network ecology in dynamic landscapes. *Proceedings of the Royal Society B*, *288*(1949), 20201889.

Frizzo, T. L., Souza, L. M., Sujii, E. R., & Togni, P. H. (2020). Ants provide biological control on tropical organic farms influenced by local and landscape factors. *Biological Control*, *151*, 104378.

Gabriel, D., Sait, S. M., Hodgson, J. A., Schmutz, U., Kunin, W. E., & Benton, T. G. (2010). Scale matters: the impact of organic farming on biodiversity at different spatial scales. Ecology letters, 13(7), 858-869.

Gardiner, M. M., Landis, D. A., Gratton, C., Schmidt, N., O'Neal, M., Mueller, E., ... & DiFonzo, C. D. (2009). Landscape composition influences patterns of native and exotic lady beetle abundance. *Diversity and Distributions*, *15*(4), 554-564.

Garratt, M. P. D., Wright, D. J., & Leather, S. R. (2011). The effects of farming system and fertilisers on pests and natural enemies: a synthesis of current research. Agriculture, Ecosystems & Environment, 141(3-4), 261-270.

Gontijo, L. M. (2019). Engineering natural enemy shelters to enhance conservation biological control in field crops. Biological control, 130, 155-163.

Gontijo, L. M., Beers, E. H., & Snyder, W. E. (2013). Flowers promote aphid suppression in apple orchards. Biological Control, 66(1), 8-15.

Gontijo, L. M., Saldanha, A. V., Souza, D. R., Viana, R. S., Bordin, B. C., & Antonio, A. C. (2018). Intercropping hampers the nocturnal biological control of aphids. Annals of Applied Biology, 172(2), 148-159.

Gordon, R. D. (1985). The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society, 93(1).

Grabarczyk, E. E., Tillman, P. G., Querejeta, M., & Schmidt, J. M. (2025). Honey, What's for Dinner? Dietary Overlap and Size Dimorphism Between Female and Male Joro Spiders (Trichonephila clavata). *Integrative Zoology*.

Grez, A. A., Zaviezo, T., Gardiner, M. M., & Alaniz, A. J. (2019). Urbanization filters coccinellids composition and functional trait distributions in greenspaces across greater Santiago, Chile. *Urban Forestry & Urban Greening*, *38*, 337-345.

Grez, A., and T. Zaviezo. 2024. "Landscape Simplification, Urbanization, Biological Invasions, and Climate Change: A Review of the Major Threats to Native Coccinellids in Central Chile." *Entomologia Experimentalis et Applicata* 172(6): 460–466.

Hagen, M., Kissling, W. D., Rasmussen, C., De Aguiar, M. A., Brown, L. E., Carstensen, D. W., ... & Olesen, J. M. (2012). Biodiversity, species interactions and ecological networks in a fragmented world. In Advances in ecological research (Vol. 46, pp. 89-210). Academic Press.

Hatt, S., & Osawa, N. (2019). The role of Perilla frutescens flowers on fitness traits of the ladybird beetle Harmonia axyridis. *BioControl*, *64*(4), 381-390.

He, X., & Sigsgaard, L. (2019). A floral diet increases the longevity of the coccinellid Adalia bipunctata but does not allow molting or reproduction. *Frontiers in Ecology and Evolution*, *7*, 6.

Hedlund, K., Griffiths, B., Christensen, S., Scheu, S., Setälä, H., Tscharntke, T., & Verhoef, H. (2004). Trophic interactions in changing landscapes: responses of soil food webs. *Basic and Applied Ecology*, *5*(6), 495-503.

Hobbs, R. J., Arico, S., Aronson, J., Baron, J. S., Bridgewater, P., Cramer, V. A., ... & Zobel, M. (2006). Novel ecosystems: theoretical and management aspects of the new ecological world order. Global ecology and biogeography, 15(1), 1-7.

Hodek, I., & Honêk, A. (1996). Ecology of coccinellidae (Vol. 54). Springer Science & Business Media.

Honek, A., Dixon, A. F., Soares, A. O., Skuhrovec, J., & Martinkova, Z. (2017). Spatial and temporal changes in the abundance and compostion of ladybird (Coleoptera: Coccinellidae) communities. *Current opinion in insect science*, *20*, 61-67.

Hood-Nowotny, R., Knols, B.G.J., 2007. Stable isotope methods in biological and ecological studies of arthropods. Entomol. Exp. Appl. 124, 3–16.

Hyodo, F. (2015). Use of stable carbon and nitrogen isotopes in insect trophic ecology. Entomological Science, 18(3), 295-312.

Iuliano, B., Gratton, C., Kim, T. N., & Spiesman, B. J. (2024). Semi-natural habitat, but not aphid amount or continuity, predicts lady beetle abundance across agricultural landscapes. *Journal of Applied Ecology*, *61*(8), 1881-1893.

Jachowicz, N., & Sigsgaard, L. (2025). Highly diverse flower strips promote natural enemies more in annual field crops: A review and meta-analysis. *Agriculture, Ecosystems & Environment*, *381*, 109412.

Janssen, A., Sabelis, M. W., Magalhães, S., Montserrat, M., & Van Der Hammen, T. (2007). Habitat structure affects intraguild predation. *Ecology*, *88*(11), 2713-2719.

Kamenova, S., Mayer, R., Rubbmark, O. R., Coissac, E., Plantegenest, M., & Traugott, M. (2018). Comparing three types of dietary samples for prey DNA decay in an insect generalist predator. *Molecular Ecology Resources*, *18*(5), 966-973.

Kerley, G. I., Landman, M., Ficetola, G. F., Boyer, F., Bonin, A., Rioux, D., ... & Coissac, E. (2018). Diet shifts by adult flightless dung beetles Circellium bacchus, revealed using DNA metabarcoding, reflect complex life histories. *Oecologia*, *188*(1), 107-115.

Kremen, C., & Ostfeld, R. S. (2005). A call to ecologists: measuring, analyzing, and managing ecosystem services. Frontiers in Ecology and the Environment, 3(10), 540-548.

Le Gal, A., Robert, C., Accatino, F., Claessen, D., & Lecomte, J. (2020). Modelling the interactions between landscape structure and spatio-temporal dynamics of pest natural enemies: Implications for conservation biological control. Ecological modelling, 420, 108912.

Leponiemi, M., Freitak, D., Moreno-Torres, M., Pferschy-Wenzig, E. M., Becker-Scarpitta, A., Tiusanen, M., ... & Wirta, H. (2023). Honeybees' foraging choices for nectar and pollen revealed by DNA metabarcoding. *Scientific Reports*, *13*(1), 14753.

Letourneau, D. K., Jedlicka, J. A., Bothwell, S. G., & Moreno, C. R. (2009). Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annual Review of Ecology, Evolution and Systematics, 40, 573.

Lichtenberg, E. M., Kennedy, C. M., Kremen, C., Batary, P., Berendse, F., Bommarco, R., ... & Crowder, D. W. (2017). A global synthesis of the effects of diversified farming systems on arthropod diversity within fields and across agricultural landscapes. Global change biology, 23(11), 4946-4957.

Liere, H., Egerer, M. H., & Philpott, S. M. (2019). Environmental and spatial filtering of ladybird beetle community composition and functional traits in urban landscapes. *Journal of Urban Ecology*, *5*(1), juz014.

Lixa, A. T., Campos, J. M., Resende, A. L., Silva, J. C., Almeida, M. M., & Aguiar-Menezes, E. L. (2010). Diversidade de Coccinellidae (Coleoptera) em plantas aromáticas (Apiaceae) como sítios de sobrevivência e reprodução em sistema agroecológico. Neotropical Entomology, 39, 354-359.

Lunde, L. F., Birkemoe, T., Kauserud, H., Boddy, L., Jacobsen, R. M., Morgado, L., ... & Maurice, S. (2022). DNA metabarcoding reveals host-specific communities of arthropods residing in fungal fruit bodies. *Proceedings of the Royal Society B*, *289*(1968), 20212622.

Maalouly, M., Franck, P., Bouvier, J. C., Toubon, J. F., & Lavigne, C. (2013). Codling moth parasitism is affected by semi-natural habitats and agricultural practices at orchard and landscape levels. Agriculture, ecosystems & environment, 169, 33-42.

Macfadyen, S., Craze, P. G., Polaszek, A., van Achterberg, K., & Memmott, J. (2011). Parasitoid diversity reduces the variability in pest control services across time on farms. Proceedings of the Royal Society B: Biological Sciences, 278(1723), 3387-3394.

Madeira, F., di Lascio, A., Costantini, M. L., Rossi, L., Rösch, V., & Pons, X. (2019). Intercrop movement of heteropteran predators between alfalfa and maize examined by stable isotope analysis. *Journal of Pest Science*, *92*(2), 757-767.

Marques, R. V., Sarmento, R. A., Oliveira, A. G., Rodrigues, D. D. M., Venzon, M., Pedro-Neto, M., ... & Janssen, A. (2018). Reciprocal intraguild predation and predator coexistence. *Ecology and evolution*, *8*(14), 6952-6964.

Martínez‐Núñez, C., Manzaneda, A. J., Lendínez, S., Pérez, A. J., Ruiz‐Valenzuela, L., & Rey, P. J. (2019). Interacting effects of landscape and management on plant–solitary bee networks in olive orchards. *Functional Ecology*, *33*(12), 2316-2326.

Medeiros, M. C., Boothe, E. C., Roark, E. B., & Hamer, G. L. (2017). Dispersal of male and female Culex quinquefasciatus and Aedes albopictus mosquitoes using stable isotope enrichment. *PLoS neglected tropical diseases*, *11*(1), e0005347.

Michaud, J. P. (2012). Coccinellids in biological control. Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). John Wiley & Sons, Chichester, 488-519.

Miller‐ter Kuile, A., Apigo, A., Bui, A., DiFiore, B., Forbes, E. S., Lee, M., ... & Young, H. (2022). Predator–prey interactions of terrestrial invertebrates are determined by predator body size and species identity. *Ecology*, *103*(5), e3634.

Mollot, G., Duyck, P. F., Lefeuvre, P., Lescourret, F., Martin, J. F., Piry, S., ... & Tixier, P. (2014). Cover cropping alters the diet of arthropods in a banana plantation: a metabarcoding approach. *PloS one*, *9*(4), e93740.

Morales‐Hojas, R. (2017). Molecular ecology of insect pests of agricultural importance: the case of aphids. *Ecological Entomology*, *42*, 18-27.

Nelson, G. C. (2005). Drivers of ecosystem change: summary chapter. Ecosystems.

Paetzold, A., Schubert, C. J., & Tockner, K. (2005). Aquatic terrestrial linkages along a braided-river: riparian arthropods feeding on aquatic insects. *Ecosystems*, *8*(7), 748-759.

Pardee, G. L., Ballare, K. M., Neff, J. L., Do, L. Q., Ojeda, D., Bienenstock, E. J., ... & Jha, S. (2023). Local and landscape factors influence plant-pollinator networks and bee foraging behavior across an urban corridor. *Land*, *12*(2), 362.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology, 83(3), 703-718.

Power, A. G. (2010). Ecosystem services and agriculture: tradeoffs and synergies. Philosophical transactions of the royal society B: biological sciences, 365(1554), 2959-2971.

Quinby, B. M., Creighton, J. C., Flaherty, E. A., 2020. Stable isotope ecology in insects: a review. Ecol. Entomol. 45, 1231–1246.

Rosado, M. C., de Araújo, G. J., Pallini, A., & Venzon, M. (2021). Cover crop intercropping increases biological control in coffee crops. Biological Control, 160, 104675.

Saldanha, A. V., Gontijo, L. M., Carvalho, R. M., Vasconcelos, C. J., Corrêa, A. S., & Gandra, R. L. (2019). Companion planting enhances pest suppression despite reducing parasitoid emergence. Basic and Applied Ecology, 41, 45-55.

Schmidt, N. M., Mosbacher, J. B., Eitzinger, B., Vesterinen, E. J., & Roslin, T. (2018). High resistance towards herbivore-induced habitat change in a high Arctic arthropod community. *Biology Letters*, *14*(5), 20180054.

Seastedt, T. R., Hobbs, R. J., & Suding, K. N. (2008). Management of novel ecosystems: are novel approaches required?. Frontiers in Ecology and the Environment, 6(10), 547-553.

Slusher, E. K., Cottrell, T., Gariepy, T., Acebes-Doria, A., Querejeta Coma, M., Toledo, P. F., & Schmidt, J. M. (2024). A molecular approach to unravel trophic interactions between parasitoids and hyperparasitoids associated with pecan aphids. *Journal of Insect Science*, *24*(4), 5.

Soares, A. O., Haelewaters, D., Ameixa, O. M., Borges, I., Brown, P. M., Cardoso, P., ... & Losey, J. E. (2022). A roadmap for ladybird conservation and recovery. *Conservation Biology*, *37*(1), e13965.

Syahidah, T., Rizali, A., Prasetyo, L. B., & Buchori, D. (2021). Composition of tropical agricultural landscape alters the structure of host-parasitoid food webs. *Heliyon*, *7*(7).

Togni, P. H. B., Venzon, M., Lagôa, A. C. G., da Silva, A. C., Assunção, R. M., Rodrigues, C. A., ... & Profa, I. F. (2021). Interações entre escalas espaciais no controle biológico conservativo: da paisagem ao cultivo. Controle alternativo de pragas e doenças: opção ou necessidade?, 66.

Togni, P. H., Venzon, M., Muniz, C. A., Martins, E. F., Pallini, A., & Sujii, E. R. (2016). Mechanisms underlying the innate attraction of an aphidophagous coccinellid to coriander plants: Implications for conservation biological control. Biological Control, 92, 77-84.

Togni, P. H., Venzon, M., Souza, L. M., Santos, J. P., & Sujii, E. R. (2019). Biodiversity provides whitefly biological control based on farm management. Journal of Pest Science, 92(2), 393-403.

Tuck, S. L., Winqvist, C., Mota, F., Ahnström, J., Turnbull, L. A., & Bengtsson, J. (2014). Land-use intensity and the effects of organic farming on biodiversity: a hierarchical meta-analysis. Journal of applied ecology, 51(3), 746-755.

Tylianakis, J. M., & Morris, R. J. (2017). Ecological networks across environmental gradients. Annual Review of Ecology, Evolution, and Systematics, 48, 25-48.

Tylianakis, J. M., T. Tscharntke, and O. T. Lewis. 2007. “Habitat Modification Alters the Structure of Tropical Host–Parasitoid Food Webs.” *Nature* 445(7124): 202–205.

Valdovinos, F. S. (2019). Mutualistic networks: moving closer to a predictive theory. Ecology letters, 22(9), 1517-1534.

Vázquez, D. P., Blüthgen, N., Cagnolo, L., & Chacoff, N. P. (2009). Uniting pattern and process in plant–animal mutualistic networks: a review. Annals of botany, 103(9), 1445-1457.

Venzon, M., Amaral, D. S. S. L., Togni, P. H. B., & Chiguachi, J. A. M. (2019a). Interactions of natural enemies with non-cultivated plants. In Natural Enemies of Insect Pests in Neotropical Agroecosystems (pp. 15-26). Springer, Cham.

Venzon, M., Togni, P. H. B., Chiguachi, J. A. M., Pantoja, G. M., da Silva Brito, E. A., & Sujii, E. R. (2019b). Agrobiodiversidade como estratégia de manejo de pragas. Informe Agropecuário, Belo Horizonte, 40(305), 21-29.

Venzon, M., Togni, P.H.B., Sujii, E.R. (2021) Controle biológico conservativo. In: Parra, J.R.P., Pinto, A.S., Oliveira, R.S., Diniz, A.J.F. (Eds.) Controle biológico com parasitoides e predadores na agricultura Brasileira. São Paulo: FEALQ. pp. 99-126.

Woltz, M. J., & Landis, D. A. (2014). *Coccinellid response to landscape composition and configuration. Agric Entomol 16: 341–349.*

Wood, J. R., Holdaway, R. J., Orwin, K. H., Morse, C., Bonner, K. I., Davis, C., ... & Dickie, I. A. (2017). No single driver of biodiversity: divergent responses of multiple taxa across land use types. *Ecosphere*, *8*(11), e01997.

Xia, P. L., Wang, X. F., Feng, Y., Huang, Y., Ji, F., Cui, W. X., & Qiao, B. M. (2024). Effect of pollen consumption on development and intraguild predation of two predatory Coccinellidae. *Journal of Asia-Pacific Entomology*, *27*(4), 102320.

Yang, F., Liu, B., Zhu, Y., Wyckhuys, K. A., van der Werf, W., & Lu, Y. (2021). Species diversity and food web structure jointly shape natural biological control in agricultural landscapes. Communications biology, 4(1), 1-11.

Yang, L., Xu, L., Liu, B., Zhang, Q., Pan, Y., Li, Q., ... & Lu, Y. (2019). Non-crop habitats promote the abundance of predatory ladybeetles in maize fields in the agricultural landscape of northern China. *Agriculture, Ecosystems & Environment*, *277*, 44-52.

CHAPTER 1

Life-stage niche partitioning and functional strategies promote predatory coccinellids' co-occurrence

*This chapter is based on a manuscript submitted to the journal *Oecologia*, which has been revised to address comments following a request for major revisions.

Abstract

Species coexistence is shaped by how individuals share limiting resources such as space, food, and shelter. Theory predicts that niche differentiation promotes coexistence, depending on habitat characteristics, behavioral traits, and the intensity of competition. However, niche partitioning may vary across life stages, as ontogenetic shifts alter habitat use and species interactions. We investigated habitat segregation, niche overlap, and the role of non-crop vegetation in promoting coccinellid coexistence across life stages through semi-controlled and field experiments in 42 sampling sites. Species showed consistent differences in habitat and microhabitat use across developmental stages, influenced by innate behaviors and plastic responses to interspecific interactions. Superior competitors (*Hippodamia convergens* and *Harmonia axyridis*) generally dominated prey-rich crop areas during egg, larval, and adult stages, but shifted to sheltered sites outside the plants during pupation. In contrast, *Eriopis connexa* consistently used soil microhabitats throughout its life cycle, reducing niche overlap with other species. *Cycloneda sanguinea*, a competitively inferior species, persisted by exploiting non-crop plants, which increased spatial heterogeneity and resource availability. Non-crop vegetation thus promoted coexistence by enabling spatial segregation and reducing presumed competitive asymmetries across life stages. These patterns highlight how the strength of species interactions and spatial partitioning changes ontogenetically, reflecting both behavioral flexibility and the influence of habitat features. We propose a plastic functional classification of species based on their behavioral responses to competition-risk scenarios across life stages, ranging from risk-tolerant generalists to conditional risk-avoiders and niche-fidelity strategists, that could be broadened and adapted to other study systems.

Keywords: Biodiversity · competition · density-dependence ·generalist predators · non-crop plants

Introduction

Understanding the influence of species interactions on community assembly has been a crucial goal in community ecology, given its central role in shaping community structure, diversity, and function (Paine 1966, Levine et al. 2017). However, the outcome of interactions on insect communities, for example, are influenced by life history traits of interacting species (Lancaster et al. 2017), predation risk (Levine et al. 2017), habitat or microhabitat use throughout species life stages (Ingels & De Clercq 2011, Da Silva et al. 2022), temporal variation in resource availability, including seasonality and bi- or multivoltinism, (Schoener 1972, Hood et al. 2021), and habitat structure (Pringle et al. 2019). Spatially heterogeneous environments shift the competitive abilities of holometabolous insects across their life stages, as they can exploit different microsites for foraging, oviposition, and pupation (Amarasekare 2003a).

In contrast, in spatially homogeneous environments, coexistence of competing species is possible if differences in species traits lead to niche differentiation (Amarasekare 2003a). In both scenarios, niche partitioning may stabilize coexistence when intraspecific competition outweighs interspecific competition, particularly when interspecific differences influence the latter in competitive ability, colonization capacity, and life-history trade-offs (Rocca et al. 2017). Such trade-offs are particularly relevant in guilds like aphidophagous coccinellids, where larval and adult stages rely mostly on prey (Sloggett, 2008), females may compete for suitable oviposition sites, and individuals need to seek refuges for pupation (Hodek et al. 2012). As aphids exhibit an aggregated distribution, different species simultaneously exploit the same resources, and intense interspecific competition may arise (Hodek et al. 2012), thereby affecting their community composition and structure (e.g., Bahlai et al. 2015).

However, several traits have evolved in this guild to reduce interspecific competition, affecting how they interact with habitat features through their life cycle (Evans, 2003). Adults can assess patch quality using visual cues, herbivore-induced plant volatiles (Yi et al. 2023), plant constitutive volatiles (Togni et al. 2016), prey pheromones (Tapia et al. 2010), and con- and heterospecific semiochemicals (Hemptinne et al. 2000), including larval tracks (Seagraves,

2009) and oviposition deterring pheromones (Evans, 2003, Soares et al. 2025). When aphid densities are high, females select optimal distances for ovipositing based on the risk of egg predation close to aphid colonies and the distance that prevents offspring starvation. This decision reflects the competition for ephemeral and aggregated resources and may be shaped by both active search and innate preferences (Evans, 2003, Hodek et al. 2012, Togni et al. 2016, Soares et al. 2025).

Another important coccinellid behavior is the distribution of pupae across microsites, which may reflect competitive interactions during the larval stage. Larvae are less mobile than adults and frequently engage in intraguild predation and cannibalism (Lucas et al. 2000). The outcomes of these interactions are influenced by the size of the individuals (Yasuda et al. 2004), their behavior (Sato et al. 2009), competitive ability, such as aggressiveness (Yasuda et al. 2001), and the availability of spatial refuges (da Silva et al. 2022). During the pupal stage, individuals are immobile and highly exposed to natural enemies, particularly parasitoids (Togni et al. 2015, Paula et al. 2021). As a result, the availability of safe pupation sites may become a critical resource at this stage. Although pupae aggregation associated with sunny surface has been observed (Sakuratani and Nakamura, 1997), it has not been reported in tropical regions, where the exposure to solar radiation and high temperatures may pose a threat to pupae survival. Also, the aggregation of adults associated with their aposematic color was observed, especially in overwintering and hibernation sites (Wheeler et al. 2014, Honek et al. 2007), but not in tropical environments where overwintering and hibernation are not recorded for this group.

Coccinellid species usually coexist in the same habitat by modulating their spatial distribution within the plant (Rocca et al. 2022), or the inferior competitor may be displaced (Martins et al. 2009). Non-crop plants, typically native species that grow spontaneously between and around crops, can provide pollen, nectar, alternative prey, and shelter to aphidophagous coccinellids (Amaral et al. 2013, Venzon et al. 2019, Sicsú et al. 2020). While it is well accepted that the coexistence of competing species requires some degree of niche differentiation (Amarasekare 2003a), it remains unclear whether the spatial distribution of coccinellids throughout life stages is primarily driven by innate responses to environmental cues or shaped by species interactions across their life stages.

We hypothesized that the use of surrounding non-crop plants (habitat scale) and the spatial segregation within microhabitats across life stages allow both superior and inferior competitors to co-occur in the same area. The use of non-crop plants and spatial segregation of species throughout their life stages is likely driven by competitive interactions among species rather than by an innate response. To test these hypotheses, we evaluated how habitat and microhabitat use and niche overlap of aphidophagous coccinellids change throughout their life stages. Specifically, we evaluated: a) how different life stages of coccinellid species segregate their habitat and microhabitat use; b) the role of non-crop plants in mediating coccinellid species co-occurrence; c) whether the niche overlap of coccinellid species changes across life stages.

Material and methods

Insect rearing for cage experiments

We first set a cage experiment in an experimental field (see below) to evaluate microhabitat use by coccinellid species at distinct life stages when no heterospecific competitors were present. We used only coccinellids reared in the laboratory for this experiment to obtain well-fed individuals of different species with similar ages, sizes, and environmental conditions. Field-collected adults of *Cycloneda sanguinea*., *Eriopis connexa*, *Harmonia axyridis*, and *Hippodamia convergens* (Coleoptera: Coccinellidae) were reared in the laboratory (25 ± 2 ºC, 70 ± 15% r.h., and 12 h of light) and provided with aphid *ad libitum* (*Myzus persicae* and *Lipaphis pseudobrassicae;* Hemiptera: Aphididae), dilute honey, and water. These aphid species are common in organic crops in the region (Paula et al. 2021). We formed couples of these species and kept them in individual plastic pots (500 ml) with food. After egg hatching, larvae were transferred to individual plastic pots (250 ml) and fed with the same diet as adults until pupation or use in the experiments.

We used 36 h molted fourth-instar larvae to evaluate microhabitat selection for pupation and recently mated adults to assess oviposition microhabitat selection. To ensure mating, recently hatched adults were kept individually and well fed for at least seven days to increase the likelihood of successful mating. Forty-eight hours before setting up the experiment in the field, we formed couples and observed them until mating began. Couples were monitored until the male exhibited a body shaking behavior, which typically indicates sperm transfer and successful mating (Omkar and Srivastava 2002, Zeni et al. 2024).

Cage experiment setup in the experimental field

The cage experiment was conducted between August and September 2019 at the Biology Experimental Station of the University of Brasília (15° 44' 09.8" S, 47° 53' 00.3" W), Federal District, Brazil. The average annual precipitation is 1,500 mm, and the driest months are between May and September. The climate is type Aw according to the Köppen-Geiger classification (dry winters and hot humid summers) (Alvares et al. 2013). We conducted experiments during the driest period of the year, when relative humidity can reach less than 15%, and coccinellids and aphids are most abundant in the field.

Collards (var. *acephala*) were cultivated in an experimental area covering 450 m^2 (10 m × 45 m), containing 30 plant rows with 10 plants per row (n = 300 plants). We kept the soil bare throughout the experiment by manually weeding it when necessary. Plants were spaced 0.5 m within the row and 1.5 m between plant rows. We obtained the seedlings from commercial growers. All plants were 50 days old after seed emergence. The seedlings were manually transplanted to the field one month before the experiment began to obtain uniformly sized plants for the experiment. Soil preparation and fertilization were carried out according to local technical recommendations. Plants were irrigated using a drip system.

Between 15 and 20 days before the experiments, we infested the collards with the aphids *Myzus persicae* or *Lipaphis pseudobrassicae* (Hemiptera: Aphididae) collected in the field. We used these species because all the coccinellids we evaluated are known to feed on these aphids (Andow et al. 2023). We removed weeds, other aphid species, and other invertebrates as much as we could one day before the experiment began. To do that, we carefully inspected the plants and the soil to collect the invertebrates we found, although we could not ensure that the plant was completely clean. After that, we covered the plants with voile fabric cages (1 m × 1 m) (Fig. S2). We kept between 150 and 200 aphids (mixed infestation of *M. persicae* and *L. pseudobrassicae*) per leaf to ensure that prey was not a limiting resource. The number of aphids per leaf was counted on alternate days in all previously infested plants until aphid populations reached the required densities for the experiments.

Pupation and oviposition in the cage experiment

To assess microhabitat selection for pupation by coccinellid species, we released three to six fourth-instar larvae (depending on larvae availability) of the same species into each cage with a fine brush. These densities were selected based on pilot experiments, which showed that larvae presented similar behaviors in these densities, due to plant architecture that reduced larvae encounters within the plant. The larvae were placed directly on collard leaves, near aphid colonies, but on different leaves to prevent cannibalism or other competitive interactions. To evaluate oviposition microhabitat selection, a single mated female (see above) of each species was released in separate cages. They were placed on the leaves, near aphids, after mating in the laboratory. We set up 10 cages containing coccinellid larvae of each species, totaling 170 individuals: 44 *C. sanguinea*, 46 *E. connexa*, 40 *H. axyridis*, and 40 *H. convergens*. For the adult stage, we established 20 cages for *C. sanguinea*, 23 for *E. connexa*, 22 for *H. axyridis*, and 23 for *H. convergens*, totaling 88 adults observed throughout the experiment.

Each cage was observed daily (once a day) for three days. During the observations, we carefully inspected the cages to record the locations of egg clusters (i.e., clusters of five or more eggs) and pupae. The egg clusters (hereafter referred to as eggs) and pupae were carefully removed immediately after being recorded to document their exact locations. If eggs or pupae were found directly on the soil surface, at the plant base touching the soil, or on the cage basis in contact with the soil, these locations were categorized as “soil”. For eggs and pupae found on the collards, we divided the plant into three equal sections—lower, middle, and upper thirds—and recorded their positions accordingly. We also recorded the eggs and pupae on the cage walls.

On the final day of observation, we removed and thoroughly inspected the cages, including their edges and buried parts, for hidden eggs or pupae. We also examined soil cracks and dead leaves. The collards were then removed, and all plant parts were meticulously inspected. We also ensured that all leaves had aphids during the observations to guarantee that larvae and adults had sufficient prey throughout the experiment and were not foraging for limited food instead of selecting pupation and oviposition sites.

Field sampling of coccinellids in organic farms

We conducted this study on 42 organic farms cropping brassicas (mostly collards) in the Brazilian Federal District (15° 46' 13" S, and 47° 44' 46" W) between 2020 and 2023, always from August to October (end of the dry season). Each farm was sampled once each year. These farms belonged to smallholder farmers, averaging 13.4 ± 7.1 ha (mean ± SE), which falls within the range of organic farm sizes in our study region (4–70 ha). All sampled farms were at least 2 km apart from one another. All farms were certified as organic for a minimum of five years. They are required to follow Brazilian legislation for organic farming when carrying out practices such as pest and weed management, disease control, and soil fertilization, eliminating synthetic products from farm management (Togni et al. 2019).

The farms also featured vegetated field margins, which were usually used as living mulch. Although there were differences among farms, these non-crop areas exhibited diverse plant compositions and comparable structural, compositional, and temporal resources. Non-crop plants were defined as those located outside the cropping area and not intentionally planted by farmers. Most were native ruderal species.

To evaluate how the coccinellids *C. sanguinea*, *E. connexa*, *H. axyridis*, and *H. convergens* partition their habitats and microhabitats in the field, we randomly sampled 1 m × 1 m quadrats in both the crop area and adjacent non-crop plant strips on each farm (n = 5 quadrats per habitat per farm). For non-crop plants, we sampled plant strips up to 5 m from the cropping area, a distance selected because farms had non-crop plant strips of varying sizes (5–10 m). At least two well-trained observers carefully inspected each quadrat, and all coccinellid life stages were manually collected. Sampling continued until no additional coccinellids were detected, with search times varying from 10 to 20 min, depending on vegetation cover within each plot.

We recorded species, life stage (egg, larva, pupa, or adult), habitat type (crop or non-crop area), and specific microhabitat location within the crop area. Microhabitats were classified in the cage experiments: lower, middle, and upper plant thirds, and soil. In this experiment, we also recorded the life stages of coccinellids found in non-crop plants. Both mobile (larvae and adults) and immobile (eggs and pupae) stages were collected and transported to the laboratory for identification. Immobile individuals that could not be identified to the species level were counted and used only for analyses that did not require species identification. For eggs, we followed the same approach as in the cage experiments, considering only egg clusters with five or more eggs clustered in the same place (hereafter referred to as eggs). We assessed predator abundance by summing the number of individuals of each species and life stage (adults and larvae) in all quadrats inside and outside the crop area across all microhabitats, and then calculating the abundance for each farm. We also counted the number of aphids on four plants within each quadrat in the cultivated area (n = 40 per farm).

The number of aphids was counted on three leaves of each plant. In non-crop plants, we counted the frequency of plants with aphids, as aphids tended to jump off these plants during sampling. Aphid samples were taken to the laboratory for identification.

Statistical analyses

To investigate whether coccinellid species differed in their use of habitat and microhabitat for oviposition and pupation sites in the cage experiments, we fitted two multinomial logistic regression models using the function *multinom* from the *nnet* package in R (Ripley et al. 2016). This type of model requires one outcome category—arbitrarily chosen, though typically the largest or a control group—to be set as the reference, with its regression coefficients fixed at zero to enable comparisons across the remaining categories (Ripley et al. 2016).

In the first model, the response variable was the microhabitat selected by each species for oviposition, and in the second model, the response variable was the microhabitat selected for pupation. In both models, the explanatory variable was predator species identity, with cages set as the reference category for sites, and *H. convergens* as the reference for species. We compared the models using the *anova* function to fit an Analysis of Deviance (ANODEV) with a chi-squared test, contrasting the null model (without predictors) with the full model (including predator species identity) to assess whether the inclusion of predator species identity significantly improved the model's ability to explain variation in the response variable.

To investigate whether coccinellid species differed in their use of habitat and microhabitat in the field experiments, we fitted a series of multinomial logistic regression models. In all models, the response variable was habitat and microhabitat used by coccinellids, while the main explanatory variable was predator species identity. We used non-crop plants as the reference category for sites and *H. convergens* for species in all models. Specifically, we tested the following models: a null model without predictors (model 1); a model including only coccinellid species (model 2); a model with additive effects of coccinellid species and mean coccinellid abundance per farm (model 3); a model including coccinellid species and its interaction with mean coccinellid abundance (model 4).

We also evaluated models assessing the potential effect of life stage: one with additive effects of coccinellid species and life stage (model 5) and another including their interaction (model 6). Additionally, we tested a model incorporating coccinellid species and its interaction with mean coccinellid abundance, along with the additive effects of predator species and life stage (model 7). We included the interaction between predator species and mean coccinellid abundance (only larvae and adults) per farm to account for potential context-dependent effects on habitat and microhabitat selection. Model comparisons were conducted as previously described, and we selected model 4 because it had the lowest AICc and a simpler structure. For detailed model comparisons, see Table S1.

We used the Pianka Niche Overlap Index to assess pairwise niche overlap among species. The index is bounded between 0 (total separation) and 1 (complete overlap). We selected the Pianka index because it is a widely used metric for assessing mutual similarity in resource use among species. Although it is a symmetrical index, we use it here because it does not imply any a priori inference of directional dependencies, direct competition, or exclusion (Pianka, 1974).

For the cage experiment, we calculated overlap separately for each life stage and by summing the total counts of eggs and pupae per species. For the field experiment, we followed the same approach, also summing the total counts of larvae and adults. We combined life stages to evaluate interspecific differences for immobile and mobile stages, representing each species as a single ecological unit. For the field data on each farm, we calculated the indices only for larvae and adults, as these were the only life stages that could be identified across all farms.

We assessed differences in niche overlap among species pairs in the cage experiment using the Pianka index in a generalized linear mixed model (GLMM) with a beta distribution, implemented via the *glmmTMB* package (Brooks et al. 2017). To avoid exact zeros (Smithson and Verkuilen, 2006), we adjusted index values by adding a small constant (ϵ = 1e-6). We treated the adjusted Pianka indices as the response variable, species pairs as fixed effects, and cages as random effects. For the field data, we applied the same approach, using farms as random effects. We tested group differences using model contrasts with the *emmeans* function and performed multiple comparisons with a Tukey post hoc test on the estimated marginal means and evaluated model assumptions using residual diagnostics from the *DHARMa* package (Hartig and Lohse, 2020).

We assessed whether niche overlap among species and life stages differed from random expectations by comparing the observed mean Pianka index for species pairs with expected values from simulated null models. To reduce algorithmic bias, we performed 10,000 Monte

Carlo permutations (Lehsten and Harmand, 2006). We generated null assemblages for both the cage experiment (including all life stages combined, only eggs, or only pupae) and the field data (all stages combined, or only eggs, larvae, pupae, or adults). We used Lawlor's RA3 algorithm to generate these randomized matrices, which assumes all species have equal probabilities of occupying any site, given their niche breadth (Lawlor 1980, Lehsten and Harmand 2006).

We randomized species-by-site associations in the resource-use matrix by shuffling species presence across sites (columns) while preserving each species' total occurrences (rows) (Lawlor, 1980). This approach maintained species-specific niche breadth (i.e., number of sites occupied) but randomized the specific sites used, disrupting any existing guild structure (Lawlor 1980). Although RA3 does not preserve site-level richness, we aimed to introduce some ecological realism to the model by preserving niche breadth (Gotelli and Ulrich, 2012). We applied no additional constraints on species identities or the total number of sites. We performed all analyses in R (R Core Team, 2025).

Results

Microhabitat use and niche overlap in the cage experiment

The likelihood of microhabitat selection by adult coccinellids for oviposition varied among species ($\chi^2 = 106.5$; d.f. = 12; $p < 0.001$). The chances of *C. sanguinea* laying eggs in the middle third were greater than on the soil or cage. In contrast, *E. connexa* showed a higher probability of ovipositing on the soil compared to any other microhabitat. For *H. axyridis*, the likelihood of oviposition on the middle and lower thirds exceeded that on the cage and soil. In the case of *H. convergens*, the probability of laying eggs on the soil was higher than on the upper and middle thirds, as well as the cage; additionally, oviposition on the lower third was more likely than on the cage (Fig. 1).

Cycloneda sanguinea exhibited a greater tendency to oviposit on the middle third than *H. convergens*. On the lower third, *H. axyridis* had a higher probability of oviposition than *E. connexa*, whereas on the soil, *E. connexa* displayed the highest likelihood of laying eggs among all species. Still referring to the soil, *H. convergens* showed a higher probability of oviposition than *C. sanguinea* and *H. axyridis*, but still lower than *E. connexa* (Fig. 1).

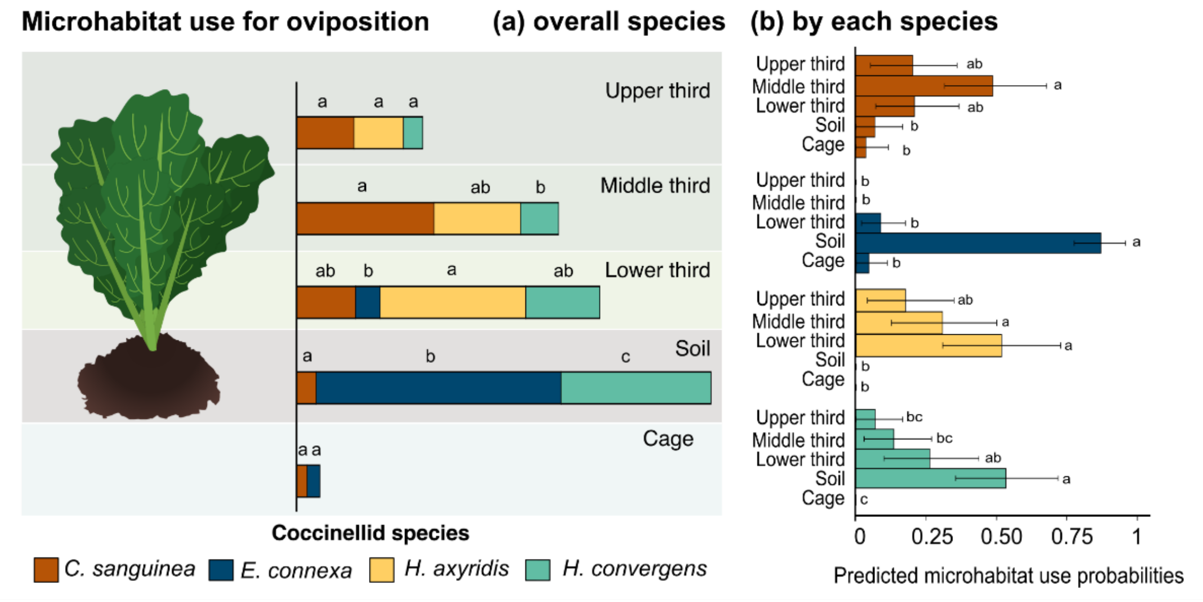

Fig. 1 Predicted probabilities of microhabitat use for oviposition by overall coccinellid species (a) and by each species (b). Error bars represent 95% confidence intervals based on 1,000 bootstrap resamples. Different letters indicate significant differences among species within the same microhabitat (a) and by each species across different microhabitats (b).

The probability of microhabitat selection by coccinellid larvae for pupation also varied among species ($\chi^2 = 69.5$; d.f. = 12; $p < 0.001$). *Cycloneda sanguinea* and *H. axyridis* showed similar pupation probabilities across all microhabitats. *Eriopis connexa* had a higher likelihood of pupating on the soil compared to the other microhabitats. For *H. convergens*, the probability of pupation on the cage walls was greater than in any other microhabitat (Fig. 2). On the soil, *E. connexa* displayed a higher pupation probability than *H. axyridis*. Conversely, on the cage, *H. convergens* exhibited a greater likelihood of pupating than both *E. connexa* and *H. axyridis* (Fig. 2).

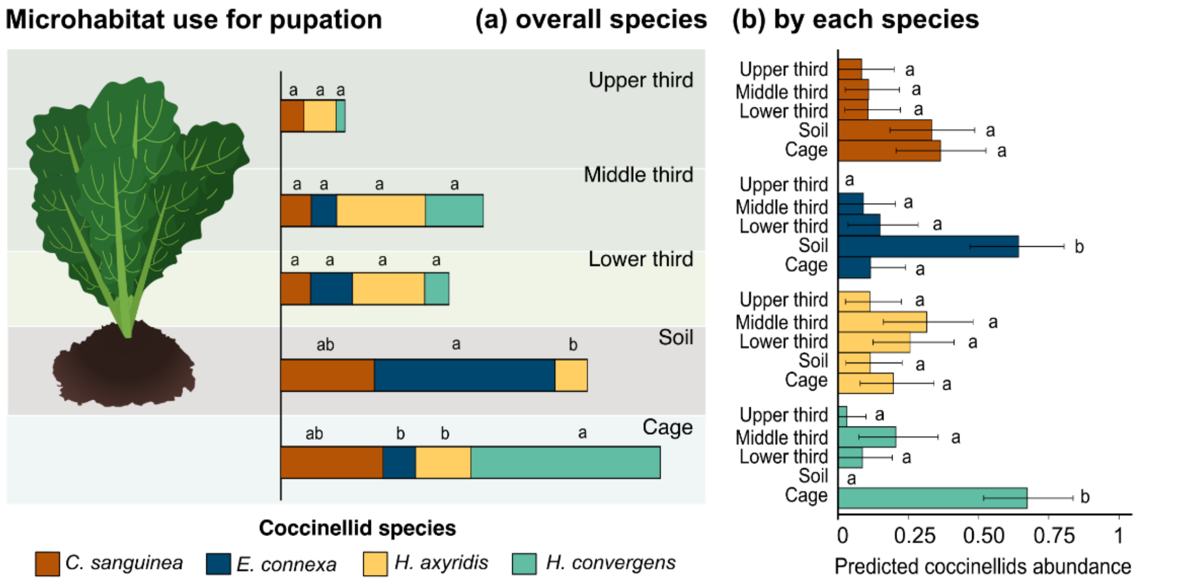

Fig. 2 Predicted probabilities of microhabitat use for pupation by overall coccinellid species (a) and by each species (b), considering 95% confidence intervals based on 1,000 bootstrap resamples. Different letters indicate significant differences among species within the same microhabitat (a) and by each species across different microhabitats (b).

The species niche highly overlapped (> 60% for eggs + pupae) among species (χ^2 = 15.5; d.f. = 5; p < 0.001). Nevertheless, the overall niche overlap for coccinellid's eggs and pupae together was not statistically different from the null models (Table 1), indicating that the niche overlap we observed can be assigned to chance. The pairwise comparisons showed that niche overlap between species included the 60% threshold (i.e., high niche overlap), except for *E. connexa* and *H. axyridis* (< 40%) (Fig. 3).

Table 1 Results from the null models for coccinellid species niche overlap in the cage and field experiments in different life stages. The observed index corresponds to the average Pianka index calculated for all individuals in the cage and field experiments data. The expected mean and variance values were derived using the RA3 algorithm. Bold values indicate statistically significant results.

Data origin	Life stage	Observed index	Expected (RA3) (10.000 iterations)				
			Mean	variance	Lower-tail P = (obs< exp)	Upper-tail P = (obs>exp)	Standardized Effect Size (SES)
Cage experiment	All	0.654	0.667	0.003	0.462	0.538	-0.245
	Eggs	0.490	0.480	0.010	0.611	0.390	0.107
	Pupae	0.611	0.581	0.006	0.707	0.293	0.378
Field experiment	All	0.836	0.437	0.013	0.992	**0.009**	3.525
	Eggs	0.289	0.372	0.015	0.278	0.722	-0.668
	Larvae	0.781	0.402	0.014	0.989	**0.011**	3.196
	Pupae	0.853	0.248	0.024	0.996	**0.016**	3.938
	Adults	0.758	0.366	0.016	0.990	**0.010**	3.130

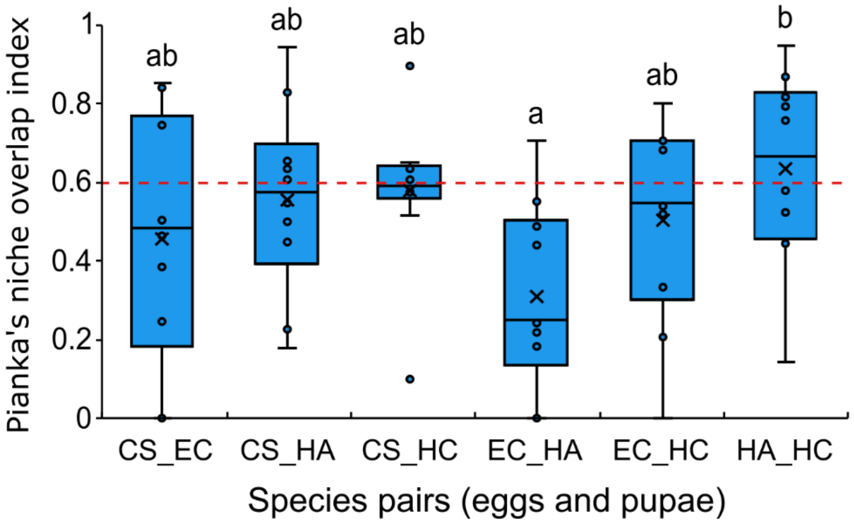

Fig. 3 Pianka’s niche overlap index between coccinellid species pairs (eggs and pupae) using the lower, middle, and upper thirds of brassicas crops and outside brassica plants in the cage

experiment. Different letters indicate significant differences based on model contrast analysis ($p < 0.05$). CS = *Cycloneda sanguinea*, EC = *Eriopis connexa*, HA = *Harmonia axyridis*, HC = *Hippodamia converges*. The horizontal line within the boxplot indicates the median and "x" denotes the mean. The dashed red line indicates where niche overlap reaches 60%.

Habitat and microhabitat use and niche overlap in the field

Across all farms, we recorded an average of 107.1 ± 16. 9 aphids per leaf on collards. The species observed were *B. brassicae*, *L. pseudobrassicae*, and *M. persicae*. On non-crop plants, only aphids from the genus *Uroleucon* were recorded. Although we did not estimate aphid density on non-crop plants, coccinellids were observed on plants hosting *Uroleucon* spp. at least 189 times across all farms. The mean abundance of coccinellids (larvae and adults) per farm ranged from 1.25 to 34.8 individuals per species. Considering all farms and only larvae and adults, *H. convergens* was the most abundant species (n = 1757), followed by *C. sanguinea* (n = 698), *E. connexa* (n = 616), and *H. axyridis* (n = 162). Egg abundance (including unidentified eggs) was highest on non-crop plants (n = 43), followed by the middle (n = 21), lower (n = 17), and upper (n = 5) brassica plant thirds, and soil (n = 3). Larvae were also most abundant on non-crop plants (n = 508), followed by soil (n = 90), lower (n = 44), upper (n = 25), and middle (n = 15) thirds of brassica plants. Pupae (including unidentified) were mainly found on non-crop plants (n = 39), with only one on soil and one in the middle third; none were found on the lower or upper thirds. Adults were most abundant on non-crop plants (n = 1662), followed by soil (n = 828), lower (n = 247), upper (n = 150), and middle (n = 114) thirds. A few eggs and pupae were identified to species level, including one *C. sanguinea* pupa on the soil (see Table S2 for details).

The probability of occurrence of coccinellids (larvae and adults) in habitat and microhabitat varied among species ($\chi^2 = 885.8$; d.f. = 12; $p < 0.001$), by the mean coccinellid abundance in farms ($\chi^2 = 72.9$; d.f. = 4; $p < 0.001$), and by the interaction between these variables ($\chi^2 = 52.2$; d.f. = 12; $p < 0.001$), demonstrating that species exhibit specific site preferences that was affected by the mean abundance of coccinellids. For the thirds of brassica plants, the predicted probabilities of species occurrence highly overlapped (predicted probability of occurrence below 25%), with a tendency to decrease when coccinellid abundance on farms was higher (Fig. 4abc). The probability of *E. connexa* using the soil was higher than for other species, ranging over 75% when mean coccinellid abundance in farms increased (Fig.

4d). Also on the soil, the probability of *H. convergens* presence was higher than that of *C. sanguinea* and *H. axyridis* (both reaching zero). Despite the increase in mean coccinellid abundance, the probability of *H. convergens* occurring on the soil remained constant, slightly above 25% (Fig. 4d).

The probability of species using non-crop plants varied notably. *Cycloneda sanguinea* had the highest probability (close to 100%), regardless of mean coccinellid abundance (Fig. 4e). For *H. axyridis*, this probability rose from under 50% to nearly 100% as coccinellid abundance increased. *Hippodamia convergens* also exhibited a consistent increase in the probability of using non-crop plants as coccinellid abundance increased, reaching approximately 60%. In contrast, *E. connexa* was more likely to use non-crop plants when coccinellid abundance was low (about 25% at fewer than a mean of 10 individuals), but this probability dropped sharply with increasing abundance, nearing zero (Fig. 4d).

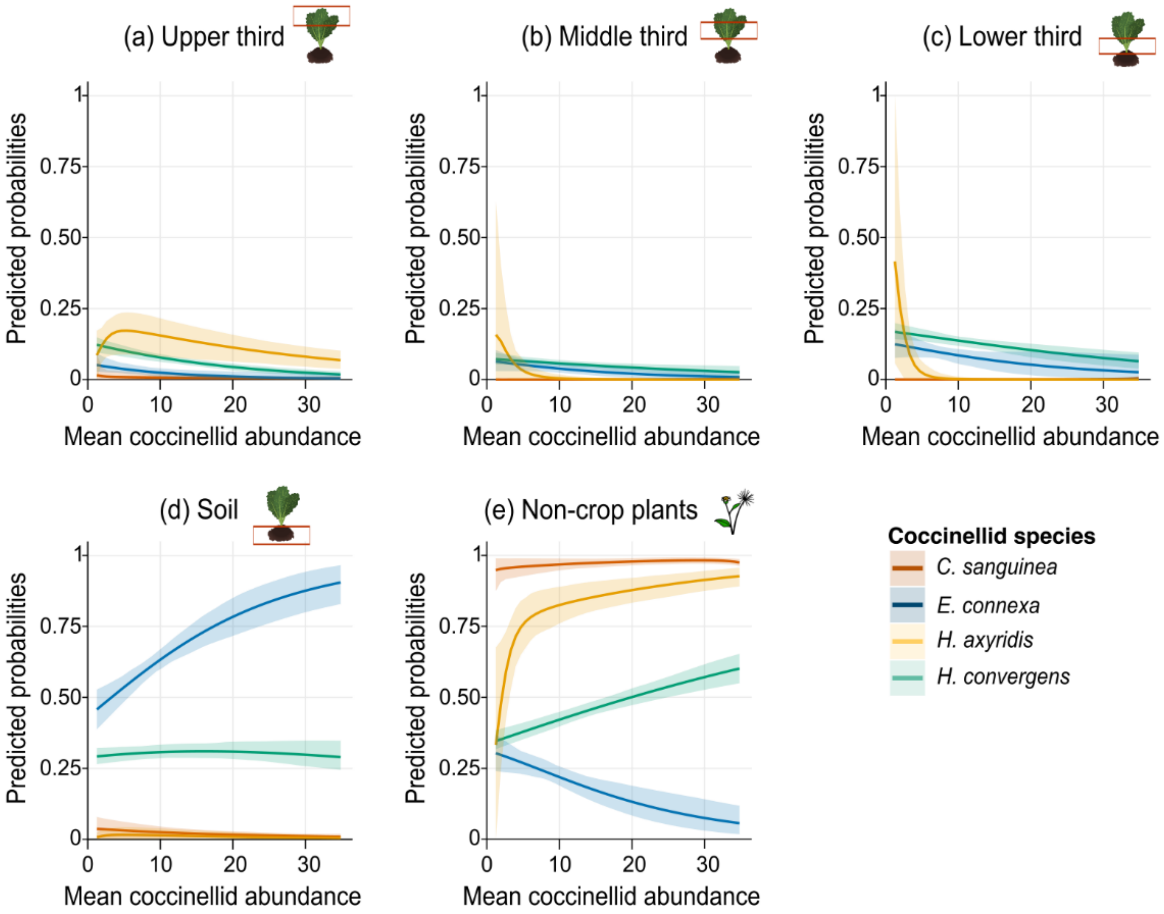

Fig. 4 Predicted probabilities of habitat and microhabitat use (upper, middle, and lower thirds; soil; and non-crop plants) by each coccinellid species at different mean coccinellid abundance per farm. Shaded areas indicate 95% bootstrap confidence intervals (1,000 resamples).

The niche overlap was higher (> 80%) between *C. sanguinea* and both *H. axyridis* and *H. convergens*, and lower (< 60%) between *C. sanguinea* and *E.* connexa, as well as between *E. connexa* and *H. axyridis*, indicating that niche overlap varied among pairs of species (χ^2 = 25.1; d.f. = 5; p < 0.001) (Fig. 5a). The niche overlap varied across life stages, with larvae showing higher overlap for *E. connexa* and *H. convergens* (79%) and lower for *E. connexa* and *C. sanguinea* (37%) (Fig. 5b). Adults had a higher overlap for *C. sanguinea* and *H. convergens* (84%) and lower for *E. connexa* and *H. axyridis* (54%) (Fig. 5c). Coccinellids niche overlap was 83% for all life stages, 28% for eggs, 78% for larvae, 85% for pupae and 75% for adults. It was statistically different from chance compared to null models, except for eggs (Table 1; Fig. S2).

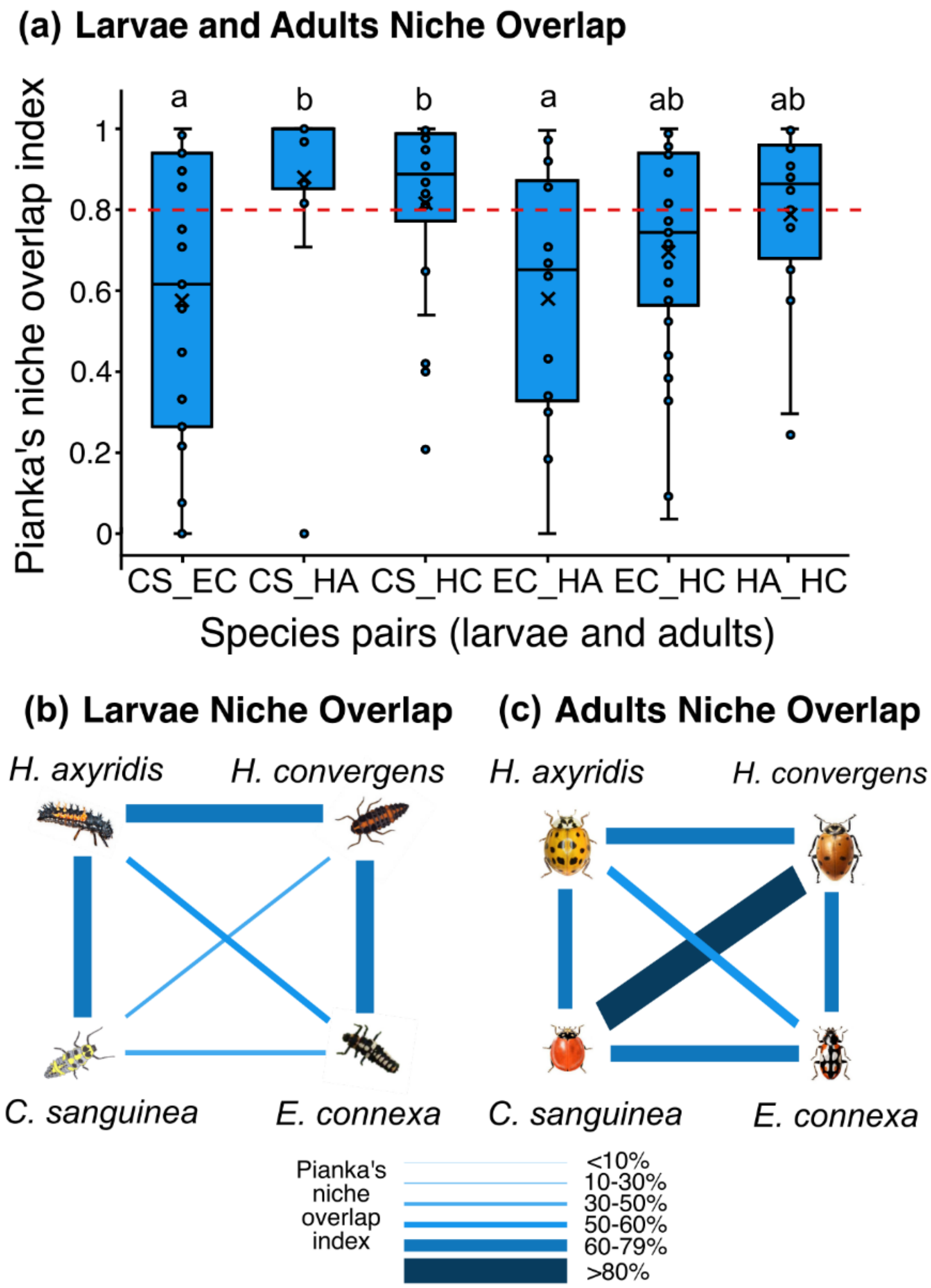

Fig. 5. a) Pianka's niche overlap index between coccinellid species pairs (larvae and adults) using the lower, middle, and upper thirds of brassica crops and the non-crop plants in the field experiments, and average Pianka's niche overlap index between larvae (b) and adults (c) species pairs. Different letter combinations indicates significance determined by model contrast analysis ($p < 0.05$). The horizontal line within the boxplot indicates the median, and the "x" the mean. Dashed red line indicates where niche overlap reaches 80%. Cs = *Cycloneda sanguinea*, Ec = *Eriopis connexa*, Ha = *Harmonia axyridis*, Hc = *Hippodamia convergens*.

Discussion

The aphidophagous coccinellids *C. sanguinea, E. connexa, H. convergens,* and *H. axyridis* exhibited distinct patterns of habitat and microhabitat use across life stages, with niche overlap varying especially during the mobile stages (larvae and adults). Species-specific traits related to space use influenced individuals' response to the increased abundance of con- and heterospecifics on farms. Non-crop plants may enable species to co-occur at the community level through shifts in niche overlap across life stages. Therefore, even in conditions of potentially strong competition, species can co-occur in spatially heterogeneous environments due to behavioral plasticity in context-dependent interactions. These behaviors include unexpected tendencies of some species to lay eggs and pupate in the soil, especially *E. connexa*.

Spatial segregation of habitat and microhabitat use across different life stages

All coccinellid species studied oviposited on collard plants, reflecting females' active search for aphid patches for their offspring, as larvae have limited mobility (Seagraves, 2009). However, oviposition behavior varied among species. While *H. axyridis* and *C. sanguinea* readily deposited eggs within each of the collard sections, *H. convergens* preferred to oviposit in the lower third of collards and on the soil. *Eriopis connexa* exhibited an unexpected tendency to lay eggs on the soil surface. Although *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) was previously reported to oviposit on the soil (Ferran, 1989), this is the first record of this behavior for *E. connexa*. These differences may reflect behavioral trade-offs involving prey access (Seagraves, 2009), predation risk (including intraguild predation) (Hodek et al. 2012), and bet-hedging strategies (Evans, 2003), influencing species-specific oviposition strategies.

Pupation site choice also varied among species, but the mechanisms underlying these changes differ from those for oviposition site selection. Pupation site selection may be more conditioned to avoid intraguild predation (Lucas et al. 2000) and reduce exposure to parasitoids (Togni et al. 2015, Paula et al. 2021). In this sense, *H. axyridis* still prefers to pupate on the plant, possibly saving energy by not moving to other areas (Ferran and Dixon 1993). Conversely, the other species employ specific strategies to minimize exposure to natural enemies (mostly pupae) or competitors (larvae, adults, and oviposition sites) (Lima and Dill 1990). *Eriopis connexa* predominantly pupates on the soil, where other coccinellid species usually do not forage, and *H. convergens* tends to avoid the plants with aphids and preferentially uses alternative sites above the soil. However, *C. sanguinea* dilutes the exposure risk to pupate by using soil and alternative sites above the soil equally. Interestingly, this is the first report of all three species pupating on the soil. Therefore, pupation site choice may be an innate behavior that has been selected in this guild in a risky environment with high exposure to natural enemies.

In field conditions, the use of plant strata by mobile stages was dependent on coccinellid abundance in the farms, possibly reflecting varying levels of competition, since both intra- and interspecific competition are density-dependent (Amarasekare 2003b). However, in our study, these effects may be restricted to the plant level or at most to the patch level. The middle third of brassica plants was used less frequently and similarly by most species. In the upper third *H. axyridis* was more frequent, particularly when coccinellid abundance was low. In contrast, in the lower third, *H. axyridis* was replaced by *H. convergens* and *E. connexa* as coccinellid abundance increased, possibly due to interference competition (Amarasekare 2003b). The increased presence of these species on the lower third and on the soil suggests spatial shifts due to heterospecific avoidance or competitive displacement (Lucas et al., 2000, Reitz and Trumble, 2002), and fine-scale microhabitat partitioning among species. In contrast, *C. sanguinea* was more closely associated with non-crop plants due to its ability to exploit prey such as *Uroleucon* spp. (Hemiptera: Aphididae) (Sicsú et al. 2020), associated with avoidance behavior or displacement by dominant species in the crop area.

On the soil surface, *E. connexa* was the only species that increased its occurrence with the increased abundance of coccinellids. This result reinforces its role as a ground-dwelling predator that opportunistically forages on plants. Probably, this species may prey on fallen aphids dislodged from the plants by other predators foraging on the plant, as demonstrated for other ground-foraging beetles (e.g., Losey and Denno, 1998). However, the use of soil as an oviposition or pupation site may be suboptimal because eggs and pupae are immobile and vulnerable to epigeal predators. This may explain why eggs on the soil were less frequent under field conditions than expected based on the cage experiment.

Conversely, *H. convergens*, despite being a superior competitor (Andow et al., 2023), became less frequent on the soil when coccinellid abundance increased, possibly outcompeted by *E. connexa*. At the same time, *C. sanguinea* and *H. axyridis* rarely used the soil. This may be due to their reliance on aphids and plant-provided food (Amaral et al. 2013), and their presumably limited ability to exploit fallen aphids in the soil, where *E. connexa* thrives. These findings highlight how microhabitat specialization and context-dependent presumed negative interactions shape spatial use in aphidophagous coccinellids.

Non-crop plants may mediate species co-occurrence at the community level

On non-crop plants, coccinellid species exhibited opposite trends to those observed in the soil. *Eriopis connexa* had the lowest probability of occurrence in these areas among all species, and its use declined with increasing coccinellid abundance. This is the opposite result for *H. convergens,* probably due to its generalist feeding habits (Michaud 2018), and flexibility in habitat use across life stages. Nevertheless, all species used non-crop plants to some extent, benefiting from habitat structural complexity, which enhances individuals' movement and favors niche partitioning among intraguild predators (da Silva et al. 2022), and provides shelter for different life stages (Togni et al. 2016).

Notably, *C. sanguinea* was consistently associated with non-crop plants irrespective of coccinellid abundance. This may be because the species either avoids, is excluded from, or does not primarily feed on the aphids on collards. Although individuals can develop on *Brevicoryne brassicae*, they prefer or are constrained to non-crop habitats due to competitive exclusion or prey specialization. Although the genus *Uroleucon* is widespread, several species are native to the Neotropical and Neartic regions, primarily found in Asteraceae and Campanulaceae plants (de Carvalho et al. 1998). In our study system, *Uroleucon* species were primarily found in the non-crop areas in *Bidens pillosa* (Asteraceae), which can have improved *C. sanguinea* fitness, whereas *B. brassicae* is a less suitable prey (Sicsú et al. 2020, Andow et al. 2023). Therefore, non-crop resources may enable native and competitively inferior species to co-occur in the community (Fonseca et al. 2017).

Harmonia axyridis, an exotic species, might not use native non-crop plant resources (aphids, pollen, and nectar) (Amaral et al. 2013), possibly relying mostly on crop aphids like *B. brassicae* (Sicsú et al. 2020). However, its increased use of non-crop plants under high coccinellid abundance suggests that superior competitors like *H. convergens* (Andow et al. 2023) displace it. However, on the non-crop plants, *C. sanguinea* can explore other resources that *H. axyridis* cannot (Amaral et al. 2013). This might explain its lower abundance in the studied farms and why this species did not successfully invade organic crops in this region (Paula et al. 2021, Andow et al. 2023).

These results point to species-specific patterns of habitat use shaped by innate preferences, prey availability, and possibly by predatory interactions (Reitz and Trumble 2002). The use of non-prey food resources (Fonseca et al. 2017, Venzon et al. 2019) and the unexpected oviposition on the soil by some species suggest a behavioral plasticity. Habitat and microhabitat use in aphidophagous coccinellids are both species- and stage-dependent but may shift in response to interspecific competition and intraguild predation on specific life stages (mostly larvae and adults), reinforcing the ecological value of behavioral plasticity (Seagraves 2009).

Coccinellids' niche overlap changes throughout their life stages

Combined, our experiments revealed that mobile stages of coccinellids (larvae and adults) exhibited greater niche overlap than immobile stages (eggs and pupae), which remained spatially segregated. Immobile stages are fixed in place until hatching/emergence. Therefore, safe sites from natural enemies may result in a higher spatial segregation to avoid natural enemy spillover due to density-dependent effects (Paula et al. 2021) and to reduce detectability by intraguild predators and competitors to avoid exclusion (Rocca et al. 2022).

The high overlap between *H. axyridis* and *C. sanguinea* suggests that such dynamics occur at the microhabitat and patch levels. In contrast, functional asymmetries appear to mediate coexistence in species that highly overlap their spatial niche. *Hippodamia convergens* exhibits higher prey capture efficiency in brassica crops (Andow et al. 2023), reinforcing its dominance along a key niche axis. However, *E. connexa* may be a more efficient ground-foraging predator than *H. convergens*, which may stabilize coexistence (Amarasekare 2003b). Similarly, while *C. sanguinea* and *H. axyridis* also overlapped with *H. convergens*, this overlap is reduced for *C. sanguinea* during the larval stage.

Our results suggest that aphidophagous coccinellid communities are structured by differences in niche overlap varying across different life stages. Although niche overlap among the immobile stages did not differ from random, overlap among mobile stages was non-random and structured, reinforcing the relevance of behavior plasticity mediating spatial partitioning. While we measured niche based on spatial distribution, niches are inherently multidimensional, and factors such as prey identity, phenology, or temporal dynamics may yield different results. Despite this limitation, our findings support previous evidence that *H. axyridis*, although a highly invasive exotic species (Martins et al. 2009, Roy et al. 2016), did not establish in the organic systems in central Brazil because other species may outcompete it due to more efficient resource use (Andow et al. 2023), especially plant-provided food (Amaral et al. 2013), associated with a high pressure of native parasitoids (Paula et al. 2021).

Conclusions

We demonstrated that aphidophagous coccinellids coexist in agroecosystems through spatial niche partitioning, which is modulated by environmental heterogeneity and life-stage differences in habitat demands. Habitat and microhabitat use varied among species and life stages, influenced by both innate behaviors and plastic responses to density-dependent effects of coccinellid abundance. We propose that the species we studied may be functionally classified based on their behavioral responses across life stages. *Hippodamia convergens* acted as a risk-tolerant species, thriving in high-density crop habitats where prey is abundant, but competition is more likely to occur when aphid densities are low. At the same time, when aphid densities are high, intraguild predation may occur due to density-dependent effects that attract more individuals to the area. Contrastingly, *C. sanguinea* acted as a risk-avoider, confined to non-crop vegetation probably due to lower interspecific competition and because it can access less abundant (mostly native) and diversified resources, at the cost of reduced access to high-prey density areas. *Eriopis connexa* acts as a niche-fidelity specialist, occupying less competitive habitats and reinforcing its soil-foraging specialization as coccinellid abundance increases, which may have reduced predation and competition over time. Notably, this species consistently foraged, oviposited, and pupated on soil, likely acting as a soil specialist. Although *H. axyridis* may be classified as a risk-tolerant species in areas where this species successfully invaded, in our study system, it behaves as a conditional risk-avoider. They thrive in suitable habitats with low interspecific competition but decline when displaced to less favorable ones, due to their strong dependence on aphids and limited resource-use flexibility in the invaded area. Those classifications could be broadened and tested for other aphidophagous coccinellids and validated for other systems. Our findings suggest that trade-offs in competitive ability, habitat use, and exposure to risk across life stages shape divergent ecological strategies of aphidophagous coccinellids and stabilize their coexistence.

References

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JL de M, Sparovek G (2013) Köppen's climate classification map for Brazil. Meteorol Z 22(6):711–728. https://doi.org/10.1127/0941-2948/2013/0507

Amaral DS, Venzon M, Duarte MVA, Sousa FF, Pallini A, Harwood JD (2013) Non-crop vegetation associated with chili pepper agroecosystems promote the abundance and survival of aphid predators. Biol Control 64(3):338–346. https://doi.org/10.1016/j.biocontrol.2012.12.006

Amarasekare P (2003a) Diversity–stability relationships in multitrophic systems: an empirical exploration. J Anim Ecol 72(5):713–724. https://doi.org/10.1046/j.1365-2656.2003.00744.x

Amarasekare P (2003b) Competitive coexistence in spatially structured environments: a synthesis. Ecol Lett 6(12):1109–1122. https://doi.org/10.1046/j.1461-0248.2003.00530.x

Andow DA, Fontes EMG, Pires CSS, Souza LM, Paula DP (2023) Invasion of the coccinellid community associated with a tropical annual agroecosystem. Ecol Entomol 48(2):174–185. https://doi.org/10.1111/een.13212

Bahlai CA, Colunga-Garcia M, Gage SH, Landis DA (2015) The role of exotic ladybeetles in the decline of native ladybeetle populations: evidence from long-term monitoring. Biol Invasions 17:1005–1024. https://doi.org/10.1007/s10530-014-0772-4

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM (2017) Modeling zero-inflated count data with glmmTMB. BioRxiv :132753. https://doi.org/10.1101/132753

Da Silva AC, Cahú RC, Cogitskei MM, Kubota KS, Sujii ER, Togni PHB (2022) Intercropping collard plants with coriander modulates behavioral interactions among aphidophagous predators by altering microhabitat structure. Biol Control 105084. https://doi.org/10.1016/j.biocontrol.2022.105084

de Carvalho RCZ, Blackman RL, Spence JM (1998) The genus *Uroleucon* Mordvilko (Insecta, Aphidoidea) in South America, with a key and descriptions of four new species. Zool J Linn Soc 123(2): 117-141. https://doi.org/10.1111/j.1096-3642.1998.tb01296.x

Evans EW (2003) Searching and reproductive behaviour of female aphidophagous ladybirds (Coleoptera: Coccinellidae): a review. Eur J Entomol 100(1):1–10. https://doi.org/10.14411/eje.2003.001

Ferran A, Dixon AFG (1993) Foraging behaviour of ladybird larvae (Coleoptera: Coccinellidae). Eur J Entomol 90(4):383–402. https://doi.org/10.14411/eje.1993.061

Ferran A, Gubanti P, Iperti G, Migeon A, Onillon J (1989) La répartition spatiale des différents stades de *Coccinella septempunctata* dans un champ de blé: variation au cours de la saison. Entomol Exp Appl 53(3):229–236. https://doi.org/10.1111/j.1570-7458.1989.tb01318.x

Fonseca MM, Lima E, Lemos F, Venzon M, Janssen A (2017) Non-crop plant to attract and conserve an aphid predator (Coleoptera: Coccinellidae) in tomato. Biol Control 115:129–134. https://doi.org/10.1016/j.biocontrol.2017.10.005

Gotelli NJ, Ulrich W (2012) Statistical challenges in null model analysis. Oikos 121(2):171–180. https://doi.org/10.1111/j.1600-0706.2011.20301.x

Hartig F, Lohse L (2020) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models

Hemptinne JL, Dixon AFG (2000) Defence, oviposition and sex: semiochemical parsimony in two species of ladybird beetles (Coleoptera: Coccinellidae)? A short review. Eur J Entomol 97(4):443–448. https://doi.org/10.14411/eje.2000.068

Hodek I, Honek A, Van Emden HF (2012) Ecology and behaviour of the ladybird beetles (Coccinellidae). John Wiley & Sons

Hood GR, Blankinship D, Doellman MM, Feder JL (2021) Temporal resource partitioning mitigates interspecific competition and promotes coexistence among insect parasites. Biol Rev 96(5): 1969-1988. https://doi.org/10.1111/brv.12735

Ingels B, De Clercq P (2011) Effect of size, extraguild prey and habitat complexity on intraguild interactions: a case study with the invasive ladybird *Harmonia axyridis* and the hoverfly *Episyrphus balteatus*. BioControl 56:871–882. https://doi.org/10.1007/s10526-011-9365-7

Yasuda, H., Kikuchi, T., Kindlmann, P., & Sato, S. (2001). Relationships between attack and escape rates, cannibalism, and intraguild predation in larvae of two predatory ladybirds. *Journal of Insect Behavior*, *14*(3), 373-384.Lancaster LT, Morrison G, Fitt RN (2017) Life history trade-offs, the intensity of competition, and coexistence in novel and evolving communities under climate change. Philos Trans R Soc B Biol Sci 372(1712):20160046. https://doi.org/10.1098/rstb.2016.0046

Lawlor LR (1980) Structure and stability in natural and randomly constructed competitive communities. The Am Nat 116(3):394–408. https://doi.org/10.1086/283634

Lehsten V, Harmand P (2006) Null models for species co-occurrence patterns: assessing bias and minimum iteration number for the sequential swap. Ecography 29(5):786–792. https://doi.org/10.1111/j.2006.0906-7590.04648.x

Levine JM, Bascompte J, Adler PB, Allesina S (2017) Beyond pairwise mechanisms of species coexistence in complex communities. Nature 546(7656):56–64. https://doi.org/10.1038/nature22898

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68(4):619–640. https://doi.org/10.1139/z90-092

Losey JE, Denno RF (1998) Positive predator–predator interactions: enhanced predation rates and synergistic suppression of aphid populations. Ecology 79(6):2143–2152. https://doi.org/10.1890/0012-9658(1998)079[2143:PPIEPR]2.0.CO;2

Lucas É, Coderre D, Brodeur J (2000) Selection of molting and pupation sites by *Coleomegilla maculata* (Coleoptera: Coccinellidae): avoidance of intraguild predation. Environ Entomol 29(3):454–459. https://doi.org/10.1603/0046-225X-29.3.454

Martins CBC, Almeida LM, Zonta-de-Carvalho RC, Castro CF, Pereira RA (2009) *Harmonia axyridis*: a threat to Brazilian Coccinellidae? Rev Bras Entomol 53(4):663–671. https://doi.org/10.1590/S0085-56262009000400018

Michaud JP (2018) Challenges to conservation biological control on the High Plains: 150 years of evolutionary rescue. Biol Control 125:65–73. https://doi.org/10.1016/j.biocontrol.2018.01.008

Michaud JP (2005) On the assessment of prey suitability in aphidophagous Coccinellidae. Eur J Entomol 102(3):385–390. https://doi.org/10.14411/eje.2005.056

Omkar, Srivastava S (2002) The reproductive behaviour of an aphidophagous ladybeetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae). Eur J Entomol 99(4):465–470. https://doi.org/10.14411/eje.2002.060

Paine RT (1966) Food web complexity and species diversity. Am Nat 100(910):65–75. https://doi.org/10.1086/282400

Paula DP, Togni PHB, Costa VA, Souza LM, Sousa AA, Tostes GM, Pires CSS, Andow DA (2021) Scrutinizing the enemy release hypothesis: population effects of parasitoids on *Harmonia axyridis* and local host coccinellids in Brazil. BioControl 66:71–82. https://doi.org/10.1007/s10526-020-10041-y

Pianka ER (1974) Niche overlap and diffuse competition. Proc Natl Acad Sci 71(5):2141–2145. https://doi.org/10.1073/pnas.71.5.2141

Pringle RM, Kartzinel TR, Palmer TM, Thurman TJ, Fox-Dobbs K, Xu CCY, Hutchinson MC, Coverdale TC, Daskin JH, Evangelista DA, Gotanda KM, Man in ’t Veld NA, Wegener JE, Kolbe JJ, Schoener TW, Spiller DA, Losos JB, Barrett RDH (2019) Predator-induced collapse of niche structure and species coexistence. Nature 570(7759):58–64. https://doi.org/10.1038/s41586-019-1264-6

R Core Team (2025) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reitz SR, Trumble JT (2002) Competitive displacement among insects and arachnids. Annu Rev Entomol 47(1):435–465. https://doi.org/10.1146/annurev.ento.47.091201.145227

Ripley B, Venables W, Ripley MB (2016) Package “nnet”

Rocca M, Díaz Lucas MF, Greco NM (2022) Effect of spatiotemporal association and trophic interactions between Aphidophagous Coccinellids Toward Aphid Control. Environ Entomol 51(1):44–51. https://doi.org/10.1093/ee/nvab122

Rocca M, Rizzo E, Greco N, Sánchez N (2017) Intra-and interspecific interactions between aphidophagous ladybirds: the role of prey in predator coexistence. Entomol Exp Appl 162(3):284–292. https://doi.org/10.1111/eea.12538

Roy HE, Brown PMJ, Adriaens T, Berkvens N, Borges I, Clusella-Trullas S, Comont RF, De Clercq P, Eschen R, Estoup A, Evans EW, Facon B, Gardiner MM, Gil A, Grez AA, others

(2016) The harlequin ladybird, *Harmonia axyridis*: global perspectives on invasion history and ecology. Biol Invasions 18:997–1044. https://doi.org/10.1007/s10530-016-1077-6

Sato S, Kushibuchi K, Yasuda H (2009) Effect of reflex bleeding of a predatory ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), as a means of avoiding intraguild predation and its cost. Appl Entomol Zool 44(2):203–206. https://doi.org/10.1303/aez.2009.203

Seagraves MP (2009) Lady beetle oviposition behavior in response to the trophic environment. Biol Control 51(2):313–322. https://doi.org/10.1016/j.biocontrol.2009.05.011

Schoener TW (1972) Resource partitioning in ecological communities. Sci 185: 27–38. DOI: 10.1126/science.185.4145.27

Sicsú PR, Macedo RH, Sujii ER (2020) Lady beetle oviposition site choices. Flor Entomol 103(2):228–235. https://doi.org/10.1653/024.103.0211

Sloggett JJ (2008) Habitat and dietary specificity in aphidophagous ladybirds (Coleoptera: Coccinellidae): explaining specialization. In: Proceedings of the Netherlands Entomological Society Meeting. pp 95–113

Smithson M, Verkuilen J (2006) A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. Psychol Methods 11(1):54. https://doi.org/10.1037/1082-989X.11.1.54

Soares AO, Vale M, Borges I, Lucas E (2025) Influence of refuge availability on the oviposition strategy of anachoretic and non-anachoretic aphidophagous ladybirds. BioControl. https://doi.org/10.1007/s10526-025-10339-9

Tapia DH, Morales F, Grez AA (2010) Olfactory cues mediating prey-searching behaviour in interacting aphidophagous predators: are semiochemicals key factors in predator-facilitation? Entomol Exp App 137(1):28–35. https://doi.org/10.1111/j.1570-7458.2010.01035.x

Togni PHB, Souza LM, Sicsú PR, Costa VA, Amaral DSSL, Franco AC, Sujii ER, Venzon M (2015) Coccinellidae parasitoids in Brazil: neglected species in a mega-diverse country. Neotrop Entomol 44:528–532. https://doi.org/10.1007/s13744-015-0309-2

Togni PHB, Venzon M, Lagôa ACG, Sujii ER (2019) Brazilian legislation leaning towards fast registration of biological control agents to benefit organic agriculture. Neotrop Entomol 48(2):175–185. https://doi.org/10.1007/s13744-018-0633-4

Togni PHB, Venzon M, Muniz CA, Martins EF, Pallini A, Sujii ER (2016) Mechanisms underlying the innate attraction of an aphidophagous coccinellid to coriander plants: Implications for conservation biological control. Biol Control 92:77–84. https://doi.org/10.1016/j.biocontrol.2015.10.001

Venzon M, Amaral DSSL, Togni PHB, Chiguachi JAM (2019) Interactions of natural enemies with non-cultivated plants. In: Natural enemies of insect pests in neotropical agroecosystems: biological control and functional biodiversity. Springer International Publishing, Cham, pp 15–26

Yasuda H, Evans EW, Kajita Y, Urakawa K, Takizawa T (2004) Asymmetric larval interactions between introduced and indigenous ladybirds in North America. Oecologia 141:722–731. https://doi.org/10.1007/s00442-004-1680-6

Yi C, Teng D, Xie J, Tang H, Zhao D, Liu X, Liu T, Ding W, Khashaveh A, Zhang Y (2023) Volatiles from cotton aphid (*Aphis gossypii*) infested plants attract the natural enemy *Hippodamia variegata*. Front Plant Sci 14:1326630. https://doi.org/10.3389/fpls.2023.1326630

Zeni V, Ricciardi R, Valicenti M, Nieri R, Mazzoni V, Canale A, Desneux N, Lucchi A, Benelli G (2024) Don’t touch me! Mating not always preceded by courtship behavior in predatory coccinellids. J Econ Entomol 117(6):2376–2383. https://doi.org/10.1093/jee/toae130

Supplementary Information

Fig. S1. Experimental collard field used for the cage experiment (August–September 2019) at the Biology Experimental Station, University of Brasília.

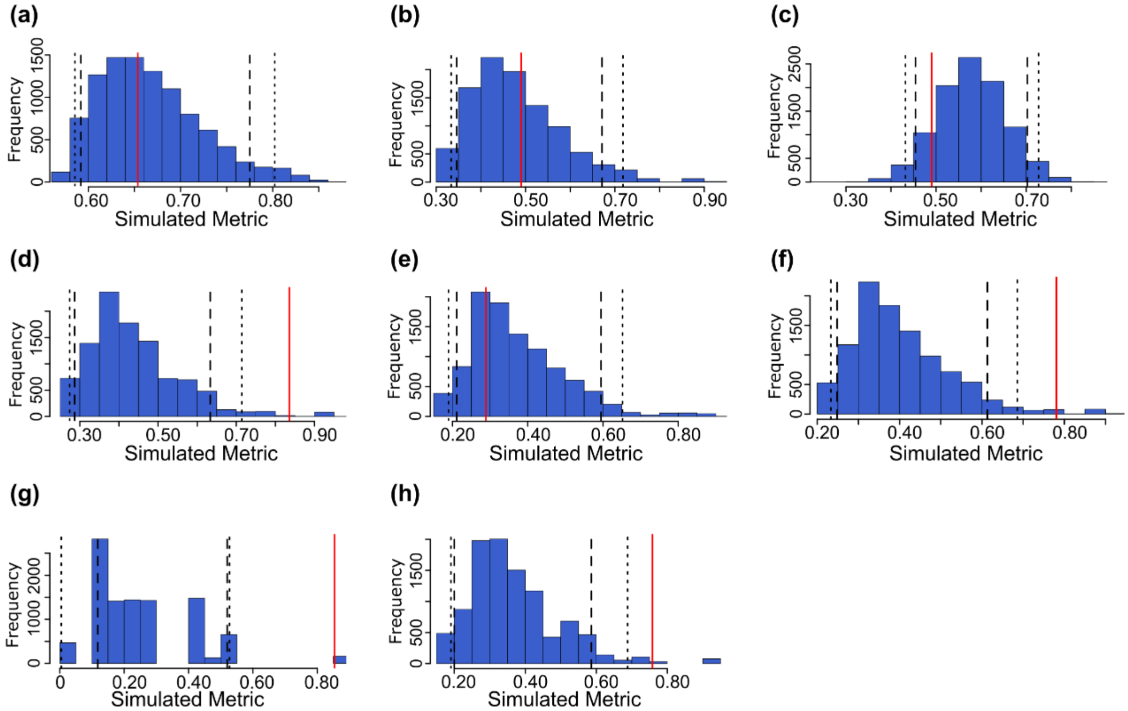

Fig. S2. Expected values of niche overlap (blue bars) of coccinellids communities based on Pianka index. Each graph represents all life stages (a), only eggs (b) and only pupae (c) for the cage experiment. All life stages (d), only eggs (e), only larvae (f), only pupae (g) and only adults (h) for the field experiments. The vertical red lines represent the observed niche values, the long-dash lines show the one-tailed 95% limits, and the short-dash lines represent the two-tailed 95% limits.

Table S1. Models and parameters from multinomial logistic regression models on the effects of coccinellid species, coccinellid life stage, and mean coccinellid abundance in the habitat and micro-habitat selection by coccinellids (the response variable used for all models) on 42 organic brassica-producing farms in the Brazilian Federal District between 2020 and 2023. Bold numbers indicate statistically significant differences at the 0.05 significance level.

Model	Explanatory variables	logLink	AICc	ΔAICc	Relative weight	χ^2	d.f.	p.value
4	**Coccinellid species**	-3186.66	6437.99	0	0.666	885.76	12	**<0.001**
	Mean coccinellid abundance					72.88	4	**<0.001**
	Interaction					52.17	12	**<0.001**
7	**Coccinellid species**	-3183.27	6439.37	1.38	0.334	856.66	12	**<0.001**
	Mean coccinellid abundance					73.82	4	**<0.001**
	Coccinellid life stage					6.79	4	0.147
	Coccinellid species: mean abundance					51.17	12	**<0.001**
3	**Coccinellid species**	-3212.75	6465.76	27.77	0	885.76	12	**<0.001**
	Mean coccinellid abundance					72.88	4	**<0.001**
2	**Coccinellid species**	-3208.85	6530.55	69.89	0	1223.7	12	**<0.001**
5	**Coccinellid species**	-3245.77	6531.79	71.14	0	1110.20	12	**<0.001**
	Coccinellid life stage					6.85	4	0.144
6	**Coccinellid species**	-3242.19	6549.04	88.38	0	1110.20	12	**<0.001**
	Coccinellid life stage					6.85	4	0.144
	Interaction					7.16	12	0.847
1	~ 1	-3861.02	7730.05	1369.58	0	N.A.	N.A.	N.A.

Table S2. Abundance of egg masses and pupae recorded across 42 organic brassica-producing farms in the Brazilian Federal District between 2020 and 2023, found on brassica plant thirds (upper, middle, and lower), soil, and non-crop plants.

Site	*Cycloneda sanguinea*	*Eriopis connexa*	*Harmonia axyridis*	*Hippodamia convergens*	Unknown	Total
Egg masses						
Upper third	1	1	0	0	3	5
Middle third	4	0	0	2	15	21
Lower third	0	0	0	5	12	17
Soil	0	0	0	0	3	3
Non-crop plants	5	0	0	0	38	43
Pupae						
Upper third	0	0	0	0	0	0
Middle third	0	0	0	0	1	1
Lower third	0	0	0	0	0	0
Soil	1	0	0	0	0	1
Non-crop plants	1	0	1	4	33	39

CHAPTER 2

Trophic plasticity in Coccinellidae: linking seasonal feeding behavior to dynamic shifts in trophic position

Abstract

Aphidophagous coccinellids are essential generalist predators and biological control agents in agroecosystems. Their ability to utilize both animal (aphids) and plant-derived supplementary resources (pollen/nectar) demonstrates trophic plasticity, which is modulated by temporal fluctuations in prey availability. Understanding how these predators adjust their diet and trophic role in response to varying prey densities is crucial for predicting the stability and effectiveness of biological control services. We investigated how temporal fluctuations in aphid density shape the coccinellid community and its associated tritrophic network structure. We employed a multifaceted approach combining ecological network analysis, stable isotope analysis (SIA), and DNA metabarcoding to unravel coccinellid interactions and trophic ecology under lower and higher aphid densities in organic brassica crops. Coccinellid abundance significantly increased with aphid density, reinforcing their reliance on this prey, but perhaps not their role as efficient natural enemies of aphids. Conversely, during lower aphid availability, coccinellid communities were sustained by non-crop plants and their associated aphids, with *Uroleucon* spp. being the only aphid species they interacted with on these plants. Prey density also affected species' isotopic trophic niche size and overlap, demonstrating plasticity through species-specific responses (specialization, generalization, or constancy), which consequently shifted niche overlap among species pairs. The overall network structure was significantly affected, for instance, network nestedness was greater at higher aphid densities compared to lower aphid densities, a pattern attributed to density-dependent changes in feeding behavior. Furthermore, metabarcoding analysis revealed a high diversity of consumed plant genera, with *Hippodamia convergens* consuming the highest diversity of plant resource. Our results demonstrate that coccinellids adjust their diet in response to seasonal prey variability, often shifting from a plant-based to an animal-based diet. These findings elucidate the temporal dynamics of coccinellid trophic ecology and highlight its consequences for the resilience and stability of agroecosystem interactions.

Keywords: Agroecosystems, DNA metabarcoding, Ecosystem services, Prey Density, Stable isotopic analysis, Trophic interactions

Introduction

Aphidophagous coccinellids are important biological control agents in agricultural systems (Evans, 2009) and are useful models for investigating mechanisms of coexistence and community assembly. These generalist predators primarily feed on aphids but also utilize plant-derived resources, such as pollen and nectar (Amaral et al., 2013). Their relatively short life cycle facilitates the monitoring of population fluctuations and community dynamics over time. Given the aggregated distribution of aphids, multiple coccinellid species frequently exploit the same resource simultaneously, resulting in high niche overlap and common competitive interactions (Hodek et al., 2012) that may change their trophic role within the food-web. These negative interactions are dynamic and are expected to change over time with prey availability. For instance, inferior competitors may be displaced to suboptimal patches where prey is scarce due to different levels of exploitative competition (Chapter 1). Consequently, these species may rely more on non-prey items, resulting in significant shifts in their trophic positions across the season.

Plant-derived resources, both alternative and supplementary, are crucial to the nutritional ecology of coccinellids (Lundgren, 2009), enhancing their survival during periods of prey scarcity (He & Sigsgaard, 2019). However, the use of floral resources, such as pollen and nectar, varies widely among species. For instance, the highly feeding generalist *Hippodamia convergens* utilizes pollen from at least ten plant families, primarily Asteraceae (Medeiros et al., 2010). Pollen from Apiaceae (e.g., coriander, dill, and fennel) supports several species, including *Coleomegilla maculata*, *Cycloneda sanguinea*, *Eriopis connexa*, and *H. convergens* (Lixa et al., 2010; D'Ávila et al., 2016; Togni et al., 2016). Coccinellid species also differ in the amounts and types of pollen they consume, a pattern that may shift across life stages (D'Ávila et al., 2016). Such trophic plasticity is often a response to fluctuations in food availability, such as prey abundance (Gliesch et al., 2023), and consequently on species interaction. Given these generalist habits, interpreting the specific roles and trophic positions of individual species within the food web is challenging. Nevertheless, studying the feeding habits of generalist coccinellids provides crucial insights into their trophic niches and overlap, particularly when prey is scarce.

To disentangle community complexity, interaction network analysis serves as a valuable tool, emphasizing the structural patterns of species interactions rather than focusing solely on community composition (Paine, 1980; Bascompte, 2009). Beyond observing these structural links, understanding the energy transfer processes is crucial. Stable isotope analysis (SIA) can complement network metrics by revealing trophic plasticity and the realized niche of species, effectively tracking energy assimilation within the ecosystem (e.g., Castro et al., 2024; Costa et al., 2025; Arribas et al., 2015; de Carvalho et al., 2019). However, while isotopes elucidate energy pathways, they often lack the resolution to identify specific prey resources. To bridge this gap and uncover the mechanisms driving these trophic links, recent advances in molecular methods, such as high-throughput sequencing (HTS) and DNA metabarcoding, have become available (Kamenova et al., 2018). By precisely identifying gut contents, these approaches clarify the dietary composition (Kerley et al., 2018; Grabarczyk et al., 2025), validate and elucidate the specific interactions that underpin the broader network structure (e.g., Lefort et al., 2017; Kennedy et al., 2020; Lunde et al., 2022; Slusher et al., 2024).

Furthermore, as coccinellids forage within the habitat, they encounter patches containing varying prey species (Dixon, 2000), but they exhibit variable preferences for particular prey (Hodek et al., 2012). For example, *C. sanguinea* performs poorly when feeding on *B. brassicae* (a dominant aphid on brassicas) but thrives on *Uroleucon* spp. found on non-crop plants (Sicsú et al., 2015, 2020), potentially restricting its reliance to the latter. Conversely, *H. axyridis* cannot reach adulthood when feeding on *Uroleucon* spp. (Sicsú et al., 2020) and is thus unlikely to compete directly with *C. sanguinea* for this resource. Such species-specific differences in prey suitability and utilization could thereby facilitate or constrain coexistence within the same habitat. However, temporal variation in prey availability may modulate foraging selectivity, as predators are predicted to become less selective under low prey density (Stephens & Krebs, 1986; Crawley & Krebs, 1992).

To understand how temporal fluctuations in aphid density shape the coccinellid community and their trophic roles, we addressed four main questions: How does prey availability drive changes in coccinellid abundance? Do the isotopic niches, niche overlap and trophic positions of these species differ between periods of lower and higher aphid density? Does the structure of predator–prey–plant networks vary according to these density regimes? How does the richness of non-prey food affect the trophic position of coccinellids? We expected that coccinellid abundance would be positively correlated with temporal changes in aphid density, as increases in prey availability promote reproduction and subsequent population growth of aphidophagous coccinellids. Therefore, aphid–coccinellid interactions were expected to be more frequent during peaks in aphid density Furthermore, depending on the substitutability of the resources, increases in aphid density will result in a measurable elevation of the trophic position of coccinellid species, reflecting an increased reliance on animal-based resources. However, this dietary shift will be species-specific, as coccinellids that feed more on non-crop floral resources or non-aphid prey are expected to show minimal or non-significant changes in their trophic position during periods of higher aphid abundance on brassicas. Consequently, we expect the plant-aphid-coccinellid interaction network to exhibit lower specialization during periods of higher aphid abundance, as increased prey availability may relax foraging constraints and promote opportunistic interactions across multiple aphid preys and plants. Moreover, the overall network complexity (e.g., connectance) is expected to increase with the arrival and incorporation of aphid species into the cropping system.

Material and Methods

Study area

We sampled 21 organic brassica farms (each ≥ 2 km apart) from May to August 2022 and from May to September 2023. The farms were in Brazil's Federal District (15°46'S, 47°44'W), within the central Cerrado biogeographic district, a recognized biodiversity hotspot (Myers et al., 2000; Francoso et al., 2020) characterized by a Köppen-Geiger Aw climate (Alvares et al., 2013). Farms had average sizes of 7.8 ± 2.4 ha (mean ± SE) and maintained 5-10 m strips of non-crop plants with consistent structural diversity, had been certified organic for at least five years (Togni et al., 2019), and shared similar phenological and resource conditions despite individual variations among farms.

Coccinellids and aphids sampling

We monitored coccinellid and aphid populations on 13 of the 21 brassica farms. Each farm was visited three to four times per year, with a two-week interval between consecutive visits, across two seasonal phases: (1) the rainy-to-dry season transition (May–July), to monitor initial colonization of prey and coccinellids, and (2) the peak dry season (August–October), when populations of aphids and coccinellids typically reach maximum abundance. This sampling design was intended to assess how changes in aphid density influenced coccinellid abundance and species interactions.

For direct insect collection, we established 1 × 1 m quadrats at 10 points spaced systematically to sample both the central and edge areas of the crop. We also randomly placed 10 additional quadrats in non-crop vegetation within 10 m of crop edges. Within these quadrats, at least two trained collectors sampled coccinellids (larvae and adults) and, for each specimen, recorded taxonomic identification (to the lowest possible level), host plant species and phenological stage, and aphid presence and identity. Sampling in each quadrat continued until no additional individuals were detected (10–20 min per quadrat).

In addition to the quadrat-based surveys, we conducted supplemental sampling to specifically quantify aphid density over time. For each farm and sampling date, we randomly selected 40 brassica plants (collards and mainly cabbages) and counted all aphids on three random leaves per plant. Representative aphid and host-plant specimens were collected and taken to the laboratory for species or morphospecies identification, with the assistance of a taxonomist and a reference collection when needed.

Plant-aphid-coccinellid interactions

We described tritrophic interactions using field observations of plants (basal level), aphids (intermediate level), and coccinellids (top level). For each farm, records from all quadrats were combined to obtain species-level weighted interaction matrices, in which cell values corresponded to observed interaction frequencies. Interactions were assigned by linking aphids to the host plants on which they occurred and coccinellids to the aphid species they were found with. In cases where coccinellids were observed interacting with plants in the absence of aphids, we also recorded direct predator–plant links. Based on these data, we assembled weighted tripartite networks, including (i) two aggregate networks pooling information from all farms, stratified by lower and higher aphid density periods, and (ii) farm-specific subnetworks, also stratified by these density regimes. All networks were analyzed using the same set of descriptors. The latter were used to capture a gradient of local interaction structures occurring within the broader regional network and shaped by both landscape and local conditions.

From each network, we extracted the following metrics: (a) connectance, defined as the fraction of realized links relative to all potential links (Bersier et al., 2002); (b) specialization (*H2*), which quantifies overall network-level specialization (Blüthgen et al., 2006); (c) modularity (*Q*), describing the extent to which species are organized into distinct interaction modules (Olesen et al. 2007); (d) nestedness (*NODF*), measuring the tendency for specialists to interact with subsets of the partners used by generalists (Almeida-Neto et al., 2008); (e) network size (*S*), given by the total number of nodes (Dunne et al., 2002); and (f) trophic generality (*G*), the average number or diversity of prey species per predator, reflecting the balance between generalists and specialists (Schoener, 1989). All network indices were computed in R using the packages *bipartite*, *igraph,* and *cheddar* (Antonov et al., 2023; Dormann et al., 2009; Hudson et al., 2013).

Stable isotopic analysis

Coccinellid individuals (n = 382) were collected for stable isotope analysis from 21 farms. The dataset comprised the four most common coccinellid species found in brassica crops: *H. convergens* (n = 137), *E. connexa* (n = 103), *C. sanguinea* (n = 90) and *H. axyridis* (n = 52). Live coccinellids were transported to the laboratory and maintained without food until death allowing for complete gut evacuation and thereby minimizing potential bias from unassimilated food residues in the tissues. Specimens were then preserved in tubes containing 70% ethanol and stored under refrigeration until preparation for isotopic analysis.

Prior to analysis, each specimen was cleaned by immersion in a 2:1 chloroform–methanol solution for 60 min and subsequently rinsed with distilled water. Cleaned specimens were oven-dried at 50 °C for 48 h, finely ground to a homogeneous powder, and transferred to tin capsules for weighing on a microanalytical balance. Each capsule contained tissue from a single individual, with sample mass ranging from 0.40 to 0.59 mg. Samples were analyzed at the Laboratory of Isotopic Ecology, Center for Nuclear Energy in Agriculture (CENA), University of São Paulo, Piracicaba, Brazil, to determine carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios using an elemental analyzer (Carlo Erba CHN-1100) coupled to a Thermo Finnigan Delta Plus mass spectrometer. We expressed isotopic ratios in delta notation (δ) as parts per thousand (‰) relative to Vienna Pee Dee Belemnite (for Carbon) and atmospheric air (for Nitrogen). The calculation followed the equation:

$$\delta^{13}C \ or \ \delta^{15}N \ (‰) = \frac{(R_{sample} - R_{standard})}{R_{standard}} \times 1000$$

with *R* representing the heavy/light molar isotope ratio. Regarding internal standards, the CENA employes sugarcane with precision of 0.2‰ for δ^{13}C and 0.3‰ for δ^{15}N.

DNA extraction

DNA was extracted from 283 individuals, including *H. convergens* (n = 185), *C. sanguinea* (n = 61), *H. axyridis* (n = 30), and *E. connexa* (n = 7), collected in November 2023 from eight of the 21 farms and used exclusively for this analysis. Whole coccinellids were processed, with elytra and legs removed to reduce the amount of predator tissue and facilitate sample cleaning. Before DNA extraction, we surface-sterilized the specimens with 10% bleach solution, molecular-grade H_2O, and 95% ethanol to minimize environmental DNA contamination (Ingrao et al. 2017, Staudacher et al. 2018). After cleaning, we dried the samples and transferred them to sterile microcentrifuge tubes. We homogenized the sterilized samples in 180 µL of phosphate-buffered saline (PBS) using a TissueLyser II (Qiagen, Chatsworth, CA, USA) with 3-mm sterile stainless steel grinding beads (OPS Diagnostics LLC, Lebanon, NJ, USA) for 30 seconds. We then extracted and purified the DNA following the animal tissue protocol of the DNeasy 96 Blood and Tissue Kit (Qiagen), eluting the final product in 75 µL of AE buffer. To control for contamination, we included a blank well with no predator tissue, negative control, in each 96-well extraction plate. Finally, we stored all extracted coccinellid DNA samples at -20°C until PCR analysis.

Diagnostic PCR, metabarcoding and bioinformatics

Diagnostic PCR analyses were performed using primers from prior work and following protocols established in prior published work (Staudacher et al. 2018, Bowers et al. 2021; Schmidt et al., 2024), screening coccinellid samples for thrips, southern green stinkbugs (*Nezara viridula*), tarnished plant bugs (*Lygus lineolaris*), whiteflies, Collembola, Diptera, Lepidoptera, spider mites (*Tetranychus urticae*) and aphids. We targeted the mitochondrial *Cytochrome Oxidase* subunit I (*COI*) gene to detect arthropods DNA (Jusino et al., 2019) and the nuclear ribosomal *Internal Transcribed Spacer* (*ITS2*) region to detect plants' DNA (Moorhouse-Gann et al., 2018) in coccinellid samples. Metabarcoding procedures, sequence processing, and taxonomic assignment followed the protocols of previous work (Slusher et al., 2024; Grabarczyk et al. 2025). Standardized pools were then submitted to the Georgia Genomics and Bioinformatics Core lab (GGBC-UGA) for sequencing on an Illumina NexSeq

2000 (Illumina, San Diego, CA, USA) with P2 chemistry, 300bp PE, and 600 cycles. Briefly, the bioinformatic pipeline followed current standards and full details are provided in previous work from the lab (e.g. see Grabarczyk et al. 2025).

Data analysis

To assess the effect of aphid density on coccinellid abundance, we fitted a generalized linear mixed model (GLMM) with a negative binomial error distribution, using aphid density per leaf as a fixed-effect predictor and farm identity as a random intercept. To assess the effects of change in aphid density over time on network metrics, we first classified aphid density as a categorical factor with two levels (lower and higher). We restricted the analysis to farms where aphid density increased over time (ten farms). For each of these farms, we identified two sampling days: the day with the lower aphid density ("lower") and the day with the higher aphid density ("higher"), which allowed us to interpret natural variations in aphid densities among farms and how coccinellids interact with within-farm variations in prey densities.

We used this categorical predictor with two levels (low vs. high aphid density) as the main fixed effect in a series of generalized linear models (GLMs), with each network metric treated as a response variable. GLMs with a Gaussian error distribution were fitted for connectance, generality, vulnerability, and nestedness. For modularity, we adjusted zero values by adding a small constant ($\varepsilon = 1e^{-6}$) to ensure that all observations fell within the interval (>0, <1) (Smithson & Verkuilen 2006) and then fitted a model with a Beta error distribution. For specialization, we used a quasi-Poisson error distribution. These last two models were fitted with the *glmmTMB* package (Brooks et al., 2017).

For the isotopic analysis, we assessed similarity in $\delta^{13}C$ and $\delta^{15}N$ signatures among coccinellid species under lower and higher aphid density conditions. For each species, we calculated the standard ellipse area corrected for small sample size (SEAc) in $\delta^{13}C$–$\delta^{15}N$ space using the SIBER package (Jackson et al., 2011) to characterize the isotopic niche of the samples. The SEAc was computed separately for data obtained under lower and higher aphid densities. We then quantified the proportional overlap between species' ellipses as a measure of isotopic niches under each aphid density level. The comparison of ellipse overlaps followed de Camargo et al. (2022), using a Markov chain Monte Carlo (MCMC) procedure with 10,000 iterations to generate Bayesian 40% credible intervals.

To evaluate whether increased prey availability affected coccinellid $\delta^{13}C$ signatures, we modelled the relationship between aphid density (predictor) and $\delta^{13}C$ (response) using a Finite Mixture Regression (FMR) implemented in the *FlexMix* package (Grün & Leisch, 2007). This approach allowed us to identify latent groups with potentially distinct relationships between aphid density and $\delta^{13}C$. The model used Gaussian components for component-specific regressions. Model selection was performed across a range of components (k = 1–5) using the *stepFlexmix* function, and the final model was chosen based on the lowest Integrated Completed Likelihood (ICL) value (Biernacki et al., 2002) (Table S1).

To evaluate whether increased prey availability affected coccinellid $\delta^{15}N$ signatures (used here as a proxy for trophic position), we fitted a Linear Mixed-Effects Model (LMM) with a Gaussian error distribution. Aphid density per leaf was included as a fixed-effect predictor, and farm identity was specified as a random intercept to account for spatial variation. To examine potential species-specific responses, we then fitted separate LMMs with Gaussian errors for each of the four coccinellid species, using the same fixed and random effects structure previously described.

In addition to isotopic analyses, we used metabarcoding data to characterize the diversity of plant resources associated with each coccinellid species. We assessed plant species diversity using plant occurrence data obtained from metabarcoding. We used a combination of rarefaction analysis and subsampling to normalize sequencing depths (Cameron et al. 2021). We created 30 subsamples of each read depth (25 to 5000 reads) to identify the sequencing depth at which plant richness reached an asymptote, indicating adequate sampling coverage. We then standardized datasets among coccinellid species by equalizing both sample size ($n \geq 19$ per species) and sequencing depth (n = 1000 reads), to reduce bias in comparisons of plant–coccinellid interaction patterns across species (Fig. S1). After the normalization, we used these data to quantify plant resource use by each coccinellid species.

For each of the 30 subsamples of each species dataset, we calculated plant abundance (number of sequence reads), plant species richness, and diversity (Shannon and Simpson indices) in coccinellid gut contents, excluding *E. connexa* due to its small sample size. We then compared these metrics among coccinellid species to assess differences in plant–coccinellid interaction patterns with a Kruskal-Wallis test and post-hoc pairwise Wilcoxon Rank Sum Tests with Bonferroni correction. To investigate whether the richness of plant genera consumed by coccinellids predicted their $\delta^{15}N$ signatures, we fitted a LMM with a Gaussian error distribution. The richness of plant genera detected in coccinellid gut contents (DNA metabarcoding data) was included as a fixed-effect predictor, and farm identity was specified as a random intercept. Model assumptions were assessed, where applicable, using residual diagnostics implemented in the *DHARMa* package (Hartig & Lohse, 2020). All statistical analyses were performed in R (R Core Team, 2025).

Results

When considering all farms across both years and sampling periods, ten coccinellid genera and at least 12 species were recorded. The coccinellid species included: *Cycloneda sanguinea* (n = 638 individuals), *Hippodamia convergens* (n = 556), *Eriopis connexa* (n = 220), *Psyllobora lenta* (n = 125), *Harmonia axyridis* (n = 90), *Scymnus* sp. (n = 28), *Psyllobora* sp. (n = 9), *Coccidophilus* sp. (n = 6), *Hyperaspis festiva* (n = 5), *Coleomegilla maculata* (n = 1), *Diomus* sp. (n = 1), and *Psyllobora confluens* (n = 1). Regarding aphids, at least for species, each of a different genus, were recorded. *Brevicoryne brassicae* was the most abundant (n = 34.928 individuals), followed by *Lipaphis pseudobrassicae* (n = 7.844), *Myzus persicae* (n = 2.535), and *Uroleucon* spp. (n = 81). In almost all farms, except for three, aphid density on brassicas increased over time. Among the farms where density increased, periods with lower aphid abundance corresponded to 1.89 ± 0.56 aphids per leaf (mean ± SE), whereas periods with higher aphid abundance corresponded to 29.41 ± 17.63 aphids per leaf, and all previously mentioned aphid species were recorded in both periods. Also, coccinellid densities was lower when aphid densities were lower and increased with higher aphid densities (β = -1.075, SE = 0.244, t = -4.416; χ^2 = 19.503, d.f. = 1, p < 0.0001; Fig. 1).

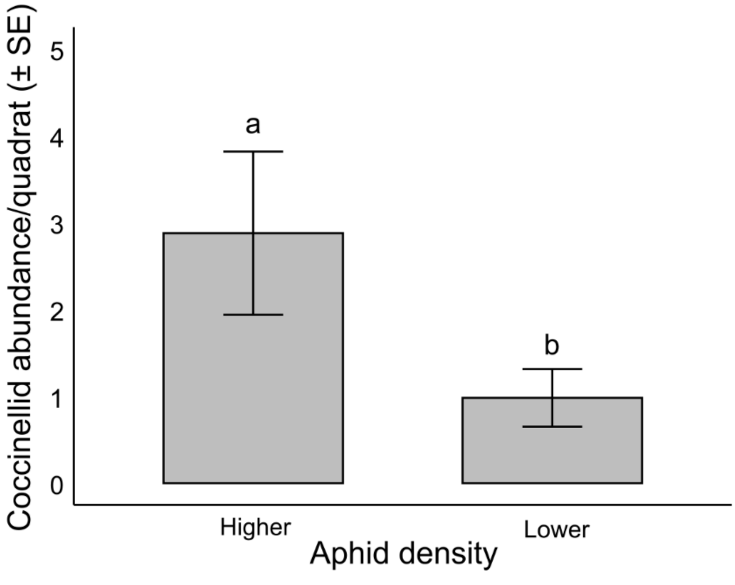

Fig. 1. Coccinellid densities under lower and higher aphid densities in brassica crops, estimated

using a generalized linear model (GLM) with negative binomial distribution. Different lowercase letters indicate statistically significant differences among aphid density levels based on model contrast analysis ($p < 0.05$).

Coccinellid species showed variation in Standard Ellipse Area (SEAc) in response to increasing aphid density, indicating shifts in their isotopic niches. *Cycloneda sanguinea* and *Hippodamia convergens* exhibited larger SEAc values at lower aphid densities, suggesting broader niches under prey scarcity. In contrast, *Harmonia axyridis* displayed larger SEAc values when aphid density was high. For *Hippodamia convergens*, the mean SEAc remained relatively constant. However, individual variation appeared lower at higher aphid densities (Fig. 2). The plotted ellipses visually illustrate these distinct patterns of isotopic niche expansion and contraction across density treatments (Fig. 3).

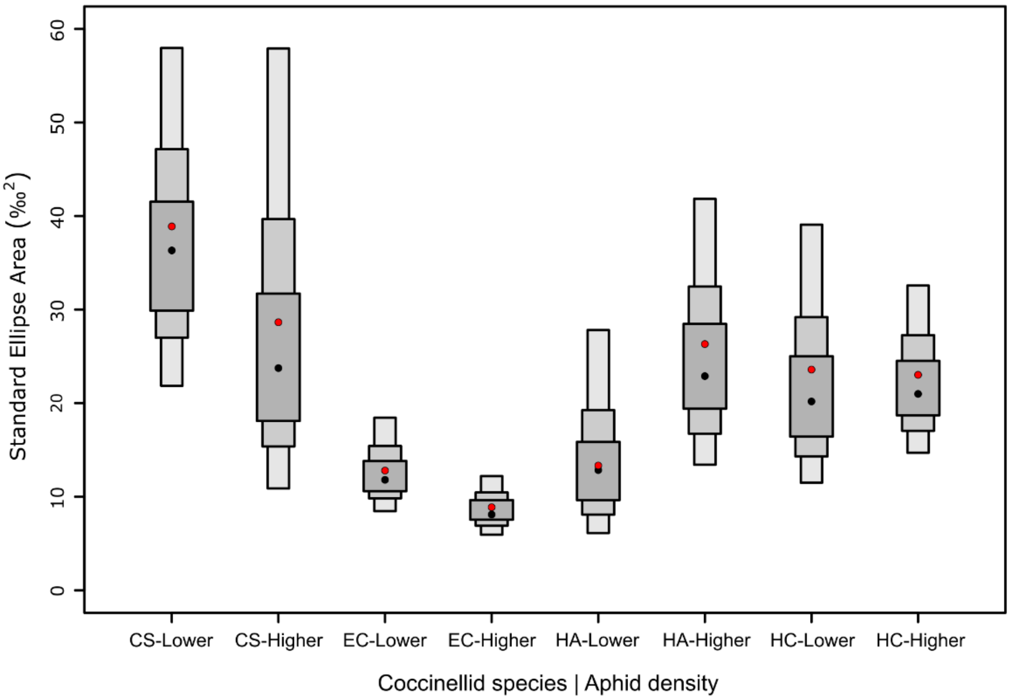

Fig. 2. Standard Ellipse Area corrected for small sample size (SEAc) for *Cycloneda sanguinea* (CS), *Eriopis connexa* (EC), *Harmonia axyridis* (HA), and *Hippodamia convergens* (HC) under low and high aphid density in brassica crops. Boxes represent Bayesian posterior distributions of SEAc. Red dots indicate posterior mean estimates, and black dots represent the corresponding maximum likelihood estimates.

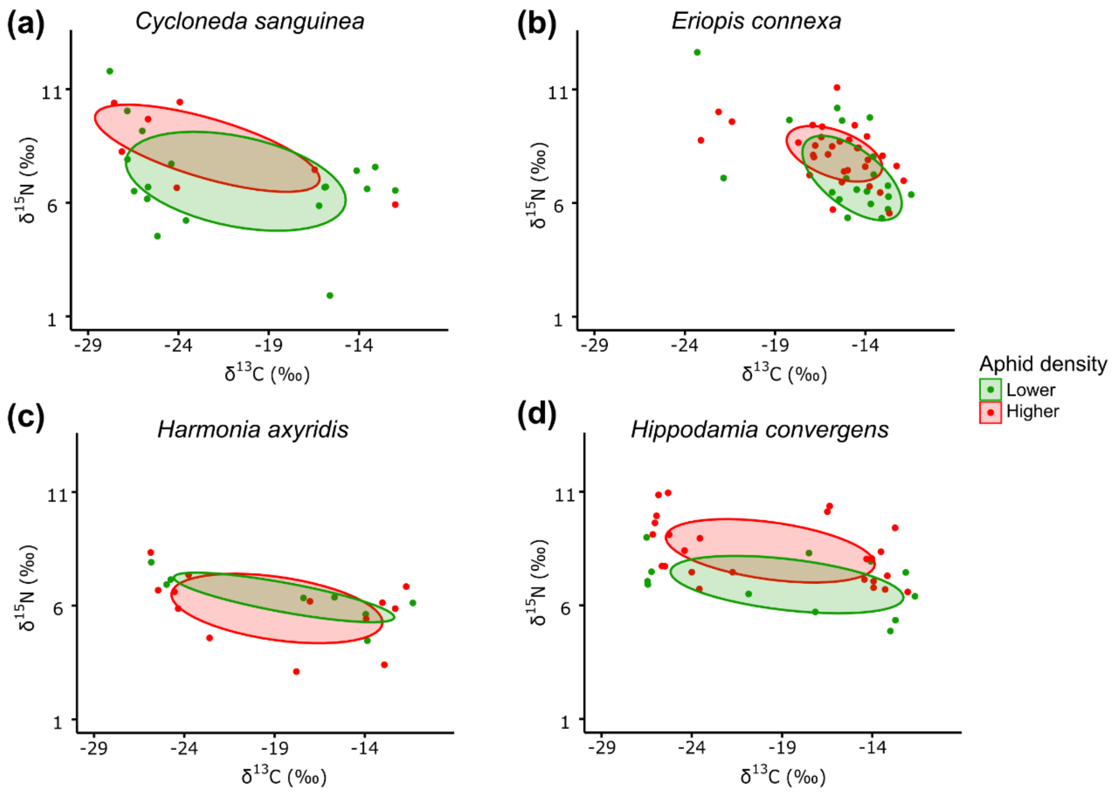

Fig. 3. Isotopic ratios overlap of (a) *Cycloneda sanguinea*, (b) *Eriopis connexa*, (c) *Harmonia axyridis*, and (d) *Hippodamia convergens* collected from brassica crops and non-crop plants in surrounding areas under lower (green) and higher (red) aphid densities. Ellipses represent 40% confidence intervals.

Interspecific overlap varied according to species pairs and aphid density. At lower aphid density, the highest isotopic niche overlaps (>40%) were observed between *C. sanguinea* and *H. convergens*, and between *H. axyridis* and *H. convergens* (Fig. 4a, b). In contrast, at higher aphid density, overlaps were highest between *C. sanguinea* and *H. convergens* and between *C. sanguinea* and *H. axyridis*. Notably, niche overlap between *C. sanguinea* and *H. convergens* increased slightly from lower to higher aphid density. However, overlap decreased for most other species pairs as aphid density increased, with a marked reduction observed between *H. axyridis* and *H. convergens* (Fig. 4).

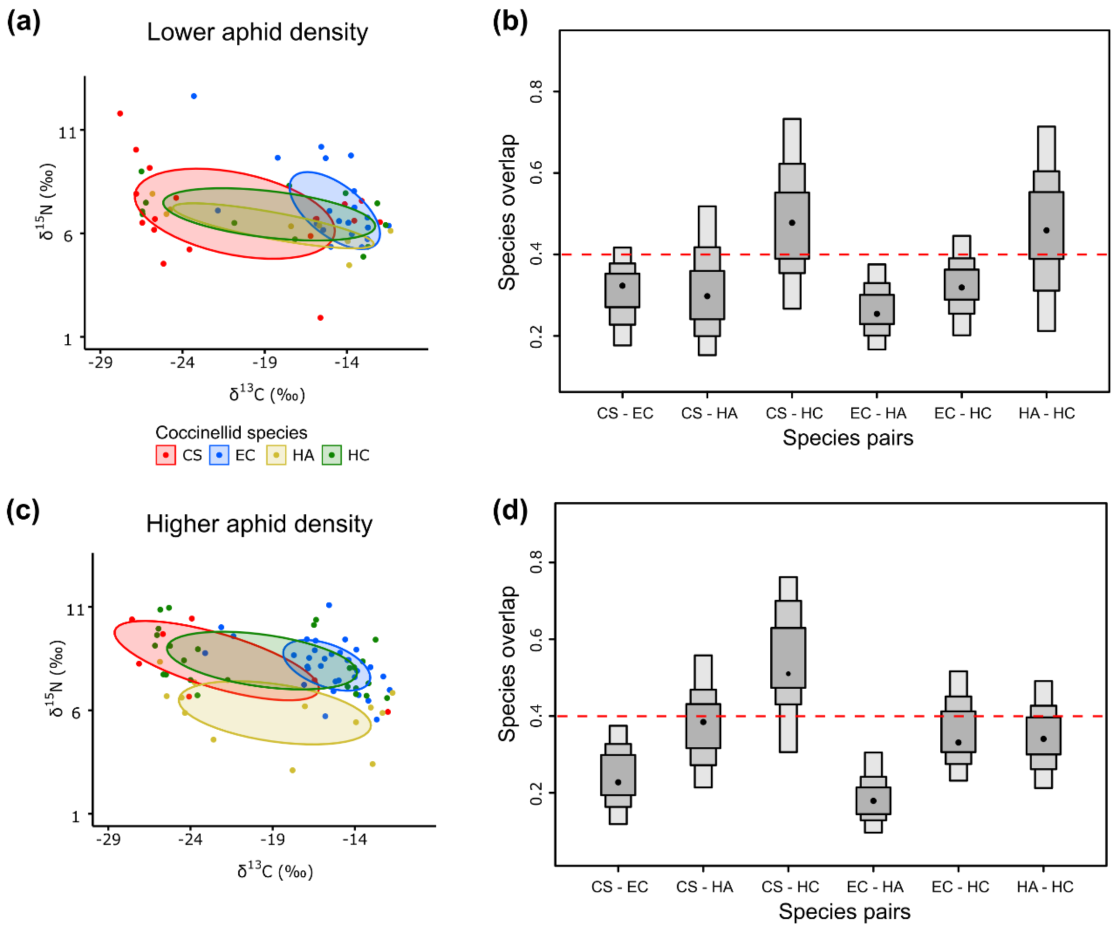

Fig. 4. (a) Isotopic ratios of coccinellid species collected in brassica crops and in non-crop plants in surrounding areas under lower aphid density on brassicas; (b) isotopic niche overlap among coccinellid species pairs under lower aphid density; (c) isotopic ratios of coccinellid species collected under higher aphid density on brassicas; (d) isotopic niche overlap among coccinellid species pairs under higher aphid density. Ellipses represent 40% confidence intervals. Red dashed lines indicate the point at which species-pair overlap reached 40%. CS = *Cycloneda sanguinea*; EC = *Eriopis connexa*; HA = *Harmonia axyridis*; HC = *Hippodamia convergens*.

Network structure at lower and higher aphid densities on brassicas

By comparing observed network metrics at lower aphid density with null-model expectations, we found that the plant–aphid–coccinellid network is non-randomly structured and reflects underlying ecological organization for all metrics except specialization ($H2_{obs}$: 0.27, $H2_{exp}$: 0.25 ± 0.06 SD, z = 0.25, p-value = 0.805). The connectance was significantly higher than random expectations (C_{obs}: 0.10, C_{exp}: 0.03 ± 0.002 SD, z = 44.85, p < 0.0001), as well as a generality (G_{obs}: 5.86; G_{exp}: 4.79 ± 0.41 SD; $z = 2.62$; $p = 0.009$) and vulnerability (V_{obs}: 2.93; V_{exp}: 2.38 ± 0.16 SD; $z = 3.42$; $p = 0.001$). Moreover, the network had a lower observed modularity (Q_{obs}: 0.05; Q_{exp}: 0.17 ± 0.05 SD; $z = -2.38$; $p = 0.017$) than would be expected by chance. The same pattern was observed for network nestedness which presented a lower observed nestedness (WNODF_{obs}: 9.76, WNODF_{exp}: 40.35 ± 2.86, $z = -10.70$, $p < 0.0001$) than would be expected by chance.

At higher aphid density, the plant–aphid–coccinellid network also deviated strongly from null-model expectations, indicating ecological structuring, with all metrics differing significantly from null means. The connectance (C_{obs}: 0.14, C_{exp}: 0.04 ± 0.00 SD, $z = 49.08$, $p < 0.0001$), generality (G_{obs}: 4.60; G_{exp}: 3.60 ± 0.25 SD; $z = 3.95$; $p < 0.0001$), vulnerability (V_{obs}: 3.83; V_{exp}: 3.00 ± 0.21 SD; $z = 4.05$; $p < 0.0001$), modularity (Q_{obs}: 0.35; Q_{exp}: 0.16 ± 0.04 SD; $z = 4.55$; $p < 0.0001$) and specialization ($H2_{obs}$: 0.35; Q_{exp}: 0.24 ± 0.05 SD; $z = 2.13$; $p = 0.033$) were significantly higher than expected at random. While the opposite was observed for network nestedness (WNODF_{obs}: 10.87, WNODF_{exp}: 59.74 ± 3.52, $z = -13.90$, $p < 0.0001$).

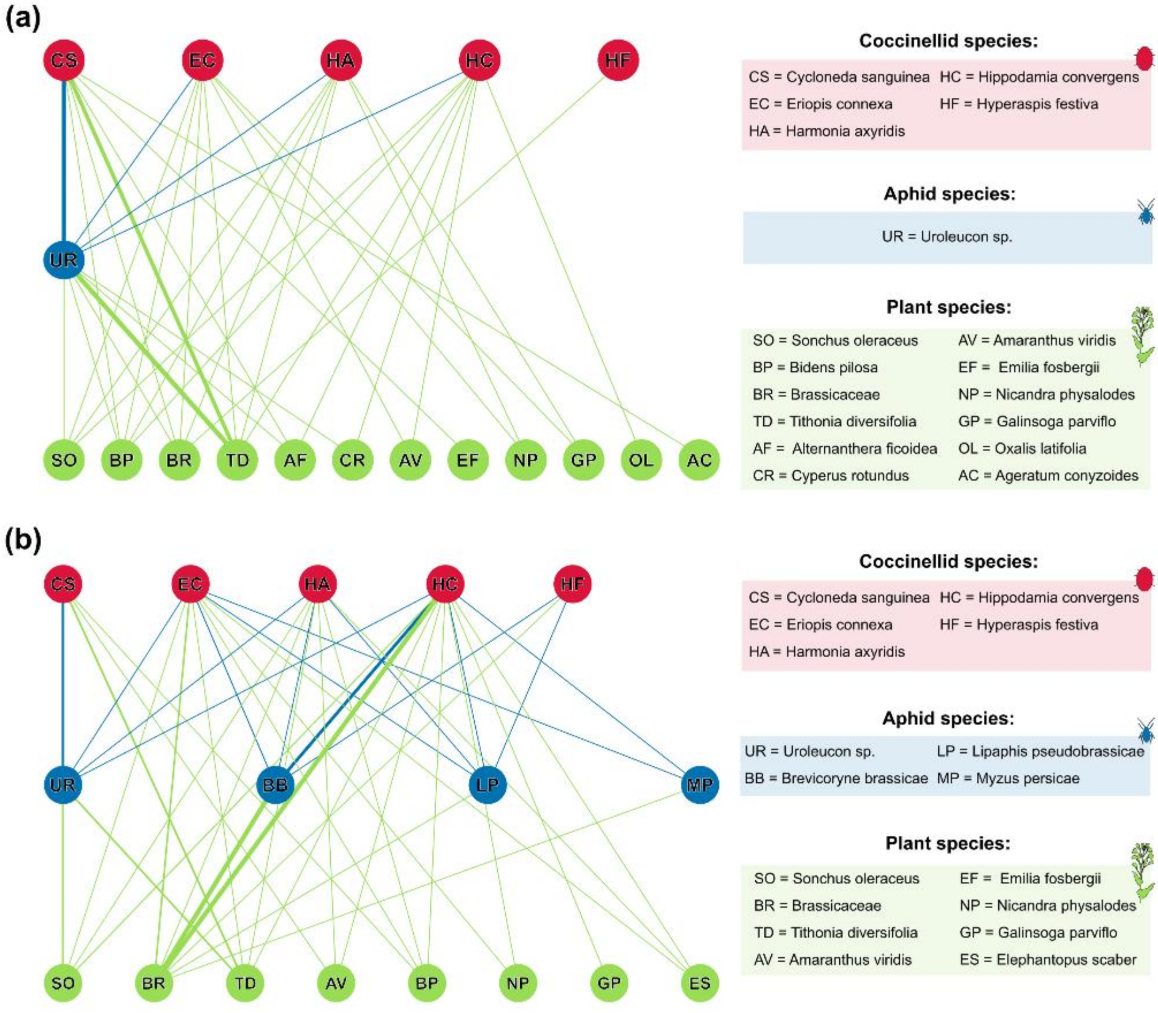

Fig. 5. Structure of the plant–aphid–coccinellid interaction network at (a) lower aphid density and (b) higher aphid density. Nodes represent plants (green), aphids (blue), and coccinellids (red). Link width is proportional to interaction frequency, and link colors denote interaction type: green = plant-aphid and plant–coccinellid, blue = aphid–coccinellid.

Network nestedness was significantly higher at higher aphid densities on farms (estimate = -10.180 ± 3.48; t = -2.93; p = 0.010; $\chi^2 = 1.74$; d.f. = 1; p = 0.187) (Fig 6a). However, network connectance did not vary between periods of lower and higher aphid densities (χ^2 = 0.20; d.f. = 1; p = 0.526), the same patter was observed for generality (χ^2 = 0.13; d.f. = 1; p = 0.720), vulnerability (χ^2 = 0.50; d.f. = 1; p = 0.479), modularity (χ^2 = 0.91; d.f. = 1; p = 0.341), and specialization (χ^2 = 1.74; d.f. = 1; p = 0.187) (Fig 6b, c, d, e, f).

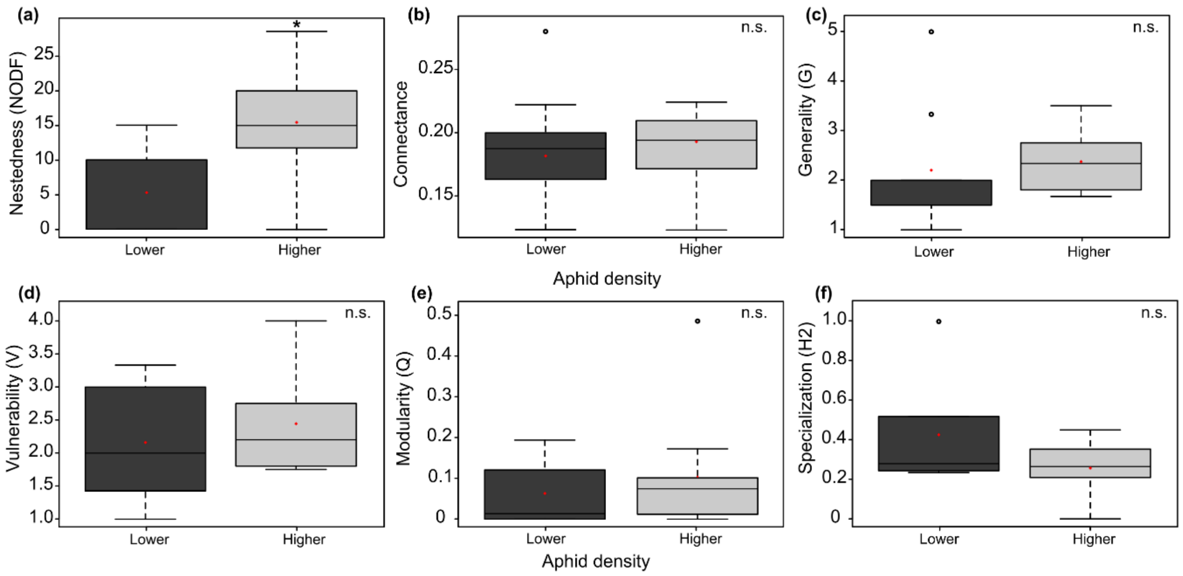

Fig. 6. Relationships between network (a) nestedness (*NODF*), (b) connectance, (c) generality (*G*), (d) vulnerability (*V*), (e) modularity (*Q*), and (f) specialization (*H2*) and aphid density on farms. Asterisks indicate statistically significant differences among aphid density levels based on model contrast analysis ($p < 0.05$). ns = non-significant.

Relationship between $\delta^{13}C$ and $\delta^{15}N$ and aphid density

The best-fitting model for coccinellids $\delta^{13}C$ signature included two components (Fig. S2). Component 1 accounted for 67% of the total observations. For this group, aphid density had no significant effect on coccinellid $\delta^{13}C$ values ($\beta = 0.427$, SE = 0.348, z = 1.228, p = 0.219; Fig. S3). In contrast, component 2 accounted for the remaining 33% of observations. The relationship between aphid density and $\delta^{13}C$ in this group was negative but was also not significant ($\beta = -0.677$, SE = 0.477, z = 1.419, p = 0.156) (Fig. S3).

Moreover, coccinellids contained lower values of $\delta^{15}N$ when aphid density was lower ($\beta = -1.041$, SE = 0.257, t = -4.047; $\chi^2 = 16.376$, d.f. = 1, $p < 0.0001$; Fig. 7a). However, when considering each species separately there was no difference on $\delta^{15}N$ for *C. sanguinea* at lower and higher aphid densities ($\chi^2 = 2.532$, d.f. = 1, p = 0.112; Fig. 7b) and the same was observed for *H. axyridis* ($\chi^2 = 1.648$, d.f. = 1, p = 0.199; Fig. 7d). In contrast, lower values of $\delta^{15}N$ when aphid density was lower was observed for *E. connexa* ($\beta = -0.989$, SE = 0.331, t = -2.989; $\chi^2 = 8.936$, d.f. = 1, p = 0.003; Fig. 7c) as well as for *H. convergens* ($\beta = -1.666$, SE = 0.456, t = -3.654; $\chi^2 = 13.35$, d.f. = 1, p = 0.0003; Fig. 7e).

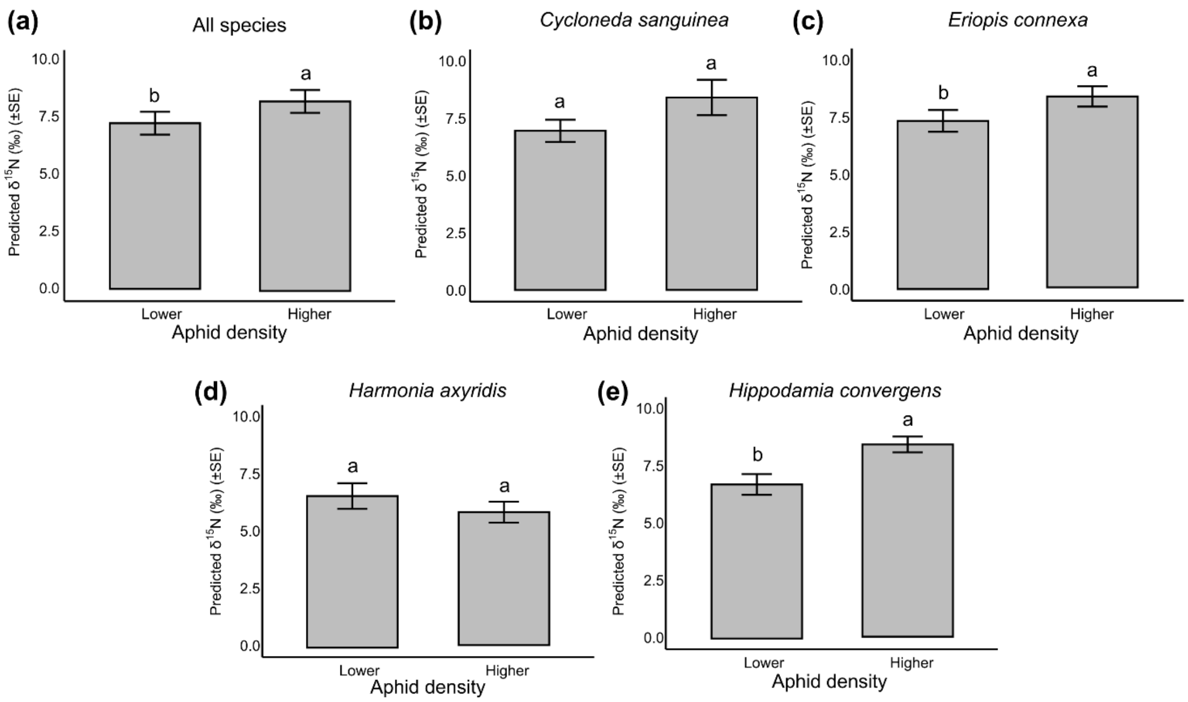

Fig. 7. Predicted δ^{15}N response to aphid density for (a) all coccinellid species, (b) *Cycloneda sanguinea*, (c) *Eriopis connexa*, (d) *Harmonia axyridis*, and (e) *Hippodamia convergens*, as predicted by Linear Mixed-Effects Model (LMM) with a Gaussian error distribution. Different lowercase letters indicate statistically significant differences among aphid density levels based on model contrast analysis ($p < 0.05$).

Coccinellid-plant network structure and the effect of plant-based resources consumption diversity on coccinellid trophic position (δ^{15}N)

Diagnostic PCRs revealed that coccinellids consumed prey from four invertebrate groups: including aphids, Diptera, Collembola, and Lepidoptera (see Table S2 for more details). The metabarcoding analysis of coccinellid gut contents revealed limited detection of animal prey DNA. Therefore, these data are not presented here nor included in any analyses. In contrast, plant DNA was well represented: metabarcoding detected interactions involving 45 plant genera across 20 families (Table S3). The six most abundant genera were *Amaranthus* (10,875 reads), *Bidens* (6,142), *Eleusine* (2,600), *Tithonia* (2,328), *Euphorbia* (2,028), and *Galinsoga* (1,496). The four most abundant plant families were Amaranthaceae (10,875 reads), Asteraceae (6,142), Poaceae (2,600), and Euphorbiaceae (2,028). At the coccinellid species level, *C. sanguinea* showed the highest number of reads for Amaranthus (3,019). *E. connexa* was most strongly associated with Citrus (200 reads), while both *H. axyridis* (2,435 reads) and *H. convergens* (5,223 reads) showed their highest read counts for *Amaranthus*. The richness of consumed plant genera differed significantly among coccinellid species ($\chi^2 = 233.16$, df = 2, $p < 0.0001$), as did plant diversity (Shannon index: $\chi^2 = 263.72$, df = 2, $p < 0.0001$; Simpson index: 259.2, df = 2, $p < 0.0001$). *Hippodamia convergens* exhibited the highest richness and diversity of plant resources, followed by *C. sanguinea* and *H. axyridis* (Fig. S1).

Network metrics derived from DNA metabarcoding data differed significantly from null expectations, except for generality (G_{obs}: 8.85, C_{exp}: 6.13 ± 0.002 SD, $z = 0.781$, $p = 0.435$) and nestedness ($WNODF_{obs}$: 47.41, $WNODF_{exp}$: 53.88 ± 3.57, $z = -1.82$, $p = 0.070$). Network connectance (C_{obs}: 0.42, C_{exp}: 0.82 ± 3.471 SD, $z = -28.52$, $p < 0.0001$) was lower than expected by chance. In contrast, network specialization ($H2_{obs}$: 0.182, $H2_{exp}$: 0.002 ± 0.06 SD, $z = 716.546$, $p < 0.0001$) and modularity (Q_{obs}: 0.196, C_{exp}: 0.010 ± 0.002 SD, $z = 134.02$, $p < 0.0001$) were higher than expected by chance (Fig. 8).

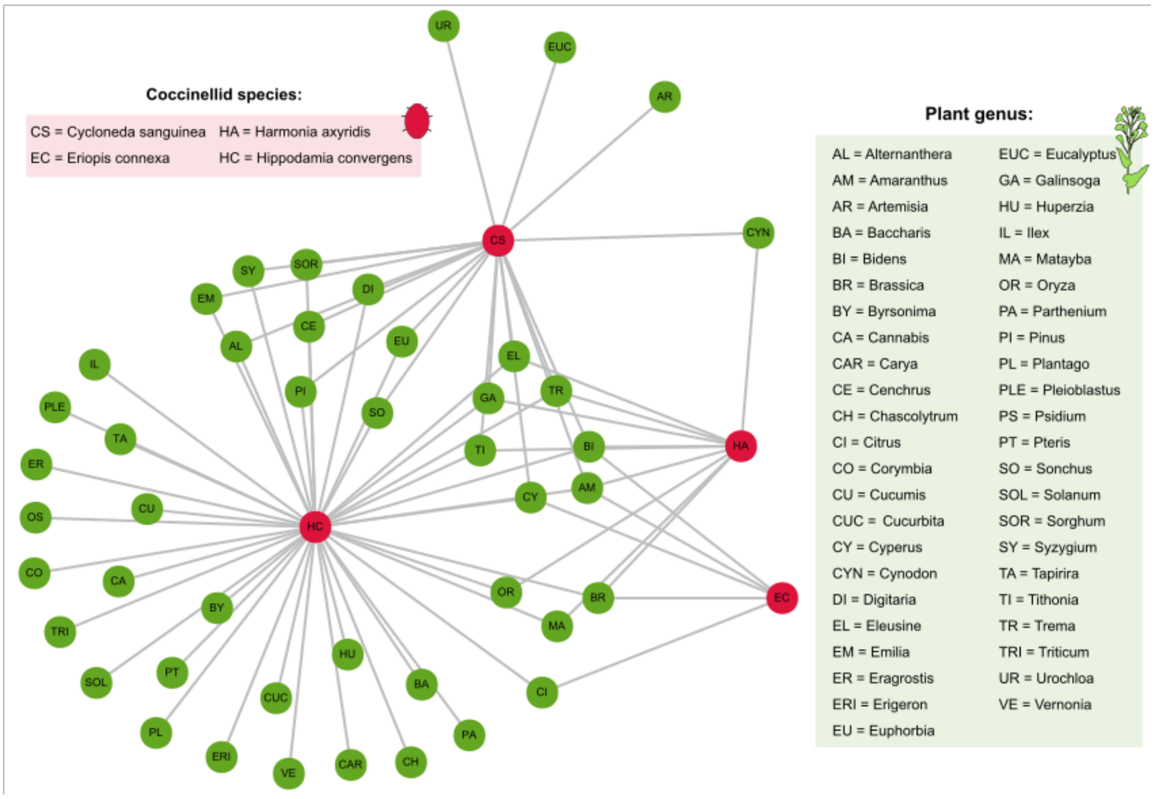

Fig. 8. Structure of the bipartite network constructed from DNA metabarcoding data of coccinellid gut content. The network visualizes the trophic interactions between the four coccinellid species and the plant taxa identified in their diet. Green nodes represent the plant genera (resource nodes) and red nodes represent the coccinellid species (consumer nodes).

Coccinellids that fed on fewer plant genera exhibited higher $\delta^{15}N$ values (β = -0.101, SE = 0.036, t = -2.781; χ^2 = 7.735, d.f. = 1, p = 0.005) (Fig. 9),indicating that coccinellids feeding on a greater number of plant genera tend to rely more heavily on plant-based resources than those feeding on fewer genera.

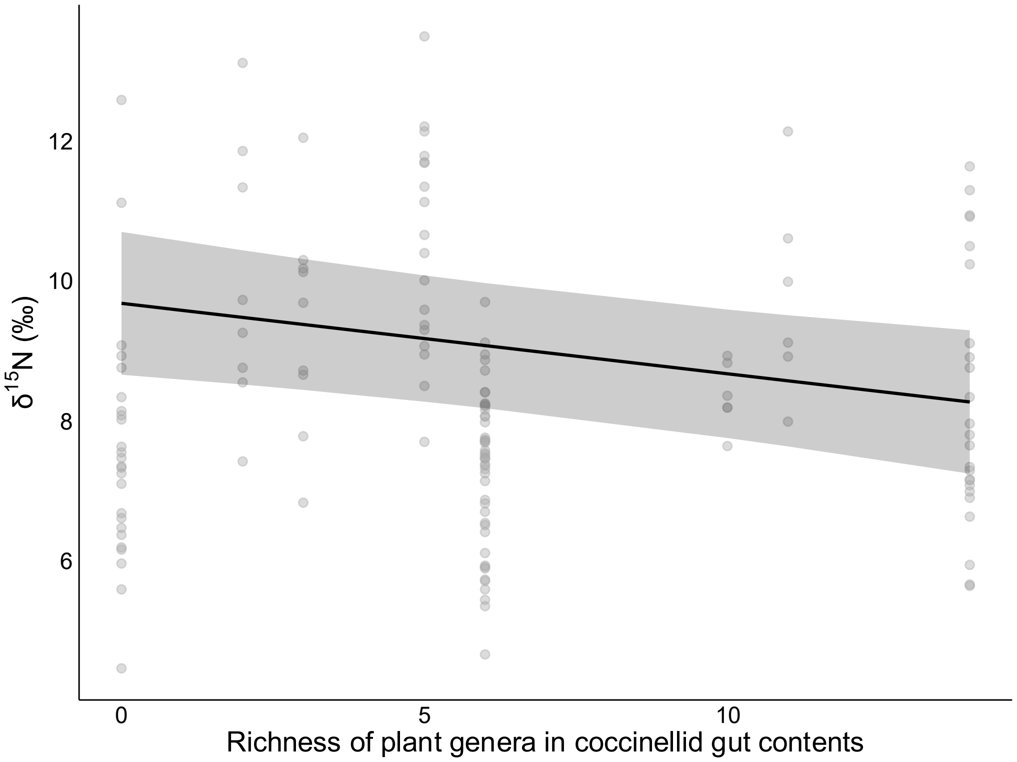

Fig. 9. Response of predicted $\delta^{15}N$ values to plant genus richness detected in the gut contents of coccinellids, as estimated by a Linear Mixed-Effects Model (LMM) with Gaussian error distribution.

Discussion

The trophic interactions of generalist aphidophagous predators are dynamic, as they respond to temporal variability in prey densities, despite the differences in response among species. We demonstrated that individuals integrate these dietary changes over time, shifting both their trophic positions and functional roles within the food web. Specifically, and contrary to Iuliano et al. (2024), aphid density was a significant predictor of coccinellid abundance at the local scale, highlighting the strong dependence of coccinellids on aphids as a food resource.

This pattern also suggests that coccinellids alone may not always be sufficient to suppress aphid populations, emphasizing the importance of other natural enemies such as parasitoid wasps, syrphid flies, and lacewings. When aphids are scarce, non-crop plants emerged as a critical alternative food source, possibly influencing differences in isotopic niche overlap and trophic position among coccinellid species through the season. However, each species responded differently to these changes due to life-history traits that dictate their preferences or reliance on prey and non-prey food (pollen). These shifts in abundance and foraging behavior had a direct effect on network topology. Therefore, as aphid density increased, interaction networks became significantly more nested, with specialist interactions forming subsets of those of generalists, consistent with the expectation that higher resource availability promotes community-level specialization.

Regarding the trophic ecology of the coccinellid species we sampled, most are historically documented as consuming an aphidophagous diet, including *Cycloneda sanguinea*, *Eriopis connexa*, *Scymnus sp.*, *Hyperaspis festiva*, and *Coleomegilla maculata* (Hagen, 1962; Wheeler & Jubb, 1979; Gordon, 1985, Gyenge et al., 1998; Giorgi et al., 2009). Conversely, some species are known to be polyphagous (or generalist predators), such as *Hippodamia convergens* and *Harmonia axyridis* (Gordon, 1985; Hodek et al., 2012; Roy et al., 2016). This spectrum of trophic guilds illustrates the remarkable diversity of feeding strategies displayed by Coccinellidae, and their inherent potential to exhibit trophic plasticity in response to varying availability of food resources.

Corroborating our expectations, coccinellids adjusted their dietary habits in response to resource availability, with the responses varying among species. *Cycloneda sanguinea*, for instance, appeared to expand its isotopic niche as aphid density increased, suggesting greater trophic generalism. This expansion was probably driven by a wider range of $\delta^{13}C$ values, indicating a shift in the use of basal resources (plants) and on distinct prey using these distinct basal resources. However, this expansion in basal resource use did not compromise *C. sanguinea* trophic position. On the contrary, the observed increase in $\delta^{15}N$ values, coupled with a narrower range, confirms the predatory role of this species (Minagawa & Wada, 1984; Post, 2002). These results are reinforced by the network analyses showing that this predatory activity was likely directed at prey inhabiting non-crop plants, mainly *Uroleucon* aphids. Notably, *C. sanguinea* interacted exclusively with this alternative prey, even in the presence of abundant brassica-associated aphids, suggesting some degree of prey specialization within the overall trophic generalism of this species.

The increase in *C. sanguinea* δ^{15}N values when crop aphid density was higher indicate a shift toward a more prey-based diet, this may be a consequence of competitive release within non-crop habitats (Bolnick et al., 2010; Andrades et al., 2019; Herrmann et al., 2021). Specifically, the high availability of aphids in the crop likely triggered a foraging shift in competitors such as *H. convergens*, *H. axyridis*, and *E. connexa*, which migrated to exploit resources in the brassica crops (e.g., Feinsinger & Swarm, 1982). This hypothesis is supported by our interaction networks, which reveal that these species (particularly *H. convergens*) expanded their trophic niche, primarily targeting brassica-associated aphids under high-density conditions. Consequently, this redistribution of foraging pressure may favored prey consumption by *C. sanguinea* on non-crop plants, as suggested by its higher δ^{15}N values at higher aphid densities. This dynamic has important implications for conservation biological control strategies aiming to maximize species diversity, because it suggests that for most species (excluding *C. sanguinea*), *Uroleucon* is not perceived as a higher-quality resource than brassica-associated aphids. This distinction is crucial because, if alternative prey were highly preferred, they could distract predators, inadvertently releasing crop pests from predation pressure in the crop area (Deere et al., 2024).

In contrast, *E. connexa* exhibited a marked contraction in its isotopic niche as aphid density increased, signaling a shift towards greater resource specialization. The observed enrichment in δ^{15}N values further suggests that *E. connexa* intensified its consumption of prey, likely brassica aphids, as indicated by interaction networks under both lower- and higher-density conditions. These results corroborate the specialized behavior previously described (Chapter 1), in which *E. connexa* was observed foraging primarily on the soil surface within crop fields. Our findings confirm that this spatial segregation extends to its realized diet. Together, these factors explain why *E. connexa* maintained the lowest isotopic niche overlap with nearly all other species, even at higher aphid densities, supporting its functional classification as a 'niche-fidelity specialist' (Chapter 1).

Hippodamia convergens maintained its isotopic niche breadth relatively constant at lower and higher aphid densities. However, we registered a significant increase in its δ^{15}N values, indicating a shift toward a more prey-based diet under higher aphid densities. This trend is reinforced by network analyses, which show that *H. convergens* interacted with a greater richness of aphid species and at higher frequencies, particularly targeting brassica-associated aphids. Remarkably, despite this intensified predation, this species also consumed the highest diversity of plants, as revealed by DNA metabarcoding. The combined results of intense predation coupled with broad omnivory reinforce the status of *H. convergens* as a highly generalist and superior competitor in organic brassica systems (Andow et al., 2022).

The increased niche overlap between *C. sanguinea* and *H. axyridis* at higher prey densities is consistent with our previous findings (Chapter 1) showing that *H. axyridis* shifts part of its foraging activity toward non-crop plants when overall coccinellid abundance increases. Periods of high aphid density led to numerical responses of coccinellids, which can promote spatial and dietary shifts that increase niche overlap. Although our interaction networks did not explicitly capture this, *H. axyridis* likely consumes aphids effectively when both prey density on brassicas and coccinellid abundance are low, suggesting it is an efficient predator under prey scarcity. However, at higher aphid densities, *H. axyridis* failed to access abundant prey resources, unlike other species (except *C. sanguinea*), which exhibited significant δ^{15}N enrichment. This result suggests that *H. axyridis* acts as an inferior competitor in our system, avoiding prey patches heavily exploited by superior competitors to minimize the risk of interference (Cosner et al., 1999; Anderson, 2010), and also helps to explain why this species did not successfully invade and does not dominate the aphidophagous coccinellid communities in organic agroecosystems in our study region.

During periods of lower pest density, coccinellid populations and network structure appeared to be supported by non-crop plants and the alternative prey they host. Supporting this observation, Romero et al. (2023) demonstrated that under conditions of low aphid density, coccinellid species significantly consumed more pollen grains compared to scenarios where aphid density was high. Notably, the aphid genus *Uroleucon* was the only alternative prey recorded interacting with coccinellids in these plants. Moreover, the higher nestedness observed at higher aphid densities supports the observation that species adopted divergent foraging strategies, with some becoming more generalist (e.g., *C. sanguinea*) and others more specialized (e.g., *E. connexa*). Indeed, the coexistence of specialist and generalist strategies often facilitates the formation of nested networks (Bascompte et al., 2003; Vázquez & Aizen, 2004). Furthermore, higher interaction density often leads to increased nestedness (Bascompte et al., 2003). Yet, unlike mutualistic networks where nestedness promotes resilience, in trophic networks, high nestedness is associated with reduced persistence, whereas modularity enhances stability (Thébault & Fontaine, 2010). Consequently, the nested pattern observed under higher aphid density is likely transient, reflecting short-term reorganizations of species interactions in this ephemeral crop system, rather than a stable property of the network, because network structure depends on the spatial and temporal availability of interacting species (Tylianakis et al., 2017).

The predominance of trophic links between coccinellids and non-crop plants suggests a heavy reliance on floral resources like pollen and nectar. This strategy is consistent with the findings that mixed diets including non-prey items (e.g., pollen, nectar, honeydew) improve coccinellid performance when prey is also available (Lundgren, 2009). Although pollen consumption is well described for several coccinellid species (Medeiros et al., 2010; D'Ávila et al., 2016), the taxonomic extent of plant consumption in these systems has often remained obscured by methodological limitations. To overcome this limitation, we employed diagnostic PCR and DNA metabarcoding, which revealed a dietary complexity that prior studies had not fully detected. Based on diagnostic PCR, we confirmed that at least some samples contained arthropod DNA, predominantly from aphids, with only rare detections of Collembola, Diptera, and Lepidoptera. However, the subsequent metabarcoding analysis failed to yield precise taxonomic resolution for these or other arthropod prey, likely due to inherent methodological limitations. DNA metabarcoding is subject to several processing biases. Specifically, the combination of general primers and high-throughput sequencing often results in the preferential amplification of the abundant consumer DNA, thereby masking the detection of scarcer or degraded prey DNA (Cuff et al., 2023). Although previous studies successfully detected arthropods in coccinellid guts using this technique (e.g., Romero et al., 2023), the authors reported that up to 95% of the recovered sequences can derive from the coccinellids themselves, significantly reducing the overall detectability of arthropod prey DNA (including aphids). Furthermore, these detection challenges are further exacerbated by the rapid digestion rate of aphids within the coccinellid gut (e.g., Rondoni et al., 2018).

Conversely, for plant DNA detection, this issue is mitigated as pollen grains possess a more robust structure (exine) that hinders digestion by predators (Roulston & Cane, 2000; Lundgren, 2009). Moreover, since the primers targeting the ITS region are designed to anneal to plant-specific sequences, they prevent the co-amplification of the consumer's arthropod DNA (e.g., Chen et al., 2010; Moorhouse-Gann et al., 2010), thereby avoiding the bias observed in prey detection. Consequently, we successfully identified 45 distinct plant genera from which coccinellids had probably consumed floral resources. This represents a significant resolution, given that a recent molecular study on coccinellid gut contents identified only 14 plant taxa, with limited genus-level resolution (Batuecas et al., 2024). The level of detail we achieved offers novel insights into the breadth of their omnivory and underscores the critical role of trophic plasticity, showing that these generalist predators exploit a wide diversity of plant resources to persist under varying prey regimes.

Furthermore, this highlights the importance of non-crop plants as alternative resource hubs, primarily due to their provision of non-prey food resources. Previous studies have demonstrated that adjacent flower strips increase the abundance and diversity of natural enemies and enhance natural pest control services across a range of contexts (Albrecht et al., 2020; Crowther et al., 2023). Notably, these floral resources have been shown to be more relevant to coccinellids in non-crop habitats than the alternative aphids they host (da Silva et al., 2025). Supporting this, our stable isotope findings demonstrate that the richness of plant-based resources consumed by coccinellids proved to be a predictor of coccinellid trophic position and indirectly their foraging strategy. Specifically, coccinellids that feed on a greater number of plant genera demonstrate a higher degree of trophic plasticity. This active exploitation of multiple plant resources suggests a generalist foraging strategy which can compensate for the stochastic availability of high-quality animal prey. By effectively incorporating numerous plant genera into their diet, these individuals maintain a lower mean $\delta^{15}N$ signature, reflecting a greater reliance on plant-based resources and a lower trophic position compared to those whose diet is less diversified. This pattern suggests an optimal strategy under periods of prey scarcity.

We employed an integrative approach to resolve the trophic interactions of predators renowned for their generalist feeding behaviors. It is well established that variations in prey availability across space and time are critical drivers that compel generalist predators to modify their foraging strategies (López-Bao et al. 2011). However, interaction networks are typically sampled only once, precluding the assessment of their inherent spatial and temporal variability (Fortin et al. 2021). For example, in Coccinellidae, prey scarcity during early or late seasons has been proposed as a selective pressure favoring the evolution of traits that allow the use of alternative diets (Sloggett & Majerus, 2000). Our findings demonstrate that coccinellids adjust their feeding behavior in response to prey availability, resulting in observable changes in species trophic positions throughout the season, which were successfully tracked using stable isotope analysis. Finally, by constructing interaction networks of coccinellids at two distinct time points during the season (corresponding to periods of lower and higher aphid densities), we move beyond the single-time-point sampling that characterizes most studies. Although still limited, this approach allows us to begin elucidating the temporal dynamics of network structure and their implications for network resilience and stability.

References

Albrecht, M., Kleijn, D., Williams, N. M., Tschumi, M., Blaauw, B. R., Bommarco, R., ... & Sutter, L. (2020). The effectiveness of flower strips and hedgerows on pest control, pollination services and crop yield: a quantitative synthesis. *Ecology letters*, *23*(10), 1488-1498.

Almeida-Neto, M., P. Guimaraes, P. R. Guimaraes, J. R. D. Loyola, and W. Ulrich. 2008. "A Consistent Metric for Nestedness Analysis in Ecological Systems: Reconciling Concept and Measurement." *Oikos* 117(8): 1227–1239.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. D. M., & Sparovek, G. 2013. "Köppen's climate classification map for Brazil". *Meteorologische zeitschrift*, *22*(6), 711-728.

Amaral, D. S., Venzon, M., Duarte, M. V., Sousa, F. F., Pallini, A., & Harwood, J. D. (2013). Non-crop vegetation associated with chili pepper agroecosystems promote the abundance and survival of aphid predators. *Biological Control*, *64*(3), 338-346. Amarasekare, P. (2002). Interference competition and species coexistence. Proceedings of the Royal Society of London. Series B: Biological Sciences, 269(1509), 2541-2550.

Andow, D. A., Fontes, E. M., Pires, C. S., Souza, L. M., & Paula, D. P. (2022). Invasion of the coccinellid community associated with a tropical annual agroecosystem. Ecological Entomology.

Andrades, R., Jackson, A. L., Macieira, R. M., Reis-Filho, J. A., Bernardino, A. F., Joyeux, J. C., & Giarrizzo, T. (2019). Niche-related processes in island intertidal communities inferred from stable isotopes data. *Ecological Indicators*, *104*, 648-658.

Arribas, R., Díaz-Paniagua, C., Caut, S., & Gomez-Mestre, I. (2015). Stable isotopes reveal trophic partitioning and trophic plasticity of a larval amphibian guild. *PLoS One*, *10*(6), e0130897.

Bascompte, J. (2009). Disentangling the web of life. Science, 325(5939), 416-419.

Bascompte, J., Jordano, P., Melián, C. J., & Olesen, J. M. (2003). The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences*, *100*(16), 9383-9387.

Batuecas, I., Alomar, O., Castañé, C., & Agustí, N. (2024). Disentangling omnivory of heteropteran and coccinellid predators present in peach and alfalfa crops by metabarcoding analysis. *Biological Control*, *194*, 105545.

Bersier, L. F., C. Banašek-Richter, and M. F. Cattin. 2002. "Quantitative Descriptors of Food-Web Matrices." *Ecology* 83(9): 2394–2407.

Biernacki, C., Celeux, G., & Govaert, G. (2002). Assessing a mixture model for clustering with the integrated completed likelihood. *IEEE transactions on pattern analysis and machine intelligence*, *22*(7), 719-725.

Blüthgen, N., F. Menzel, and N. Blüthgen. 2006. "Measuring Specialization in Species Interaction Networks." *BMC ecology* 6(1): 9.

Bolnick, D. I., Ingram, T., Stutz, W. E., Snowberg, L. K., Lau, O. L., & Paull, J. S. (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1689), 1789-1797.

Bowers, C., Toews, M. D., & Schmidt, J. M. (2021). Winter cover crops shape early-season predator communities and trophic interactions. *Ecosphere*, *12*(7), e03635.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, . . . and B. M. Bolker. 2017. "Modeling Zero-Inflated Count Data with glmmTMB." *BioRxiv* 132753.

Cameron, E. S., Schmidt, P. J., Tremblay, B. J. M., Emelko, M. B., & Müller, K. M. (2021). Enhancing diversity analysis by repeatedly rarefying next generation sequencing data describing microbial communities. Scientific reports, 11(1), 22302. https://doi.org/10.1038/s41598-021-01636-1

Castro, É. C., Camargo, N. F., Novaes, D. R., Pereira, M. A., Nardoto, G. B., & Togni, P. H. (2024). Large-scale crops are sources of polyphagous pest populations for small farmers at the landscape level: an isotope tracing model. *Journal of Pest Science*, *97*(2), 561-574.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., ... & Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PloS one*, *5*(1), e8613.

Cosner, C., DeAngelis, D. L., Ault, J. S., & Olson, D. B. (1999). Effects of spatial grouping on the functional response of predators. *Theoretical population biology*, *56*(1), 65-75.

Costa, F. J., Abdalla-Filho, A. L., Boesing, A. L., Marques, T. S., Augusto, F. G., Reis, T. D., ... & Martinelli, L. A. (2025). Stable isotope analysis reveals dietary and foraging ecology in Neotropical birds. *Ornithological Applications*, duaf058.

Crawley, M. J., Krebs, R. (1992) Foraging theory. In: Crawley, M. J. (eds) Natural enemies. Blackwell Scientific Publications, Oxford, pp 90–114.

Crowther, L. I., Wilson, K., & Wilby, A. (2023). The impact of field margins on biological pest control: a meta-analysis. *BioControl*, *68*(4), 387-396.

D'Ávila, V. A., Aguiar-Menezes, E. L., Gonçalves-Esteves, V., Mendonça, C. B. F., Pereira, R. N., & Santos, T. M. (2016). Morphological characterization of pollens from three Apiaceae species and their ingestion by twelve-spotted lady beetle (Coleoptera: Coccinellidae). Brazilian Journal of Biology, 76, 796-803.

da Silva, A. C., Oliveira, L. C., de Lacerda, J. G., Venzon, M., Frizzas, M. R., Sujii, E. R., & Togni, P. H. (2025). Floral availability outweighs aphid presence in supporting coccinellid abundance across life stages and promotes aphid predation. *Basic and Applied Ecology*.

de Camargo, N. F., dos Reis, G. G., Mendonça, A. F., Laumann, R. A., Nardoto, G. B., de Camargo, A. J., & Vieira, E. M. (2022). Native marsupial acts as an in situ biological control agent of the main soybean pest (Euschistus heros) in the Neotropics. European Journal of Wildlife Research, 68(5), 1-16.

de Carvalho, D. R., de Castro, D. M. P., Callisto, M., Chaves, A. J. D. M., Moreira, M. Z., & Pompeu, P. S. (2019). Stable isotopes and stomach content analyses indicate omnivorous habits and opportunistic feeding behavior of an invasive fish. *Aquatic Ecology*, *53*(3), 365-381.

Deere, J. A., Beretta, G. M., van Rijn, P. C., Messelink, G. J., Leman, A., & Janssen, A. (2024). Does alternative food for predatory arthropods improve biological pest control? A meta-analysis. *Biological Control*, *198*, 105605.

Dunne, J. A., R. J. Williams, and N. D. Martinez. 2002. "Food-Web Structure and Network Theory: The Role of Connectance and Size." *Proceedings of the National Academy of Sciences* 99(20): 12917–12922.

Dixon, A. F. G., & Dixon, A. E. (2000). Insect predator-prey dynamics: ladybird beetles and biological control. Cambridge University Press.

Evans, E. W. (2009). Lady beetles as predators of insects other than Hemiptera. *Biological Control*, *51*(2), 255-267.

Feinsinger, P., & Swarm, L. A. (1982). " Ecological Release," Seasonal Variation in Food Supply, and the Hummingbird Amazilia Tobaci on Trinidad and Tobago. *Ecology*, *63*(5), 1574-1587.

Fortin, M. J., Dale, M. R., & Brimacombe, C. (2021). Network ecology in dynamic landscapes. *Proceedings of the Royal Society B*, *288*(1949), 20201889.

Giorgi, J. A., Vandenberg, N. J., McHugh, J. V., Forrester, J. A., Ślipiński, S. A., Miller, K. B., ... & Whiting, M. F. (2009). The evolution of food preferences in Coccinellidae. *Biological control*, *51*(2), 215-231.

Gliesch, L., de Andrade Linhares, B., Ozorio, C. P., Ott, P. H., Jacoby, J., Bugoni, L., & Nunes, G. T. (2023). Specialist shorebird respond to prey and habitat availability through trophic plasticity. *Food Webs*, *36*, e00300.

Gordon, R. D. (1985). The Coccinellidae (Coleoptera) of America north of Mexico.

Grabarczyk, E. E., Tillman, P. G., Querejeta, M., & Schmidt, J. M. (2025). Honey, What's for Dinner? Dietary Overlap and Size Dimorphism Between Female and Male Joro Spiders (Trichonephila clavata). *Integrative Zoology*.

Grün, B., & Leisch, F. (2007). Fitting finite mixtures of generalized linear regressions in R. *Computational Statistics & Data Analysis*, *51*(11), 5247-5252.

Gyenge, J. E., Edelstein, J. D., & Salto, C. E. (1998). Efectos de la temperatura y la dieta en la biología de Eriopis connexa (Germar) (Coleoptera: Coccinellidae). *Anais da Sociedade Entomológica do Brasil*, *27*, 345-356.

Hagen, K. S. (1962). Biology and ecology of predaceous Coccinellidae. *Annual review of entomology*, *7*(1), 289-326.

Hartig, F., & Lohse, L. (2020). *DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. Version 0.3. 3.0.*

He, X., & Sigsgaard, L. (2019). A floral diet increases the longevity of the coccinellid *Adalia bipunctata* but does not allow molting or reproduction. *Frontiers in Ecology and Evolution*, *7*, 6.

Herrmann, N. C., Stroud, J. T., & Losos, J. B. (2021). The evolution of 'ecological release'into the 21st century. *Trends in Ecology & Evolution*, *36*(3), 206-215.

Hodek, I., Honek, A., & Van Emden, H. F. (Eds.). (2012). Ecology and behaviour of the ladybird beetles (Coccinellidae). *John Wiley & Sons*.

Ingrao, A. J., Schmidt, J., Jubenville, J., Grode, A., Komondy, L., VanderZee, D., & Szendrei, Z. (2017). Biocontrol on the edge: Field margin habitats in asparagus fields influence natural enemy-pest interactions. *Agriculture, Ecosystems & Environment*, *243*, 47-54.

Iuliano, B., Gratton, C., Kim, T. N., & Spiesman, B. J. (2024). Semi-natural habitat, but not aphid amount or continuity, predicts lady beetle abundance across agricultural landscapes. *Journal of Applied Ecology*, *61*(8), 1881-1893.

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*(3), 595-602.

Jusino, M. A., Banik, M. T., Palmer, J. M., Wray, A. K., Xiao, L., Pelton, E., ... & Lindner, D. L. (2019). An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. *Molecular ecology resources*, *19*(1), 176-190.

Kamenova, S., Mayer, R., Rubbmark, O. R., Coissac, E., Plantegenest, M., & Traugott, M. (2018). Comparing three types of dietary samples for prey DNA decay in an insect generalist predator. *Molecular Ecology Resources*, *18*(5), 966-973.

Kennedy, S. R., Prost, S., Overcast, I., Rominger, A. J., Gillespie, R. G., & Krehenwinkel, H. (2020). High-throughput sequencing for community analysis: the promise of DNA barcoding to uncover diversity, relatedness, abundances and interactions in spider communities. *Development Genes and Evolution*, *230*(2), 185-201.

Kerley, G. I., Landman, M., Ficetola, G. F., Boyer, F., Bonin, A., Rioux, D., ... & Coissac, E. (2018). Diet shifts by adult flightless dung beetles Circellium bacchus, revealed using DNA metabarcoding, reflect complex life histories. *Oecologia*, *188*(1), 107-115.

Lefort, M. C., Wratten, S., Cusumano, A., Varennes, Y. D., & Boyer, S. (2017). Disentangling higher trophic level interactions in the cabbage aphid food web using high-throughput DNA sequencing. *Metabarcoding and Metagenomics*, *1*, 13709.

Lixa, A. T., Campos, J. M., Resende, A. L., Silva, J. C., Almeida, M. M., & Aguiar-Menezes, E. L. (2010). Diversidade de Coccinellidae (Coleoptera) em plantas aromáticas (Apiaceae) como sítios de sobrevivência e reprodução em sistema agroecológico. Neotropical Entomology, 39, 354-359.

López-Bao, J. V., Palomares, F., Rodríguez, A., & Ferreras, P. (2011). Intraspecific interference influences the use of prey hotspots. *Oikos*, *120*(10), 1489-1496.

Lunde, L. F., Birkemoe, T., Kauserud, H., Boddy, L., Jacobsen, R. M., Morgado, L., ... & Maurice, S. (2022). DNA metabarcoding reveals host-specific communities of arthropods residing in fungal fruit bodies. *Proceedings of the Royal Society B*, *289*(1968), 20212622.

Lundgren, J. G. (2009). Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. *Biological Control*, *51*(2), 294-305.

Medeiros, M. A. D., Ribeiro, P. D. A., Morais, H. C. D., Castelo Branco, M., Sujii, E. R., & Salgado-Laboriau, M. L. (2010). Identification of plant families associated with the predators Chrysoperla externa (Hagen)(Neuroptera: Chrysopidae) and Hippodamia convergens Guérin-Menéville (Coleoptera: Coccinelidae) using pollen grain as a natural marker. Brazilian Journal of Biology, 70, 293-300.

Minagawa, M., & Wada, E. (1984). Stepwise enrichment of 15N along food chains: further evidence and the relation between δ15N and animal age. *Geochimica et cosmochimica acta*, *48*(5), 1135-1140.

Moorhouse-Gann, R. J., Dunn, J. C., De Vere, N., Goder, M., Cole, N., Hipperson, H., & Symondson, W. O. (2018). New universal ITS2 primers for high-resolution herbivory analyses using DNA metabarcoding in both tropical and temperate zones. *Scientific Reports*, *8*(1), 8542.

Olesen, J. M., J. Bascompte, Y. L. Dupont, and P. Jordano. 2007. "The Modularity of Pollination Networks." *Proceedings of the national academy of sciences* 104(50): 19891–19896.

Paine, R. T. (1980). Food webs: linkage, interaction strength and community infrastructure. *Journal of animal ecology*, *49*(3), 667-685.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, *83*(3), 703-718.

R Core Team (2025) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Romero, V., Zaviezo, T., Sánchez, R., & Grez, A. A. (2023). More than aphids in the guts of coccinellids revealed by molecular and visual analyses. *BioControl*, *68*(4), 435-446.

Rondoni, G., Fenjan, S., Bertoldi, V., Ielo, F., Djelouah, K., Moretti, C., ... & Conti, E. (2018). Molecular detection of field predation among larvae of two ladybird beetles is partially predicted from laboratory experiments. *Scientific reports*, *8*(1), 2594.

Roulston, T. A. H., & Cane, J. H. (2000). Pollen nutritional content and digestibility for animals. *Plant systematics and Evolution*, *222*(1), 187-209.

Roy, H. E., Brown, P. M., Adriaens, T., Berkvens, N., Borges, I., Clusella-Trullas, S., ... & Zhao, Z. (2016). The harlequin ladybird, Harmonia axyridis: global perspectives on invasion history and ecology. *Biological invasions*, *18*(4), 997-1044.

Schmidt, J. M., Russell, K., Bowers, C., Coffin, A. W., Thompson, M., Grabarczyk, E. E., ... & Olson, D. (2024). Resource overlap and infrequent predation on key pests show vulnerability in cotton biological control services. *Agriculture, Ecosystems & Environment*, *374*, 109164.

Sicsú, P. R., Macedo, R. H., & Sujii, E. R. (2020). Lady beetle oviposition site choices. The Florida Entomologist, 103(2), 228-235.

Sicsú, P. R., Macedo, R. H., Sujii, E. R. (2015). Oviposition site selection structures niche partitioning among coccinellid species in a tropical ecosystem. Neotropical Entomology, 44, 430-438.

Sloggett, J. J., & Majerus, M. E. (2000). Habitat preferences and diet in the predatory Coccinellidae (Coleoptera): an evolutionary perspective. *Biological Journal of the linnean Society*, *70*(1), 63-88.

Slusher, E. K., Cottrell, T., Gariepy, T., Acebes-Doria, A., Querejeta Coma, M., Toledo, P. F., & Schmidt, J. M. (2024). A molecular approach to unravel trophic interactions between parasitoids and hyperparasitoids associated with pecan aphids. *Journal of Insect Science*, *24*(4), 5.

Smithson, M., & Verkuilen, J. (2006). A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. Psychological Methods, 11(1), 54.

Staudacher, K., Rennstam Rubbmark, O., Birkhofer, K., Malsher, G., Sint, D., Jonsson, M., & Traugott, M. (2018). Habitat heterogeneity induces rapid changes in the feeding behaviour of generalist arthropod predators. *Functional Ecology*, *32*(3), 809-819.

Stephens, D. W., & Krebs, J. R. (1989). Foraging theory. In Foraging theory. Princeton university press.

Thébault, E., & Fontaine, C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science*, *329*(5993), 853-856.

Togni, P. H. B., Venzon, M., Lagôa, A. C. G., & Sujii, E. R. (2019). Brazilian legislation leaning towards fast registration of biological control agents to benefit organic agriculture. Neotropical entomology, 48(2), 175-185.

Togni, P. H., Venzon, M., Muniz, C. A., Martins, E. F., Pallini, A., & Sujii, E. R. (2016). Mechanisms underlying the innate attraction of an aphidophagous coccinellid to coriander plants: Implications for conservation biological control. Biological Control, 92, 77-84.

Tylianakis, J. M., & Morris, R. J. (2017). Ecological networks across environmental gradients. *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 25-48.

Vázquez, D. P., & Aizen, M. A. (2004). Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology*, *85*(5), 1251-1257.

Wheeler Jr, A. G., & Jubb Jr, G. L. (1979). Scymnus cervicalis Mulsant, a predator of grape phylloxera, with notes on S. Brullei Mulsant as a predator of woolly aphids on elm (Coleoptera: Coccinellidae). *The Coleopterists' Bulletin*, 199-204.

Supplementary material

Table S1. Results from the model selection to determine the optimal number of components (*K*) for the mixture model. Models were fitted across a range of *K* (1 to 5) and compared using the Integrated Completed Likelihood (ICL) as the primary selection criterion. Other fit criteria are also presented: Log-Likelihood, the Akaike Information Criterion (AIC), and the Bayesian Information Criterion (BIC). The model with the lowest ICL value was chosen as the best-fitting and most parsimonious solution. The Number of Components (*K*) indicates the number of latent classes, while Iterations denotes the number of steps the Expectation-Maximization (EM) algorithm took to converge.

Interactions	converged	K	ICL	logLik	AIC	BIC
32	TRUE	2	755.9	-360.604	735.2	755.8
48	TRUE	3	775.9	-357.023	736.0	768.5
126	TRUE	4	802.1	-352.246	734.5	778.7
168	TRUE	4	802.1	-352.246	734.5	778.7
2	TRUE	1	880.2	-432.665	871.3	880.2

Table S2. Number of positive detections of arthropods in the gut content of four coccinellid species using diagnostic PCR analysis.

		Positive detections				
Coccinellid species	**N. of samples**	**Aphid**	**Collembola**	**Diptera**	**Lepdoptera**	**Total**
Cycloneda sanguinea	61	16	1	3	0	20

Eriopis connexa	7	1	0	0	0	1
Harmonia axyridis	31	16	0	0	1	17
Hippodamia convergens	141	71	1	12	0	84
Total	240	104	2	15	1	122

Table S3. Number of sequences reads corresponding to plant genera detected in the gut content of four coccinellid species using DNA metabarcoding analysis. CS = *Cycloneda sanguinea*, EC = *Eriopis connexa*, HA = *Harmonia axyridis*, HC = *Hippodamia convergens*.

Genus	**Family**	**CS**	**EC**	**HA**	**HC**	**Total**
Amaranthus	Amaranthaceae	3019	198	2435	5223	10875
Bidens	Asteraceae	2559	185	212	3186	6142
Tithonia	Asteraceae	1007	0	422	899	2328
Galinsoga	Asteraceae	294	0	345	857	1496
Eleusine	Poaceae	245	0	162	2193	2600
Sonchus	Asteraceae	225	0	0	194	419
Pinus	Pinaceae	203	0	0	38	241
Artemisia	Asteraceae	199	0	0	0	199
Digitaria	Poaceae	173	0	0	585	758
Euphorbia	Euphorbiaceae	107	0	0	1921	2028
Alternanthera	Amaranthaceae	78	0	0	664	742
Trema	Cannabaceae	53	0	11	796	860
Cynodon	Poaceae	24	0	6	0	30
Syzygium	Myrtaceae	13	0	0	59	72
Cenchrus	Poaceae	10	0	0	178	188
Eucalyptus	Myrtaceae	10	0	0	0	10
Emilia	Asteraceae	2	0	0	137	139
Sorghum	Poaceae	1	0	0	8	9
Cyperus	Cyperaceae	1	15	0	212	228
Urochloa	Poaceae	1	0	0	0	1
Plantago	Plantaginaceae	0	0	0	159	159
Oryza	Poaceae	0	0	41	159	200
Ilex	Aquifoliaceae	0	0	0	17	17
Vernonia	Asteraceae	0	0	0	1	1
Eragrostis	Poaceae	0	0	0	105	105
Tapirira	Anacardiaceae	0	0	0	4	4
Psidium	Myrtaceae	0	0	0	27	27
Citrus	Rutaceae	0	200	0	5	205
Corymbia	Myrtaceae	0	0	0	219	219
Cucumis	Cucurbitaceae	0	0	0	41	41
Cannabis	Cannabaceae	0	0	0	18	18
Triticum	Poaceae	0	0	0	143	143
Pteris	Pteridaceae	0	0	0	59	59
Matayba	Sapindaceae	0	0	1	1	2
Erigeron	Asteraceae	0	0	0	1	1

Cucurbita	Cucurbitaceae	0	0	0	4	4
Byrsonima	Malpighiaceae	0	0	0	18	18
Carya	Juglandaceae	0	0	0	5	5
Solanum	Solanaceae	0	0	0	2	2
Brassica	Brassicaceae	0	2	5	394	401
Chascolytrum	Poaceae	0	0	0	200	200
Parthenium	Asteraceae	0	0	0	18	18
Huperzia	Lycopodiaceae	0	0	0	12	12
Baccharis	Asteraceae	0	0	0	144	144
Pleioblastus	Poaceae	0	0	0	2	2
Total		8224	600	3640	18908	

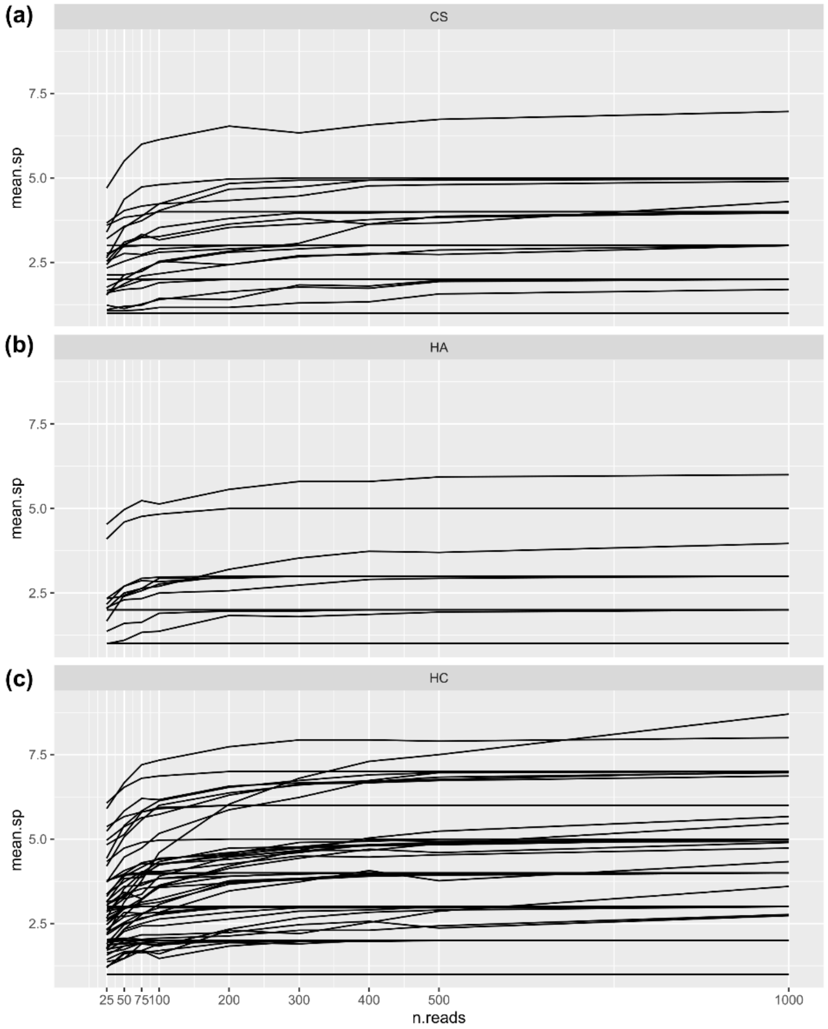

Fig. S1. Curves showing the mean number of plant genera identified (taxonomic richness) as a function of the number of sequences reads subsampled from the gut content of each coccinellid species (CS = *Cycloneda sanguinea*, HA = *Harmonia axyridis*, HC = *Hippodamia convergens*).

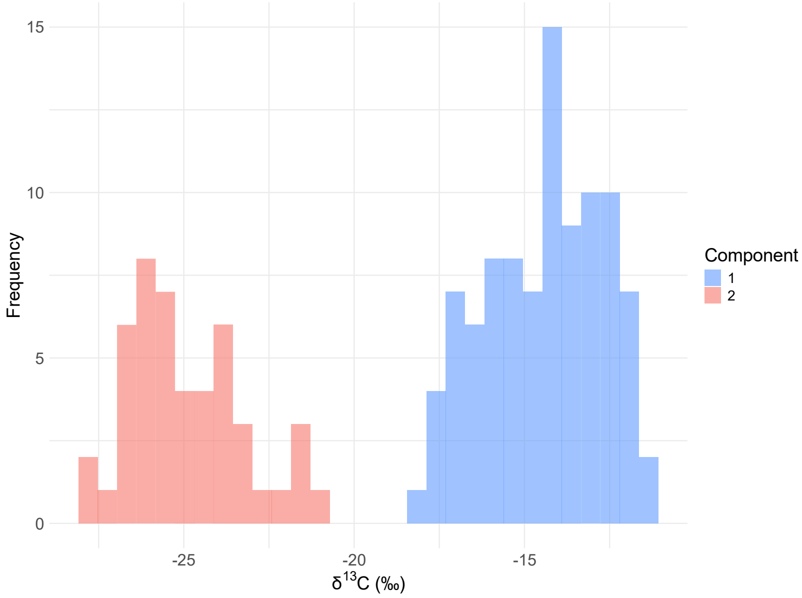

Fig. S2. Frequency distribution (histograms) of the predicted δ^{13}C signatures for the two latent components (*K*=2) identified by the Finite Mixture Regression (FMR) model. These components represent distinct groups with potentially different relationships between aphid density (predictor) and δ^{13}C (response), as modeled using Gaussian components.

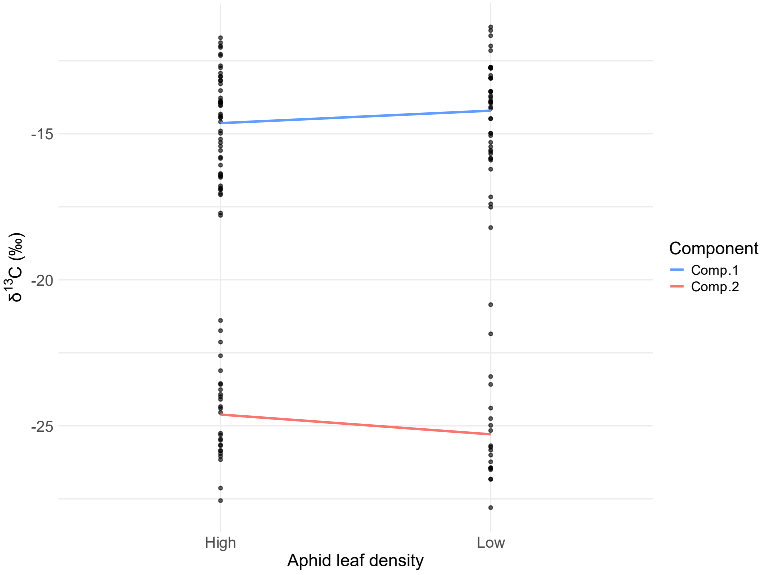

Fig. S3. Predicted component-specific regression lines showing the relationship between aphid density (predictor) and coccinellid δ^{13}C signatures (response). The distinct regression lines correspond to the two latent groups (*K*=2) identified by the Finite Mixture Regression (FMR**)** model, highlighting their potentially different ecological responses to prey availability.

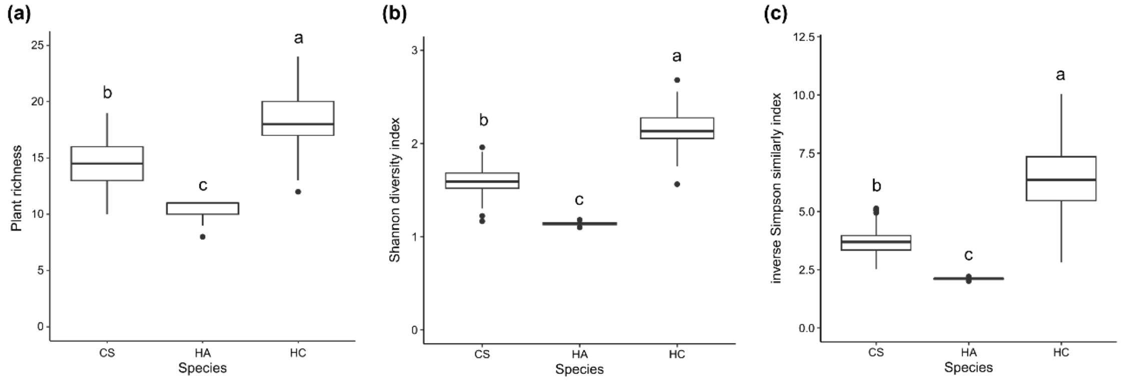

Fig. S4. Plant genus richness and diversity in the gut contents of three coccinellid species. Richness (a), Shannon index (b), and Simpson index (c) of plant genera identified via metabarcoding are compared among *Cycloneda sanguinea* (CS), *Harmonia axyridis* (HA), and *Hippodamia convergens* (HC). Statistical differences ($p < 0.05$) between species are denoted by different lower-case letters (Kruskal–Wallis rank-sum test).

CHAPTER 3

Different natural vegetation types in the landscape shape coccinellid trophic interactions and biological control

* This chapter is based on a manuscript submitted to the journal *Ecological Applications* and is formatted according to its guidelines.

Abstract

Organic agroecosystems rely on biodiversity-based services, such as biological control, which are influenced by local management and the surrounding landscape. Both local and landscape characteristics have the potential to shape aphidophagous coccinellid communities and affect biological control. Using a multiscale approach, we aimed to assess how different natural vegetation types in the landscape influence the architecture of plant-aphid-coccinellid networks, and how these interactions affect biological control. The study was conducted in 28 farms that grew organic collard from 2022 to 2024 in the Federal District, Brazil. Direct sampling and field observations enabled us to model the plant-aphid-coccinellid networks, considering both crop and non-crop plants in the field margins across different landscape contexts. We also quantified biological control services through exclusion experiments in the crop area. The plant-aphid-coccinellid network showed high connectance, generality, and vulnerability but low nestedness, reflecting the generalist behavior of coccinellids in agricultural systems. Non-crop plants provided prey, plant-based food, and shelter for coccinellids. At the landscape level, forests reduced coccinellid abundance due to the adverse effects on aphid densities. In contrast, the proportion of agricultural cover favored coccinellid abundance because it promoted aphid infestation at broad spatial scales. Grasslands (semi-natural habitats) increased network generality, while the amount of savannas favored network modularity and positively impacted aphid predation. Nevertheless, the landscape effects on community abundance and network architecture varied among spatial scales. According to a structural equation model, prey availability, network structure, and landscape composition jointly influenced aphid control, with outcomes depending on the combined effects of these factors. Our results demonstrate that landscapes mediate species interactions, which in turn shape biological control, thereby reinforcing the value of multiscale interaction-based approaches in agroecosystem management. This approach also revealed the need for different vegetation types to support key ecosystem services in agricultural areas.

KEYWORDS: Agroecology; conservation biological control; ecosystem services; landscape ecology; natural enemies; predation; non-crop plants; tropical

INTRODUCTION

Human-induced land-cover changes have led to the degradation and fragmentation of natural habitats (Boivin et al., 2016; Norder et al., 2020), creating "novel ecosystems" characterized by homogenized and altered communities assembling in local habitats (Macdonald & King, 2018; Newbold et al., 2018). Coupled with environmental changes, such transformations pose significant challenges to essential ecosystem services. This issue is particularly critical for organic agriculture in tropical regions, which relies on biodiversity-driven ecosystem services to sustain productivity (Noriega et al., 2018; Ramos et al., 2020). In this context, nature-based solutions, which leverage natural processes to address environmental and societal goals (Kabisch et al., 2016), emerge as a promising approach to reconcile biodiversity conservation with sustainable food production in fragmented landscapes.

Landscape changes can modify habitat structure, reducing spatial and temporal overlap between pests and their natural enemies, and thereby lowering natural enemy searching efficiency (Tylianakis & Morris, 2017). Consequently, landscape composition and configuration are key determinants of the structure and dynamics of natural enemy communities and their interactions in local habitats (Fahrig et al., 2011; Hass et al., 2018; Tscharntke et al., 2012). Structurally complex landscapes can enhance local diversity and mitigate the effects of intensive management, depending on the species pool available to colonize crop areas (Novaes et al., 2024; Tscharntke et al., 2005). Therefore, remnants of natural vegetation may serve as reservoirs of beneficial insects that disperse to adjacent crop fields, sustaining community diversity and the stability of trophic interactions (Assunção et al., 2022; Marins et al., 2024).

Landscape-mediated changes in species composition and abundance can alter how organisms interact within ecological networks, as network architecture responds to landscape features (Lami et al., 2021; Moreira et al., 2018; Tylianakis et al., 2007).

Habitat homogenization and disturbance, for instance, may increase network generalization and reduce modularity (Aizen et al., 2008; Tylianakis et al., 2007), while decreasing nestedness across spatial scales (Moreira et al., 2015). Which can potentially reduce the stability and reliability of ecosystem services such as biological pest control, depending on how these structural changes influence interaction complementarity and functional redundancy. Species at higher trophic levels often operate at broader spatial scales than those at lower levels (Holt, 1996), which can lead to scale-dependent responses to landscape features and thereby influence patterns of species encounters and interactions at local scales. Additionally, each land cover type may have different effects on community patterns, affecting how species assemble in different habitats. For example, a recent study on tropical organic agroecosystems demonstrated that grasslands affect primarily parasitoid abundance, while forests favor species richness and both mediate aphid parasitism rates (Marins et al., 2024).

Aphidophagous coccinellids may be suitable models for understanding such multiscale effects on biodiversity because they respond to both landscape and local scales (Gardiner et al., 2009a,b). Landscape simplification threatens native species by reducing habitat quality and connectivity (Grez & Zaviezo, 2024; Soares et al., 2023), while fragmentation can lead to a decline in their abundance (Taranto et al., 2022). In contrast, natural and semi-natural habitats enhance coccinellid populations because species can use different habitat types in the landscape (Grez et al., 2021). Consequently, the structural and functional composition of the landscape can determine the effectiveness of biological control provided by them (e.g., Gardiner et al., 2009a; Grez & Zaviezo, 2024). At the local scale, organic farming promotes greater evenness within natural enemy assemblages (Crowder et al., 2010). In tropical smallholder organic systems, for example, farmers often maintain strips of diverse non-crop plants that provide food and shelter for several species (Amaral et al., 2013), increasing habitat permeability for coccinellids (Venzon et al., 2019).

Therefore, evidence that landscape composition affects coccinellid communities and related biological control services in well-established (e.g., Elliott et al., 2002; Gardiner et al., 2009a; Grez et al., 2014; Grez & Zaviezo, 2024; Yang et al., 2018, 2019). While these studies have advanced our understanding of community-level responses, less is known about how landscape-mediated effects on species pool influence the structure of local trophic interactions, ultimately affecting aphid predation (e.g., Tylianakis et al., 2007). More importantly, it is necessary to know how each natural vegetation type in the landscape mediates community patterns and species interactions. Here, we move beyond species richness and abundance to test how landscape composition influences both community- and network-level properties of plant–aphid–coccinellid interactions across multiple spatial scales and their combined effects on biological control. Specifically, we examined: (a) the effects of landscape composition on coccinellid abundance, richness, and diversity; (b) on aphid density; (c) on network metrics across scales; and (d) on biological control services. We hypothesize that agricultural landscapes increase aphid densities, thereby favoring generalist coccinellid species, that are more tolerant of homogeneous and disturbed environments. This shift toward generalists is expected to reduce network modularity and increasing nestedness, leading to greater overlap and redundancy in predator–prey interactions. Therefore, intensified intraguild predation and competitive interference among predators may reduce aphid suppression and ultimately weaken biological control. We also expect land-use types to influence coccinellids and network structure at distinct spatial scales, reflecting scale-dependent responses influenced mainly through different vegetation types at the landscape level. This integrated framework advances our understanding of how landscape composition shapes multitrophic interactions and their cascading effects on ecosystem functioning.

MATERIAL AND METHODS

Landscape context and study area

We conducted the study in the Brazilian Federal District (15°46'13.140" S, 47°44'46.408" W), a region located within Cerrado biome (Francoso et al., 2020). The climate is characterized by dry winters, hot humid summers, with an annual rainfall of 1400 mm concentrated in the rainy season (November to April) (Alvares et al., 2013). The Cerrado harbors forests, savanna, and grasslands. Savanna formations consist of scattered trees and shrubs in a grass-dominated landscape, lacking continuous canopy (Ribeiro & Walter 2008).

We sampled 28 organic farms with collard crops, at least 2 km apart, between 2022 and 2024 during the dry season (August–October), when coccinellids and aphids are most abundant. Farms averaged 10.4 ± 2.4 ha (mean ± SE), belonged to smallholders, and had organic certification for at least the last five years (Togni et al., 2019). Across all farms, collard crops covered an average area of 4035 ± 1345 m² (mean ± SE) and were bordered by 5 to 10-m-wide strips of spontaneous non-crop vegetation, mainly consisting of native or naturalized herbaceous species commonly considered weeds. For instance, the main non-crop plants found in these farms were from Asteraceae family: *Bidens pilosa*, *Galinsoga parviflora*, *Sonchus oleraceus*, and *Tithonia diversifolia*. Here, we defined the crop and adjacent non-crop area (up to 10 m) as our local scale. Farmers used manual methods for weeding in the inter-row areas. Aphids were controlled with water sprinklers, the removal of heavily infested plants, and, occasionally, plant-based extracts permitted by Brazilian legislation for organic crops. The latter method was not applied during the sampling period of this study. Despite differences among farms, non-crop habitats consistently harbored diverse plant communities at various phenological stages and provided similar structural and temporal resources.

Coccinellids and aphids sampling

Coccinellids and aphids were sampled once per farm, and each farm was considered a separate sampling unit. We randomly established five 1 × 1 m quadrats per habitat type (crop and adjacent non-crop strips) on each farm, with a total of 140 quadrats per habitat. The quadrats in the non-crop strips were located up to 5 m from the crop edge, as strip widths ranged from 5 to 10 m across farms. Each quadrat was inspected by at least two trained observers. All coccinellids (and life stages) were recorded and identified to the lowest possible taxonomic level. When possible, their behavior was observed to determine whether they preyed on aphids or used plants as refuges or alternative food resources. Individuals were then manually collected to avoid resampling. Observation and sampling continued until no additional individuals were detected (10–20 min/quadrat). Aphids on both crop and non-crop plants were also identified to the lowest taxonomic level. Representative insect and plant samples were taken to the laboratory for identification, assisted by a taxonomist and a reference collection when needed.

Aphid density and biological control in farms

To estimate aphid density on crops, we randomly selected 40 collard plants per farm and counted the total number of aphids on three leaves per plant. Each plant was treated as a sampling unit, and we did not differentiate between winged and wingless aphids. To assess aphid biological control, we conducted an exclusion experiment following Gardiner et al. (2009a). On each farm, we randomly selected 30 brassica plants in the vegetative stage (30–45 days after transplanting). We enclosed aphid-infested leaves in mesh bags (sizing 30 × 20 cm) to exclude predators (treatment), pairing each with an open, tape-marked leaf (control). This paired design controlled for plant-level variation affecting aphids and their natural enemies.

During setup, we removed all non-aphid organisms and standardized aphid numbers on treatment and control leaves within each plant, maintaining densities between 10 and 100 aphids according to natural variation in each plant. Five days later, we recounted aphids on both leaves. Since no rainfall occurred, we ruled out dislodgement as a contributing factor to mortality. We quantified the impact of natural enemies using the Biological Control Service Index (BSI) (Gardiner et al., 2009a), based on final aphid counts on treatment and control leaves. We calculated BSI as the relative difference in aphid abundance between treatments and controls, averaged across replicates within each farm. Values range from 0 (no control) to 1 (complete control). The index was calculated as follows:

$$\mathrm{BSI} = \frac{\sum_{p=1}^{4} \frac{(A_{c,p} - A_{o,p})}{A_{c,p}}}{n}$$

where *Ac* represents the number of aphids counted on the caged plant on day five, *Ao* is the number of aphids on the open (control) plant on day five, *p* denotes the plot, and *n* corresponds to the number of replicates within each site. Higher BSI values indicate stronger effects of natural enemies. Parasitized aphids were excluded to isolate predator-induced mortality, as we were interested only in predation effects. However, this approach did not allow us to distinguish the effects of coccinellids from those of other predators.

Plant-aphid-coccinellid network architecture

To characterize tritrophic species interactions, we used field data on plants (bottom level), aphids (middle level), and coccinellids (top level), pooled across all quadrants in each farm, to construct a species-level weighted matrix representing the frequency of interactions across all farms. Interactions were identified through direct and behavioral observations of coccinellids and aphids on plants, including cases where coccinellids used plants as shelter or alternative food sources. Aphids were linked to their host plants, and coccinellids to their aphid prey. For methodological consistency, aphid-coccinellid interactions were considered to occur when coccinellids were observed on plants infested with aphids, assuming that their primary interaction was with the aphids. Direct coccinellid–plant interactions were recorded only when aphids were absent or when coccinellids were observed using floral resources. The frequency of coccinellid–aphid interactions was quantified based on the presence of aphids on the plants. Thus, when a coccinellid was recorded on an aphid-infested plant, it was counted as a single interaction event. Consequently, these records represent potential, rather than individual, coccinellid–aphid interactions occurring within the same plant.

We then constructed weighted tripartite networks, a general network by pooling data across all farms and analyzed using the same metrics and partial networks for each farm. These partial networks were constructed to represent a continuum of interaction patterns within the general network, influenced by both landscape-level and local contexts. We extracted ecological and topological metrics from these networks, including network-level metrics: a) connectance: the proportion of established interactions relative to the total possible realized (Bersier et al., 2002); b) specialization (*H2'*): the overall degree of network specialization, indicating how selectively species interact, with higher values reflecting stronger specialization (Blüthgen et al., 2006); c) modularity (*Q*): the degree to which the network is divided into clearly delimited modules of species (Olesen et al., 2007); d) nestedness (*NODF*): which quantifies how much specialist species interact with subsets of the partners that generalist species interact with (Almeida-Neto et al., 2008); e) vulnerability (*V*) average number of predators per prey, reflecting how strongly species function as prey (Schoener, 1989).

We also extracted node-level metrics: a) network size (*S*): the number of nodes (i.e., species and resource types) in the network (Dunne et al., 2002b); b) trophic generality (*G*): the average number of preys per predator, indicating the prevalence of generalists versus specialists in the system (Schoener, 1989). We calculated all network metrics using the *bipartite*, *igraph*, and *cheddar* packages in R (Antonov et al., 2023; Dormann et al., 2009; Hudson et al., 2013). We used these metrics to assess how trophic structure and network organization relate to aphid biological control efficiency across farms.

Landscape characterization

We characterized the landscape based on 30 m resolution satellite imagery from the MapBiomas Collection 9 (Souza Jr. et al., 2020), which distinguishes over 30 land cover types. We reclassified the original MapBiomas land-use into six broader categories: Forests, Savannas, Grasslands, Pasture, Agriculture and Urban areas. This reclassification was performed by grouping the original MapBiomas classes as follows: (1) Forests, which included Forest Formation, Mangrove, and Wooded Sandbank Vegetation; and (2) Agriculture, which included Agriculture, Soybean, Sugarcane, Rice, Cotton, Other Temporary Crops, Perennial Crop, Coffee, Citrus, and Other Perennial Crops. We performed these analyses using the free software QGIS (QGIS, 2025). In our study area, grasslands comprise disturbed native vegetation interspersed with both natural and anthropogenic pastures and therefore represent semi-natural habitats. We used the *Scalescape* package to estimate distance-weighted landscape effects on response variables (Lowe et al., 2022). This approach identified the buffer size that best explained each response and quantified how landscape influence decayed with distance. It also enabled us to assess how multiscale factors influenced predator communities, prey density, and network metrics, while mitigating issues associated with fixed or multiple-buffer approaches (Lowe et al., 2022) (Appendix S1: supplementary methods).

Statistical analysis

To test whether the observed network metrics differed from random expectations, we compared them to null models generated from pooled species interaction data across all farms using the data pooled data of all farms. We randomized the interaction matrices 1,000 times using the r2dtable method in the *bipartite* package, which preserves species' interaction frequencies by fixing marginal totals and prevents artifacts related to model construction (Dormann et al., 2009). We then calculated the metrics for each randomized network and assessed deviations from the null distributions using Z-scores and one-sample t-tests (Vanbergen et al., 2017).

To evaluate the direction and strength of the effects of landscape composition (within the spatial scale identified a priori from the *Scalescape* analysis – See Appendix S1: supplementary methods) on coccinellid community attributes (abundance, richness, and diversity), aphid density, and network variables, we fitted Generalized Linear Models (GLMs) using the *lme4* and *glmmTMB* packages (Bates et al., 2015; Brooks et al., 2017). We performed model selection to identify the best combination of predictors explaining variation in coccinellid abundance and network modularity. Interaction terms among predictors previously not tested in the *Scalescape* models (Appendix S1: Supplementary Methods) were included. Model selection was based on the Akaike Information Criterion corrected for small samples (AICc; Burnham and Anderson, 2002).

To evaluate the effects of landscape composition on coccinellid abundance, we fitted a GLM with a negative binomial distribution, with aphid density and the proportions of forest and savanna as predictors. Although the forest alone provided the best fit in the previous *Scalescape* model selection (Appendix S1: Table S1), we also included the savanna because aphid density was introduced as a new predictor. Therefore, we expected these two land-cover types to interact with aphid density in opposite ways, potentially exerting contrasting effects on coccinellid abundance. The global model included all three predictors and their interactions, and the final model was selected based on the lowest AICc value (Appendix S1: Table S3).

To test the effects of landscape composition on coccinellid diversity (Shannon and Simpson diversity indices), we fitted separate GLMs with a Gaussian distribution. The proportion of agricultural land was included as the predictor, following the best-supported models identified in the *Scalescape* analysis (Appendix S1: Table S1). To evaluate the effect of landscape composition on aphid density, we fitted a GLM with a negative binomial distribution, with the proportion of forest as the sole predictor (see Appendix S1: Table S1).

Similarly, we assessed the influence of landscape composition on network properties by fitting separate models for each metric. For nestedness, generality, and connectance, we fitted GLMs with a Gaussian distribution. For nestedness and generality, the proportion of grassland was used as the sole predictor, whereas connectance was modeled using the proportion of savanna (Appendix S1: Table S2). For network modularity, we first adjusted zero values by adding a small constant ($\varepsilon = 1e{-6}$; Smithson & Verkuilen, 2006) and then fitted a GLM with a beta distribution. We included the proportions of agriculture and savanna as predictors, as this combination was best supported in the previous *Scalescape* analysis (Appendix S1: Table S2). The global model comprised both predictors and their interaction term and the final model was selected based on the lowest AICc value (Appendix S1: Table S4).

To evaluate the direct and indirect effects of network, prey, and landscape features on biological control (BSI) we used Structural Equation Models (SEMs), implemented with the *piecewiseSEM* package (Lefcheck, 2016). We first fitted the component models separately. Network modularity and aphid density were modeled as previously described, but for modularity we used a Generalized Additive Model (GAM), as *piecewiseSEM* does not support *glmmTMB* models. We then modeled the BSI using a GAM with a beta distribution, including modularity and aphid density as predictors (see Appendix S1: Table S5). These component models were then combined in the first SEM that included models for modularity, aphid density, and BSI.

In the second SEM, we replaced modularity with connectance as the network metric. Connectance and aphid density were modeled as previously described, but based on the analysis of independence claims, we also included network connectance as a predictor for aphid density. The BSI was then modeled using a GAM with a beta distribution, with connectance and aphid density as predictors. Model adequacy was assessed through d-separation tests (Shipley, 2009). When these tests indicated missing paths, additional links were incorporated into the initial model to enhance model fit, provided they were biologically plausible (Bouchard et al., 2018). We extracted standardized path coefficients using the *parameters* package (Lüdecke et al., 2020) to compare effect sizes across models. This step was necessary because *piecewiseSEM* does not provide standardized coefficients for all distribution types included in our models.

RESULTS

Community composition

In total, seven coccinellid species, four aphid species, and 17 plant species were recorded. The mean coccinellid abundance was 34.7 ± 6.6 individuals/farm (mean ± SE). The coccinellid species observed were *Hippodamia convergens* (n = 947 individuals), *E. connexa* (n = 114), *Cycloneda sanguinea* (n = 86), *H. axyridis* (n = 71), *Hyperaspis festiva* (n = 7), *Scymnus* sp. (n = 5) and *Coleomegilla maculata* (n = 2). Coccinellids were observed to interact mainly with *Brevicoryne brassicae* (n = 294), *Uroleucon* spp. (n = 187), *Myzus persicae* (n = 75), and *Lipaphis pseudobrassicae* (n = 41). Coccinellids were most frequently observed on four non-crop plant species: *B. pilosa* (Asteraceae) (n = 279), *S. oleraceus* (Asteraceae) (n = 179), *G. parviflora* (Asteraceae) (n = 68), and *Cyperus rotundus* (Cyperaceae) (n = 62). The interaction of coccinellids with *B. pilosa* occurred on 86% of the farms, with *S. oleraceus* on 71%, and with both *G. parviflora* and *C. rotundus* on 39%. Similarly, coccinellids interacted with *B. brassicae* on 78% of the farms, with aphids of the genus *Uroleucon* on 68%, with *L. pseudobrassicae* on 29%, and with *M. persicae* on 25%. (Appendix S1: Table S4).

Landscape effects on community parameters

The proportion of savanna had a significant positive effect on coccinellid abundance (estimate = 2.311 ± 0.91; z = 2.53; p = 0.011; χ^2 = 6.58; d.f. = 1; p = 0.011) (Figure 1a) and peaked at a scale of 10.9 km (Appendix S1: Figure S1a, b). Aphid density on farms had a statistically significant, though modest, positive effect on coccinellid abundance (estimate =

0.007 ± 0.002; z = 3.83; p = 0.0001; χ^2 = 11.68; d.f. = 1; p = 0.0006) (Figure 1b). Similarly, the proportion of agriculture had a positive effect on coccinellid diversity (Simpson index: estimate = 2.146 ± 0.71; t = 3.03; p = 0.005; χ^2 = 9.2; d.f. = 1; p = 0.002; Shannon index: estimate = 2.1439 ± 0.82; t = 2.60; p = 0.015; χ^2 = 6.7; d.f. = 1; p = 0.009) (Figure 1c), but at smaller spatial scales (5.7 and 4.8 km, respectively) (Appendix S1: Figure S1c, d, e, f). However, the amount forest negatively affected aphid density (estimate = -11.697 ± 4.48; z = -2.61; p = 0.009; χ^2 = 6.3; d.f. = 1; p = 0.011) (Figure1d) at a 1.6 km radius buffer (Appendix S1: Figure S1g, h). Moreover, coccinellid richness was not significantly influenced by any land-use type.

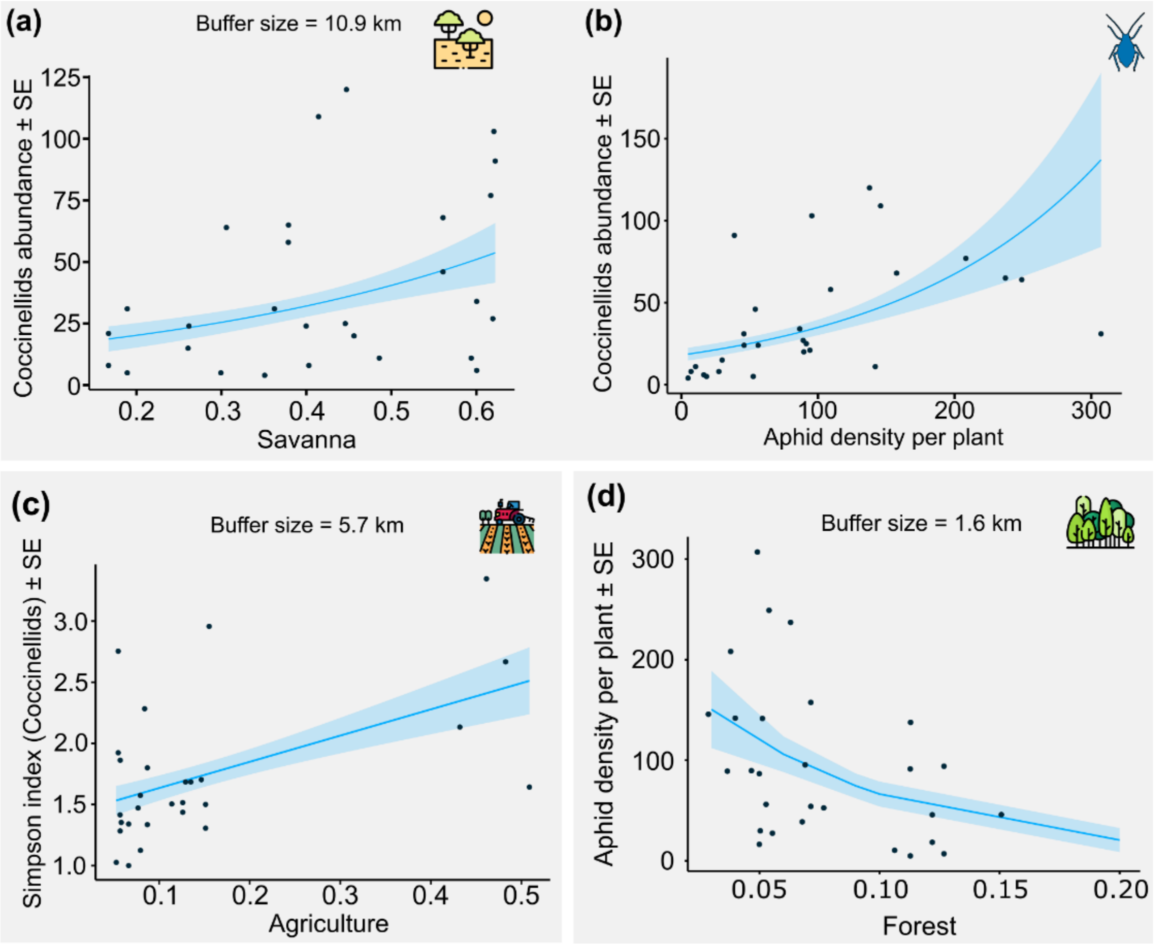

FIGURE 1. Effects of land covers within the optimal buffer on coccinellid and aphids' communities, based on generalized mixed-effects models (see Appendix S1: Table S1 and S3 for model selection details). Proportion of savanna and aphid density on coccinellid abundance (a, b); agriculture on coccinellid diversity (Simpson index) (c); and forest (d) on aphid density. Shaded areas indicate standard errors.

When comparing the observed general network metrics with null models, we observed that plant-aphid-coccinellid networks were not randomly structured, since almost all metrics, except for modularity (Observed *Q*: 0.15, Expected *C*: 0.17 ± 0.03, z = -0.66, p = 0.51) and specialization (Observed *H2'*: 0.26, Expected *C*: 0.22 ± 0.03, z = 1.12, p = 0.26) were significantly different from the null model means (Figure2b). The network had a high connectance (Observed *C*: 0.10, Expected *C*: 0.03 ± 0.001, z = 77.63, p < 0.0001) and generality (Observed *G*: 7.73, Expected *G*: 5.97 ± 0.23, z = 7.54; p < 0.0001). Nevertheless, the network exhibited lower observed nestedness (Observed WNODF: 16.47, Expected WNODF: 43.92 ± 2.35, z = -11.69, p < 0.0001) and high vulnerability (Observed *V*: 3.86, Expected *V*: 2.99 ± 0.12, z = 7.24, p < 0.0001).

Partial networks exhibited high variability in their network sizes metric values. For instance, network size (*S*), varied from a minimum of 4 nodes to a maximum of 15. This variability in network size was also reflected in other metrics, such as connectance (0.10 - 0.25), generality (1.33 - 4.00), modularity (0 - 0.36), specialization (0 - 0.43), and nestedness (6.67 - 27.27). However, while the network structure varied among farms, the interacting partners remained largely consistent. Consequently, the observed differences likely reflect variations in both landscape-level and local contexts. Despite these differences, our partial networks proved to be representative parts of the general network.

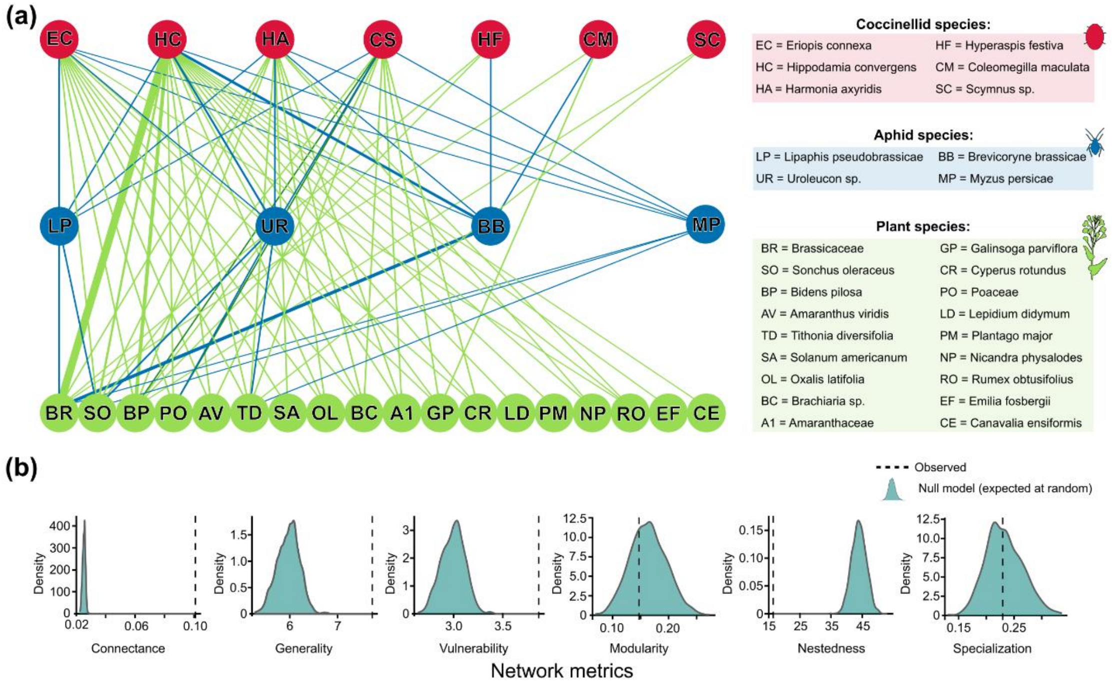

FIGURE 2. Structure and metrics of the tritrophic interaction network. (a) Network among plants (green nodes), aphids (blue), and coccinellids (red). Links are colored by trophic level: green = plant–coccinellid, blue = aphid–coccinellid or aphid–plant. Link width indicates interaction frequency. (b) Observed network metrics (vertical dashed lines) compared with null model distributions (density plots) from 1,000 randomizations.

Landscape effects on network structure and biological control

Savannas had a negative effect on network connectance (estimate = -0.111 ± 0.05; t = -2.16; p = 0.040; $\chi^2 = 4.7$; d.f. = 1; p = 0.03; Figure 3a) with the strongest impact observed at a small spatial scale (0.89 km radius buffer) (Figure S2a, b), while grasslands negatively affected nestedness (estimate = -132.284 ± 22.66; t = -5.84; p < 0.0001; $\chi^2 = 34.1$; d.f. = 1; p < 0.001; Figure 3b) at a higher spatial scale (2.3 km radius buffer; Figure S2c, d). Conversely, grasslands had a positive effect on generality (estimate = 7.624 ± 3.71; t = 2.05; p = 0.050; $\chi^2 = 4.2$; d.f. = 1; p = 0.040) (Figure 3c), with the strongest impact observed at a small spatial scale of 0.56 km radius buffer (Figure S2e, f). Similarly, the proportions of agriculture (estimate = 3.853 ± 1.23; z = 3.14; p = 0.002; $\chi^2 = 9.9$; d.f. = 1; p = 0.002; Figure 3d) and savanna (estimate = 3.819 ± 1.37; z = 2.79; p = 0.005; $\chi^2 = 7.8$; d.f. = 1; p = 0.005; Figure 3e) had positive effects on network modularity. The strongest impact observed for agriculture was at moderate spatial scale (3.6 km radius buffer) (Figure S2g, h), and for savannas it was at a higher spatial scale (10.9 km buffer radius) (Figure S2i, j).

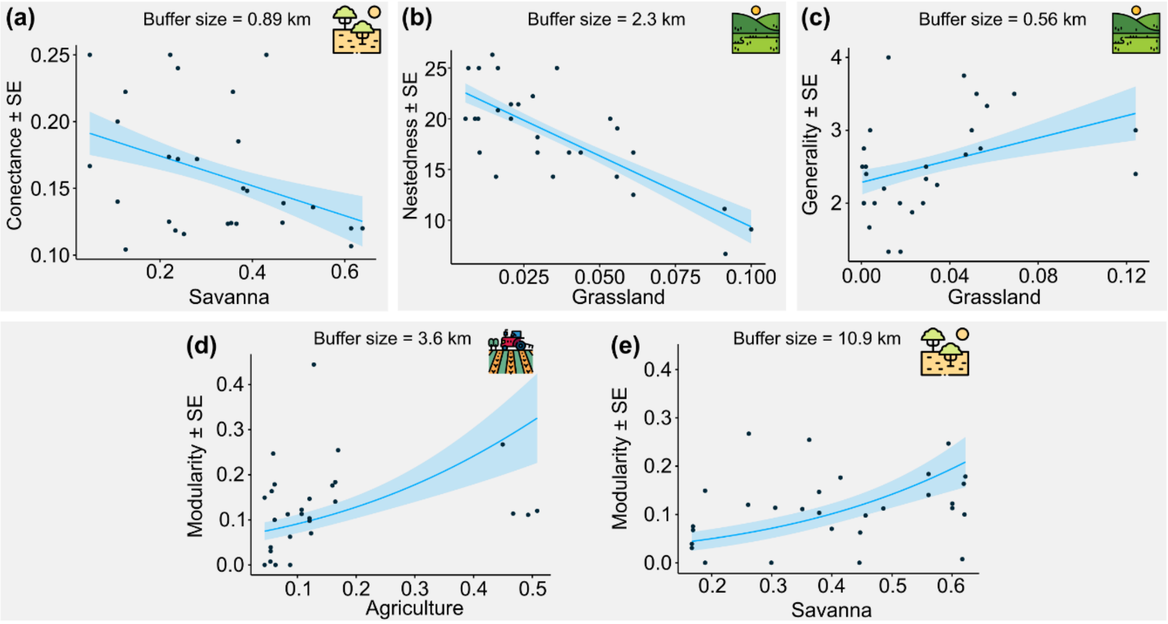

FIGURE 3. Effects of land covers within the optimal buffer on metrics of tritrophic interaction network, based on generalized mixed-effects models (see Appendix S1: Table S2 and S4 for model selection details). Proportion of savanna (a) on network connectance; grassland (b) on network nestedness; grassland (c) on network generality; agriculture (d) and savanna (e) on network modularity (see Appendix S1: Table S2 e S4 for model selection details). Shaded areas indicate standard errors.

Regarding the variables that directly affected biological control, aphid density (estimate = -0.004 ± 0.001; z = -3.67; p = 0.0002; χ^2 = 13.5; d.f. = 1; p = 0.0002; Figure 4a) and network connectance (estimate = -5.422 ± 2.14; z = -2.53; p = 0.011; χ^2 = 6.4; d.f. = 1; p = 0.011; Figure 4b) had negative effects. Contrary, network modularity (estimate = 2.061 ± 0.79; z = 2.60; p = 0.009; χ^2 = 6.8; d.f. = 1; p = 0.009; Figure 4d) had a positive effect.

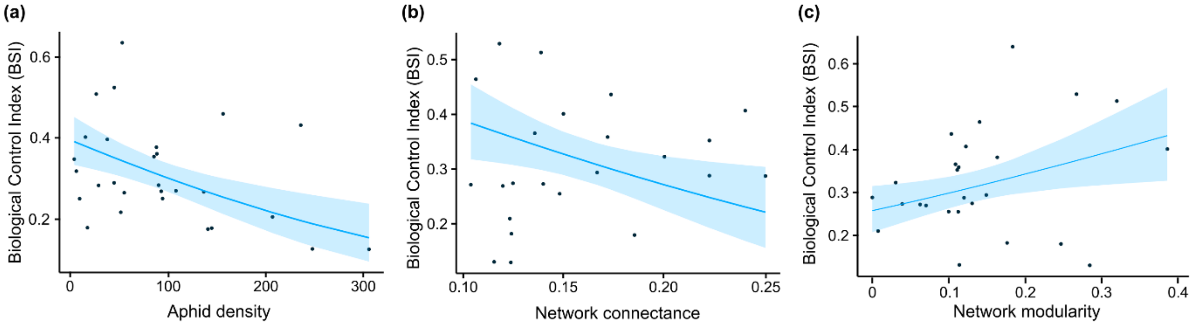

FIGURE 4. Effects of (a) aphid density per plant, (b) network connectance and (c) modularity on aphid control measured through the Biological Control Index (BSI). See Appendix S1: Table S5 for model selection details. Shaded areas indicate standard errors.

Spatial and ecological drivers of biological control

The first SEM showed a good overall fit to the data (χ^2 = 5.41, d.f. = 6, p = 0.492; Fisher's C = 7.69, d.f. = 12, p = 0.809), indicating that the specified causal relationships are are consistent with the observations and that no relevant paths were missing from the model. The proportion of agriculture (standardized coefficient = 0.54; 95% CI = 0.19, 0.89) and savanna (standardized coefficient = 0.57; 95% CI = 0.20, 0.95) had significant positive direct effects on network modularity. Agriculture (standardized coefficient = 0.10) and savanna (standardized coefficient = 0.11) proportions also had indirect positive effects on biological control through their influence on network modularity (Figure 5). Aphid density had a significant negative direct effect on biological control (standardized coefficient = -0.253; 95% CI =-0.44, -0.06) (Figure 5). Meanwhile, forest proportion had a significant negative direct effect on aphid density (standardized coefficient = -0.41; 95% CI = -0.70, -0.10), therefore indirectly and positively affecting aphid biological control (standardized coefficient = 0.11) (Figure 5).

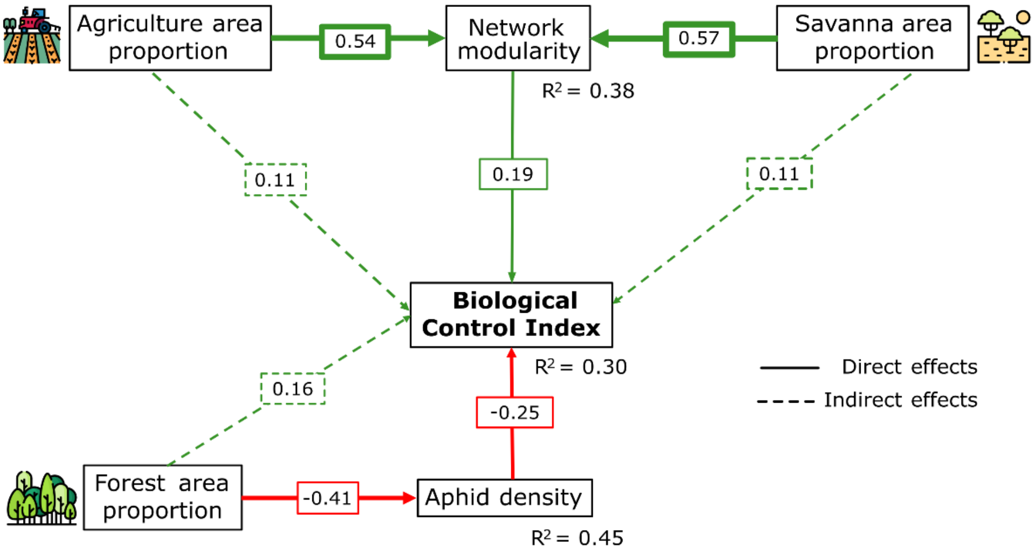

FIGURE 5. Path diagram of the Structural Equation Model (SEM) testing the direct and indirect pathways through which landscape composition mediated by network modularity and aphid density affects aphid biological control, quantified by the Biological Control Index (BSI). Green arrows indicate positive effects and red arrows negative effects. All estimated direct paths from predictor variables to network modularity and BSI were statistically significant ($p < 0.05$).

The second SEM also showed a good overall fit to the data ($\chi^2 = 6.06$, p = 0.195, df = 4; Fisher's C = 10.10, d.f. = 8, p = 0.195). In this model, the proportion of savanna had a direct negative effect on network connectance (standardized coefficient = -0.38; 95% CI = -0.74, 0.04), and connectance, in turn, had a direct negative effect on biological control (standardized coefficient = -0.26; 95% CI = -0.46, -0.05). Network connectance also exerted a direct negative effect on aphid density (standardized coefficient = 0.45; 95% CI = -0.72, -0.17). Because aphid density negatively affected biological control (standardized coefficient = -0.38; 95% CI = -0.59, -0.16), network connectance consequently had an indirect positive effect on it (standardized coefficient = 0.17). Similarly, the proportion of forest negatively affected aphid density (standardized coefficient = -0.34; 95% CI = -0.61, -0.06), thereby exerting a positive indirect effect on biological control (standardized coefficient = 0.13) (Figure S3).

DISCUSSION

Landscape composition strongly influenced not only coccinellid abundance and diversity but also the structure of plant–aphid–coccinellid networks. High connectance and generality indicated that species exploit multiple plant and aphid resources simultaneously, whereas low nestedness and high vulnerability revealed limited redundancy and greater sensitivity to disturbance (Biggs et al., 2020; Piazzon et al., 2011; Sanders et al., 2018). However, these effects varied across land-cover types and scales. Savannas enhanced coccinellid abundance at broader scales, agricultural areas increased diversity, and grasslands promoted generality. Aphid availability, network structure, and landscape composition jointly influenced aphid control, with outcomes depending on the combined effects of these factors.
influenced aphid control, with outcomes depending on the combined effects of these factors.
Overall, our results demonstrate that landscapes mediate species interactions, which in turn shape biological control, thereby reinforcing the value of multiscale interaction-based approaches in agroecosystem management.

The main species in our study — *C. sanguinea*, *E. connexa*, *H. axyridis* and *Hippodamia convergens* — are all generalist predators well adapted to agricultural habitats (Hodek et al., 2012), yet they differ in their functional traits and habitat affinities. For instance, *H. axyridis* is closely tied to agriculture, whereas *C. sanguinea* and *H. convergens* inhabit a broader range of environments (Grez et al., 2021). Trait differences likely explain uneven responses to local and landscape features (Perović et al., 2025; Poveda et al., 2025) and modulate their trophic interactions, helping to account for the effects of landscape composition on the plant–aphid–coccinellid network topology observed here.

Landscape composition modulated these communities in distinct ways. Forests reduced coccinellid abundance, in contrast to temperate systems where they serve as overwintering refuges (Elliott et al., 1999; Gardiner et al., 2009a). In tropical systems, where overwintering does not occur, forests may instead limit aphid colonization, by acting as a barrier, and reducing prey availability. Conversely, savannas increased coccinellid abundance, likely due to their intermediate structural complexity and resource diversity and continuity (Ribeiro & Walter, 2008; Honek et al., 2017). Generalist species, such as *H. convergens* and *C. sanguinea*, are likely favored in these heterogeneous environments (Mendoza-Arroyo & del-Val, 2024; Sloggett & Majerus, 2000).

Agricultural cover positively affected coccinellid diversity, reflecting the dominance of species adapted to crop habitats (Sloggett & Majerus, 2000). High prey availability in agricultural landscapes may relax competitive exclusion, allowing species to coexist despite resource overlap (Chesson, 2000; Holt, 1984) thereby promoting higher coccinellid diversity. Forest cover, in contrast, reduced aphid density, a pattern consistent with other studies in tropical agroecosystems (Marins et al., 2024, Novaes et al., 2024). This likely reflects dispersal limitation, as they are mainly dispersed by wind currents (Riley et al., 1995), and the stratified forest canopy may act as a physical barrier to their colonization, as demonstrated for whiteflies (Machado et al., 2024). Although agricultural areas provide abundant and conspicuous resources (Root, 1973; O'Rourke et al., 2017), the proportion of agricultural cover in the landscape had no effect on aphid densities, suggesting that forest barriers may play a more important role in modulating aphids populations. Additionally, the typical high disturbance and temporal variability of agricultural systems (Urruty et al., 2016) may further constrain their establishment.

Network structure reflected these dynamics in mediating species interactions. High connectance and generality, but low specialization and nestedness, reflect the coccinellids' generalized feeding habits on plants and aphids (Romero et al., 2023). Although this flexibility promotes interaction richness, it also increases vulnerability, as consumers rely on overlapping resources (Schmidt et al., 2024). Simplified landscapes may amplify this vulnerability (Tylianakis et al., 2007), as homogeneous prey conditions heighten the risk of cascading failures when local resources decline (Liere et al., 2015). Thus, the apparent robustness of highly connected networks (Dunne et al., 2002a) may mask instability driven by ephemeral crop resources (Morrison et al., 2020; Kortsch et al., 2023; Wissinger, 1997).

Savannas and grasslands shaped network architecture in different ways. Savannas reduced connectance, while grasslands decreased nestedness but increased generality, likely reflecting disturbed semi-natural habitats that favor generalist dominance (Gámez-Virués et al., 2015). The loss of specialists disrupts nestedness, whereas persistent generalists inflate generality (Bascompte et al., 2003), broadening functional roles but lowering redundancy and complementary effects on aphid predation (Dehling et al., 2021). Agriculture and savannas also increased modularity, likely due to spatial compartmentalization of interactions among crop and non-crop plants (Assunção et al., 2022; Melo-Cerón et al., 2025). Although agricultural areas support higher diversity, if competition or intraguild interactions arise, it may be higher within modules, also limiting the functional complementarity of species.

As expected, landscape effects on coccinellid abundance, diversity, and network structure were scale-dependent. Savannas influenced coccinellid abundance up to 10.9 km, while agriculture affected diversity at ~5.7 km. Connectance and generality responded to scales below 1 km, and modularity operated at broader scales (3.5–10.6 km). Patterns of abundance and diversity appear to be driven by large-scale processes such as resource tracking or dispersal limitation, while local predator–prey dynamics determine interaction structure. These findings parallel scale dependence observed for pest–enemy (Novaes et al., 2024) and bee–plant networks (Assunção et al., 2022), contrasting with temperate agroecosystems (Gardiner et al., 2009a).

Modularity positively affected aphid biological control, contrary to parasitoid-based networks (Macfadyen et al., 2011; Yang et al., 2021). Here, modularity likely reflects habitat-driven specialization mediated by non-crop plants that provide floral resources and alternative prey (Amaral et al., 2013; da Silva et al., 2025), promoting differential use of non-crop habitats by coccinellid species and, consequently, the emergence of distinct interaction modules. Such resource diversification reduces niche overlap, which may enhance biological control (Svanbäck & Bolnick, 2007) thereby increasing the effectiveness of predation through complementary resource use (Greenop et al., 2018). In parallel, higher modularity lowers the likelihood of cascading disturbances by compartmentalizing species interactions, confining perturbations within modules and slowing their spread across the network (Hermann et al., 2023; Olesen et al., 2007; Tylianakis et al., 2010), thereby strengthening network robustness (Gilarranz et al., 2017; Stouffer & Bascompte, 2011). Together, these processes probably contribute to more stable and effective ecosystem service provision.

Conversely, high connectance reduced biological control, suggesting that dense networks may intensify competition and intraguild predation (De Heij & Willenborg, 2020; Traugott et al., 2012). Yet, connectance also indirectly benefited aphid control by lowering aphid density, highlighting its context-dependent nature. At higher aphid densities on brassicas, biological control was less effective, likely because aphid recruitment exceeded the removal capacity by natural enemies (Latham & Mills, 2010; Zhao et al., 2024). This pattern further underscores the role of landscape composition, particularly forests, in suppressing aphid populations. However, BSI values cannot be solely attributed to coccinellids, as aphid removal likely reflects the collective contributions of coccinellids and other aphidophagous species (e.g., Marins et al., 2024). Altogether, our findings highlight the importance of multiscale integration of network structure, resource dynamics, and landscape context to unravel the mechanisms underlying effective biological control in agroecosystems, where local habitat conditions interact with broader-scale processes such as species pool composition and dispersal (Gordon et al., 2024; Neumüller et al., 2020).

CONCLUSION

Our results indicate that biological control in tropical smallholder systems dependents not only on predator abundance or diversity but also on the structure of plant–aphid–coccinellid interaction networks. Non-crop plants sustained these networks by providing alternative prey and floral resources, enhancing modularity and stability, and should be recognized as key nature-based solutions for maintaining predator populations and interactions. At the same time, agricultural areas provide abundant prey, whereas savannas offer complementary resources and refuges for coccinellids, thereby increasing their abundance and network modularity, and indirectly strengthening aphid predation. Altogether, these findings reveal that distinct vegetation types play complementary roles in predator–prey interactions, underscoring the importance of conserving heterogeneous mosaics of forests, savannas, and grasslands in agricultural landscapes, particularly in non-forest regions such as the Cerrado biome. This perspective supports landscape designs that integrate natural vegetation as a functional component of agroecosystems during agricultural expansion.

REFERENCES

Aguiar, L. M., U. M. Diniz, I. D. Bueno-Rocha, L. R. Filomeno, L. S. Aguiar-Machado, P. A. Gomes, and P. H. Togni. 2024. "Untangling Biodiversity Interactions: A Meta Network on Pollination in Earth's Most Diverse Tropical Savanna." *Ecology and Evolution* 14(3): e11094. https://doi.org/10.1002/ece3.11094.

Aizen, M. A., C. L. Morales, and J. M. Morales. 2008. "Invasive Mutualists Erode Native Pollination Webs." *PLoS Biology* 6(2): e31. https://doi.org/10.1371/journal.pbio.0060031.

Almeida-Neto, M., P. Guimaraes, P. R. Guimaraes, J. R. D. Loyola, and W. Ulrich. 2008. "A Consistent Metric for Nestedness Analysis in Ecological Systems: Reconciling Concept and Measurement." *Oikos* 117(8): 1227–1239. https://doi.org/10.1111/j.0030-1299.2008.16644.x.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. D. M., & Sparovek, G. 2013. "Köppen's climate classification map for Brazil". *Meteorologische zeitschrift*, *22*(6), 711-728. https://doi.org/10.1127/0941-2948/2013/0507

Amaral, D. S., M. Venzon, M. V. Duarte, F. F. Sousa, A. Pallini, and J. D. Harwood. 2013. "Non-Crop Vegetation Associated with Chili Pepper Agroecosystems Promote the Abundance and Survival of Aphid Predators." *Biological Control* 64(3): 338–346. https://doi.org/10.1016/j.biocontrol.2012.12.006.

Andow, D. A., E. M. Fontes, C. S. Pires, L. M. Souza, and D. P. Paula. 2023. "Invasion of the Coccinellid Community Associated with a Tropical Annual Agroecosystem." *Ecological Entomology* 48(2): 174–185. https://doi.org/10.1111/een.13212

Antonov, M., G. Csárdi, S. Horvát, K. Müller, T. Nepusz, D. Noom, . . . and F. Zanini. 2023. "igraph Enables Fast and Robust Network Analysis across Programming Languages." *arXiv preprint arXiv:2311.10260*. https://doi.org/10.48550/arXiv.2311.10260.

Assunção, R. M., N. F. Camargo, L. S. Souza, E. M. Rocha, G. M. Tostes, E. R. Sujii, . . . and P. H. Togni. 2022. "Landscape Conservation and Local Interactions with Non-Crop Plants Aid in Structuring Bee Assemblages in Organic Tropical Agroecosystems." *Journal of Insect Conservation* 26(6): 933–945. https://doi.org/10.1007/s10841-022-00438-8.

Audino, L. D., S. J. Murphy, L. Zambaldi, J. Louzada, and L. S. Comita. 2017. "Drivers of Community Assembly in Tropical Forest Restoration Sites: Role of Local Environment, Landscape, and Space." *Ecological Applications* 27(6): 1731–1745. https://doi.org/10.1002/eap.1562.

Bascompte, J. 2009. "Disentangling the Web of Life." *Science* 325(5939): 416–419. https://doi.org/10.1126/science.1170749

Bascompte, J., and P. Jordano. 2007. "Plant-Animal Mutualistic Networks: The Architecture of Biodiversity." *Annu. Rev. Ecol. Evol. Syst.* 38(1): 567–593. https://doi.org/10.1146/annurev.ecolsys.38.091206.095818

Bascompte, J., P. Jordano, C. J. Melián, and J. M. Olesen. 2003. "The Nested Assembly of Plant–Animal Mutualistic Networks." *Proceedings of the National Academy of Sciences* 100(16): 9383–9387. https://doi.org/10.1073/pnas.1633576100

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of statistical software* 67: 1–48. https://doi.org/10.48550/arXiv.1406.5823.

Bersier, L. F., C. Banašek-Richter, and M. F. Cattin. 2002. "Quantitative Descriptors of Food-Web Matrices." *Ecology* 83(9): 2394–2407. https://doi.org/10.1890/0012-9658(2002)083[2394:QDOFWM]2.0.CO;2

Biggs, C. R., L. A. Yeager, D. G. Bolser, C. Bonsell, A. M. Dichiera, Z. Hou, . . . and B. E. Erisman. 2020. "Does Functional Redundancy Affect Ecological Stability and Resilience? A Review and Meta-Analysis." *Ecosphere* 11(7): e03184. https://doi.org/10.1002/ecs2.3184

Blassioli-Moraes, M. C., M. Venzon, L. C. P. Silveira, L. M. Gontijo, P. H. B. Togni, E. R. Sujii, . . . and M. Birkett. 2022. "Companion and Smart Plants: Scientific Background to Promote Conservation Biological Control." *Neotropical Entomology* 1–17. https://doi.org/10.1007/s13744-021-00939-2

Blüthgen, N., F. Menzel, and N. Blüthgen. 2006. "Measuring Specialization in Species Interaction Networks." *BMC ecology* 6(1): 9. https://doi.org/10.1186/1472-6785-6-9

Boivin, N. L., M. A. Zeder, D. Q. Fuller, A. Crowther, G. Larson, J. M. Erlandson, . . . and M. D. Petraglia. 2016. "Ecological Consequences of Human Niche Construction: Examining Long-Term Anthropogenic Shaping of Global Species Distributions." *Proceedings of the National Academy of Sciences* 113(23): 6388–6396. https://doi.org/10.1073/pnas.152520011

Bouchard, M., V. Martel, J. Régnière, P. Therrien, and D. L. P. Correia. 2018. "Do Natural Enemies Explain Fluctuations in Low-Density Spruce Budworm Populations?" *Ecology* 99(9): 2047–2057. https://doi.org/10.1002/ecy.2417

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, . . . and B. M. Bolker. 2017. "Modeling Zero-Inflated Count Data with glmmTMB." *BioRxiv* 132753. https://doi.org/10.1101/132753

Brown, C., D. F. R. P. Burslem, J. B. Illian, L. Bao, W. Brockelman, M. Cao, . . . and R. Law. 2013. "Multispecies Coexistence of Trees in Tropical Forests: Spatial Signals of Topographic Niche Differentiation Increase with Environmental Heterogeneity." *Proceedings of the Royal Society B: Biological Sciences* 280(1764): 20130502. https://doi.org/10.1098/rspb.2013.0502

Burnham, K. P., and D. R. Anderson, editors. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer New York, New York, NY. https://doi.org/10.1007/978-0-387-22456-5_6

Carvalheiro, L. G., Veldtman, R., Shenkute, A. G., Tesfay, G. B., Pirk, C. W. W., Donaldson, J. S., & Nicolson, S. W. (2011). Natural and within-farmland biodiversity enhances crop productivity. *Ecology letters*, *14*(3), 251-259.

Chesson, P. 2000. "Mechanisms of Maintenance of Species Diversity." *Annual review of Ecology and Systematics* 31(1): 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343

Connor, E. F., A. C. Courtney, and J. M. Yoder. 2000. "Individuals–Area Relationships: The Relationship between Animal Population Density and Area." *Ecology* 81(3): 734–748. https://doi.org/10.1890/0012-9658(2000)081[0734:IARTRB]2.0.CO;2

Crowder, D. W., T. D. Northfield, M. R. Strand, and W. E. Snyder. 2010. "Organic Agriculture Promotes Evenness and Natural Pest Control." *Nature* 466(7302): 109–112. https://doi.org/10.1038/nature09183

da Silva, A. C., L. C. Oliveira, J. G. de Lacerda, M. Venzon, M. R. Frizzas, E. R. Sujii, and P. H. Togni. 2025. "Floral Availability Outweighs Aphid Presence in Supporting Coccinellid Abundance Across Life Stages and Promotes Aphid Predation." *Basic and Applied Ecology*. https://doi.org/10.1016/j.baae.2025.10.008

da Silva, A. C., R. C. Cahú, M. M. Cogitskei, K. S. Kubota, E. R. Sujii, and P. H. Togni. 2022. "Intercropping Collard Plants with Coriander Modulates Behavioral Interactions among Aphidophagous Predators by Altering Microhabitat Structure." *Biological Control* 105084. https://doi.org/10.1016/j.biocontrol.2022.105084

De Heij, S. E., and C. J. Willenborg. 2020. "Connected Carabids: Network Interactions and Their Impact on Biocontrol by Carabid Beetles." *BioScience* 70(6): 490–500. https://doi.org/10.1093/biosci/biaa039

Dehling, D. M., I. M. Bender, P. G. Blendinger, K. Böhning-Gaese, M. C. Muñoz, E. L. Neuschulz, . . . and D. B. Stouffer. 2021. "Specialists and Generalists Fulfil Important and Complementary Functional Roles in Ecological Processes." *Functional Ecology* 35(8): 1810–1821. https://doi.org/10.1111/1365-2435.13815

Dormann C, J. Fruend, N. Bluethgen, and B. Gruber. 2009. "Indices, Graphs and Null Models: Analyzing Bipartite Ecological Networks." *The Open Ecology Journal* 2: 7–24. Available in https://resolver.sub.uni-goettingen.de/purl?gro-2/61194

Duchenne, F., R. O. Wüest, and C. H. Graham. 2022. "Seasonal Structure of Interactions Enhances Multidimensional Stability of Mutualistic Networks." *Proceedings of the Royal Society B* 289(1982): 20220064. https://doi.org/10.1098/rspb.2022.0064

Dunne, J. A., R. J. Williams, and N. D. Martinez. 2002a. "Network Structure and Biodiversity Loss in Food Webs: Robustness Increases with Connectance." *Ecology letters* 5(4): 558–567. https://doi.org/10.1046/j.1461-0248.2002.00354.x

Dunne, J. A., R. J. Williams, and N. D. Martinez. 2002b. "Food-Web Structure and Network Theory: The Role of Connectance and Size." *Proceedings of the National Academy of Sciences* 99(20): 12917–12922. https://doi.org/10.1073/pnas.192407699

Elliott, N. C., R. W. Kieckhefer, and D. A. Beck. 2002. "Effect of Aphids and the Surrounding Landscape on the Abundance of Coccinellidae in Cornfields." *Biological Control* 24(3): 214–220. https://doi.org/10.1016/S1049-9644(02)00036-1

Elliott, N. C., R. W. Kieckhefer, J. H. Lee, and B. W. French. 1999. "Influence of Within-Field and Landscape Factors on Aphid Predator Populations in Wheat." *Landscape Ecology* 14(3): 239–252. https://doi.org/10.1023/A:1008002528345

Fahrig, L., J. Baudry, L. Brotons, F. G. Burel, T. O. Crist, R. J. Fuller, . . . and J. L. Martin. 2011. "Functional Landscape Heterogeneity and Animal Biodiversity in Agricultural Landscapes." *Ecology letters* 14(2): 101–112. https://doi.org/10.1111/j.1461-0248.2010.01559.x

Francoso, R. D., K. G. Dexter, R. B. Machado, R. T. Pennington, J. R. Pinto, R. A. Brandao, and J. A. Ratter. 2020. "Delimiting Floristic Biogeographic Districts in the Cerrado and Assessing Their Conservation Status." *Biodiversity and Conservation* 29(5): 1477–1500. https://doi.org/10.1007/s10531-019-01819-3

Gámez-Virués, S., D. J. Perović, M. M. Gossner, C. Börschig, N. Blüthgen, H. De Jong, . . . and C. Westphal. 2015. "Landscape Simplification Filters Species Traits and Drives Biotic Homogenization." *Nature communications* 6(1): 8568. https://doi.org/10.1038/ncomms9568

Gardiner, M. M., D. A. Landis, C. Gratton, C. D. DiFonzo, M. O'Neal, J. M. Chacon, et al. 2009a. "Landscape Diversity Enhances Biological Control of an Introduced Crop Pest in the North-Central USA." *Ecological Applications* 19: 143–154. https://doi.org/10.1890/07-1265.1

Gardiner, M. M., D. A. Landis, C. Gratton, N. Schmidt, M. O'Neal, E. Mueller, . . . and C. D. DiFonzo. 2009b. "Landscape Composition Influences Patterns of Native and Exotic Lady Beetle Abundance." *Diversity and Distributions* 15(4): 554–564. https://doi.org/10.1111/j.1472-4642.2009.00563.x

Gilarranz, L. J., B. Rayfield, G. Liñán-Cembrano, J. Bascompte, and A. Gonzalez. 2017. "Effects of Network Modularity on the Spread of Perturbation Impact in Experimental Metapopulations." *Science* 357(6347): 199–201. https://doi.org/10.1126/science.aal4122

Gordon, S. C., J. G. Martin, and J. T. Kerr. 2024. "Dispersal Mediates Trophic Interactions and Habitat Connectivity to Alter Metacommunity Composition." *Ecology* 105(2): e4215. https://doi.org/10.1002/ecy.4215

Grez, A. A., T. Zaviezo, F. Casanoves, R. Oberti, and P. Pliscoff. 2021. "The Positive Association between Natural Vegetation, Native Coccinellids and Functional Diversity of Aphidophagous Coccinellid Communities in Alfalfa." *Insect Conservation and Diversity* 14(4): 464–475. https://doi.org/10.1111/icad.12473

Grez, A. A., T. Zaviezo, J. Hernández, A. Rodríguez-San Pedro, and P. Acuña. 2014. "The Heterogeneity and Composition of Agricultural Landscapes Influence Native and Exotic Coccinellids in Alfalfa Fields." *Agricultural and Forest Entomology* 16(4): 382–390. https://doi.org/10.1111/afe.12068

Grez, A., and T. Zaviezo. 2024. "Landscape Simplification, Urbanization, Biological Invasions, and Climate Change: A Review of the Major Threats to Native Coccinellids in Central Chile." *Entomologia Experimentalis et Applicata* 172(6): 460–466. https://doi.org/10.1111/eea.13407

Greenop, A., Woodcock, B. A., Wilby, A., Cook, S. M., & Pywell, R. F. (2018). Functional diversity positively affects prey suppression by invertebrate predators: a meta-analysis. *Ecology*, *99*(8), 1771-1782.

Grün, B., Kosmidis, I., & Zeileis, A. (2012). Extended beta regression in R: Shaken, stirred, mixed, and partitioned. *Journal of Statistical Software*, *48*, 1-25. https://doi.org/10.18637/jss.v048.i11

Guimaraes, J. P. R. 2020. "The Structure of Ecological Networks across Levels of Organization." *Annual Review of Ecology, Evolution, and Systematics* 51(1): 433–460. https://doi.org/10.1146/annurev-ecolsys-012220-120819

Hartig, F., & Lohse, L. (2020). *DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. Version 0.3. 3.0.*

Hass, A. L., U. G. Kormann, T. Tscharntke, Y. Clough, A. B. Baillod, C. Sirami, . . . and P. Batáry. 2018. "Landscape Configurational Heterogeneity by Small-Scale Agriculture, Not Crop Diversity, Maintains Pollinators and Plant Reproduction in Western Europe." *Proceedings of the Royal Society B: Biological Sciences* 285(1872): 20172242. https://doi.org/10.1098/rspb.2017.2242

Herrmann, J., S. Buchholz, and P. Theodorou. 2023. "The Degree of Urbanisation Reduces Wild Bee and Butterfly Diversity and Alters the Patterns of Flower-Visitation in Urban Dry Grasslands." *Scientific Reports* 13(1): 2702. https://doi.org/10.1038/s41598-023-29275-8

Hodek, I., A. Honek, and H. F. Van Emden, editors. 2012. *Ecology and Behaviour of the Ladybird Beetles (Coccinellidae)*. John Wiley & Sons.

Holt, R. D. 1984. "Spatial Heterogeneity, Indirect Interactions, and the Coexistence of Prey Species." *The American Naturalist* 124(3): 377–406. https://doi.org/10.1086/284280

Holt, R. D. 1996. "Food Webs in Space: an Island Biogeographic Perspective." In *Food Webs: Integration of Patterns & Dynamics*, 313–323. Boston, MA: Springer US. https://doi.org/10.1007/978-1-4615-7007-3_30

Honek, A., A. F. Dixon, A. O. Soares, J. Skuhrovec, and Z. Martinkova. 2017. "Spatial and Temporal Changes in the Abundance and Compostion of Ladybird (Coleoptera: Coccinellidae) Communities." *Current opinion in insect science* 20: 61–67. https://doi.org/10.1016/j.cois.2017.04.001

Hudson, L. N., R. Emerson, G. B. Jenkins, K. Layer, M. E. Ledger, D. E. Pichler, . . . and D. C. Reuman. 2013. "Cheddar: Analysis and Visualisation of Ecological Communities in R." *Methods in Ecology and Evolution* 4(1): 99–104. Available in http://wileyonlinelibrary.com/onlineopen#OnlineOpen_Terms

Kabisch, N., N. Frantzeskaki, S. Pauleit, S. Naumann, M. Davis, M. Artmann, . . . and A. Bonn. 2016. "Nature-Based Solutions to Climate Change Mitigation and Adaptation in Urban Areas: Perspectives on Indicators, Knowledge Gaps, Barriers, and Opportunities for Action." *Ecology and society* 21(2). Available in https://www.jstor.org/stable/26270403

Karp, D. S., R. Chaplin-Kramer, T. D. Meehan, E. A. Martin, F. DeClerck, H. Grab, . . . and J. B. Wickens. 2018. "Crop Pests and Predators Exhibit Inconsistent Responses to Surrounding Landscape Composition." *Proceedings of the National Academy of Sciences* 115(33): E7863–E7870. https://doi.org/10.1073/pnas.1800042115

Kortsch, S., L. Saravia, A. R. Cirtwill, T. Timberlake, J. Memmott, L. Kendall, . . . and G. Strona. 2023. "Landscape Composition and Pollinator Traits Interact to Influence Pollination Success in an Individual-Based Model." *Functional Ecology* 37(7): 2056–2071. https://doi.org/10.1111/1365-2435.14353

Lami, F., I. Bartomeus, D. Nardi, T. Beduschi, F. Boscutti, P. Pantini, . . . and L. Marini. 2021. "Species–Habitat Networks Elucidate Landscape Effects on Habitat Specialisation of

Natural Enemies and Pollinators." *Ecology Letters* 24(2): 288–297. https://doi.org/10.1111/ele.13642

Latham, D. R., and N. J. Mills. 2010. "Quantifying Aphid Predation: The Mealy Plum Aphid Hyalopterus pruni in California as a Case Study." *Journal of Applied Ecology* 47(1): 200–208. https://doi.org/10.1111/j.1365-2664.2009.01749.x

Lefcheck, J. S. 2016. "piecewiseSEM: Piecewise Structural Equation Modelling in R for Ecology, Evolution, and Systematics." *Methods in Ecology and Evolution* 7(5): 573–579. https://doi.org/10.1111/2041-210X.12512

Liere, H., T. N. Kim, B. P. Werling, T. D. Meehan, D. A. Landis, and C. Gratton. 2015. "Trophic Cascades in Agricultural Landscapes: Indirect Effects of Landscape Composition on Crop Yield." *Ecological Applications* 25(3): 652–661. https://doi.org/10.1890/14-0570.1

Lowe, E. B., B. Iuliano, C. Gratton, and A. R. Ives. 2022. "'Scalescape': an R Package for Estimating Distance-Weighted Landscape Effects on an Environmental Response." *Landscape Ecology* 37(7): 1771–1785. https://doi.org/10.1007/s10980-022-01437-5

Lüdecke, D., M. S. Ben-Shachar, I. Patil, and D. Makowski. 2020. "Extracting, Computing and Exploring the Parameters of Statistical Models Using R." *Journal of Open Source Software* 5(53): 2445. https://doi.org/10.21105/joss.02445

Macdonald, E., and E. G. King. 2018. "Novel Ecosystems: A Bridging Concept for the Consilience of Cultural Landscape Conservation and Ecological Restoration." *Landscape and Urban Planning* 177: 148–159. https://doi.org/10.1016/j.landurbplan.2018.04.015

Macfadyen, S., R. H. Gibson, W. O. Symondson, and J. Memmott. 2011. "Landscape Structure Influences Modularity Patterns in Farm Food Webs: Consequences for Pest Control." *Ecological Applications* 21(2): 516–524. https://doi.org/10.1890/09-2111.1

Machado, A. F., N. F. Camargo, N. B. de Souza, E. Y. Nakasu, A. K. Inoue-Nagata, and P. H. Togni. 2024. "Spread Your Wings: Insect–Plant–Landscape Interactions Mediate the Wing Shape of a Multivoltine Polyphagous Insect." *Oikos* 2024(2): e10318. https://doi.org/10.1111/oik.10318

Marins, G., M. F. S. de Aquino, A. C. da Silva, H. A. C. de Queiroz, R. A. Laumann, and P. H. B. Togni. 2024. "Through the Green Mosaic: Different Tropical Vegetation Types Have Complementary Effects on Parasitoid Diversity and Biological Control in Organic Agroecosystems." *Agriculture, Ecosystems & Environment* 374: 109162. https://doi.org/10.1016/j.agee.2024.109162

Melo-Cerón, C. I., A. P. Martínez-Falcón, J. Quijano, D. N. Duque-Gamboa, N. Toro-Perea, and M. R. Manzano. 2025. "Invasive and Native Non-Crop Plants Support Aphidophagous Insects in Chilli Pepper Agroecosystems." *Journal of Applied Entomology* 149(4): 637–651. https://doi.org/10.1111/jen.13415

Mendoza-Arroyo, W., and E. del-Val. 2024. "Ladybird Predators in Natural and Agricultural Landscapes in Mexico: Diversity and Parasitism." *Arthropod-Plant Interactions* 18(4): 741–751. https://doi.org/10.1007/s11829-024-10075-4

Morales, J. M., and D. P. Vázquez. 2008. "The Effect of Space in Plant–Animal Mutualistic Networks: Insights from a Simulation Study." *Oikos* 117(9): 1362–1370. https://doi.org/10.1111/j.0030-1299.2008.16737.x

Moreira, E. F., D. Boscolo, and B. F. Viana. 2015. "Spatial Heterogeneity Regulates Plant-Pollinator Networks across Multiple Landscape Scales." *PloS one* 10(4): e0123628. https://doi.org/10.1371/journal.pone.0123628

Moreira, E. F., P. A. Ferreira, L. E. Lopes, R. G. S. Soares, and D. Boscolo. 2018. "Ecological Networks in Changing Tropics." In *Ecological Networks in the Tropics*, edited by W. Dáttilo and V. Rico-Gray, 155–169. Springer, Cham. https://doi.org/10.1007/978-3-319-68228-0_11

Morrison, B. M., B. J. Brosi, and R. Dirzo. 2020. "Agricultural Intensification Drives Changes in Hybrid Network Robustness by Modifying Network Structure." *Ecology Letters* 23(2): 359–369. https://doi.org/10.1111/ele.13440

Neumüller, U., H. Burger, S. Krausch, N. Blüthgen, and M. Ayasse. 2020. "Interactions of Local Habitat Type, Landscape Composition and Flower Availability Moderate Wild Bee Communities." *Landscape Ecology* 35(10): 2209–2224. https://doi.org/10.1007/s10980-020-01096-4

Newbold, T., L. N. Hudson, S. Contu, S. L. Hill, J. Beck, Y. Liu, . . . and A. Purvis. 2018. "Widespread Winners and Narrow-Ranged Losers: Land Use Homogenizes Biodiversity in Local Assemblages Worldwide." *PLoS biology* 16(12): e2006841. https://doi.org/10.1371/journal.pbio.2006841

Norder, S. J., R. F. de Lima, L. de Nascimento, J. Y. Lim, J. M. Fernández-Palacios, M. M. Romeiras, . . . and P. A. Borges. 2020. "Global Change in Microcosms: Environmental and Societal Predictors of Land Cover Change on the Atlantic Ocean Islands." *Anthropocene* 30: 100242. https://doi.org/10.1016/j.ancene.2020.100242

Noriega, J. A., J. Hortal, F. M. Azcárate, M. P. Berg, N. Bonada, M. J. Briones, . . . and A. M. Santos. 2018. “Research Trends in Ecosystem Services Provided by Insects.” *Basic and Applied Ecology* 26: 8–23. https://doi.org/10.1016/j.baae.2017.09.006

Novaes, D. R., P. S. Sujii, C. A. Rodrigues, K. M. Silva, A. F. Machado, A. K. Inoue-Nagata, . . . and P. H. Togni. 2024. “Natural Habitat Connectivity and Organic Management Modulate Pest Dispersal, Gene Flow, and Natural Enemy Communities.” *Ecological Applications* 34(2): e2938. https://doi.org/10.1002/eap.2938

Olesen, J. M., J. Bascompte, Y. L. Dupont, and P. Jordano. 2007. “The Modularity of Pollination Networks.” *Proceedings of the national academy of sciences* 104(50): 19891–19896. https://doi.org/10.1073/pnas.0706375104

O'Rourke, M. E., and M. J. Petersen. 2017. “Extending the ‘Resource Concentration Hypothesis’ to the Landscape-Scale by Considering Dispersal Mortality and Fitness Costs.” *Agriculture, Ecosystems & Environment* 249: 1–3. https://doi.org/10.1016/j.agee.2017.07.022

Perović, D. J., A. A. Grez, and T. Zaviezo. “Traits Drive Responses of Aphidophagous Coccinellid Communities to Landscape Complexity.” *Insect Conservation and Diversity*. https://doi.org/10.1111/icad.70034

Piazzon, M., A. R. Larrinaga, and L. Santamaría. 2011. “Are Nested Networks More Robust to Disturbance? A Test Using Epiphyte-Tree, Comensalistic Networks.” *PLoS One* 6(5): e19637. https://doi.org/10.1371/journal.pone.0019637

Poveda, K., D. S. Karp, R. Chaplin-Kramer, M. Centrella, T. Luttermoser, R. Perez-Alvarez, M. E. O'Rourke, E. A. Martin, and H. Grab. 2025. “The Importance of Landscape Composition for Pest Control and Crop Yield: A Global Quantitative Synthesis.” *Ecology Letters* 28: e70250. https://doi.org/10.1111/ele.70250.

QGIS Development Team. 2025. *QGIS Geographic Information System. Open Source Geospatial Foundation Project*. Versão 3.42.3. Available in https://qgis.org.

Ramos, D. L., W. L. Cunha, J. Evangelista, L. A. Lira, M. V. C. Rocha, P. A. Gomes, . . . and P. H. B. Togni. 2020. “Ecosystem Services Provided by Insects in Brazil: What Do We Really Know?” *Neotropical Entomology* 49(6): 783–794. https://doi.org/10.1007/s13744-020-00781-y

Ribeiro, J. F., B. M. B. Walter, and E. C. José Felipe Ribeiro. 2008. “As Principais Fitofissionomias do Bioma Cerrado.” Available in http://www.alice.cnptia.embrapa.br/alice/handle/doc/186922

Riede, J. O., B. C. Rall, C. Banasek-Richter, S. A. Navarrete, E. A. Wieters, M. C. Emmerson, . . . and U. Brose. 2010. "Scaling of Food-Web Properties with Diversity and Complexity across Ecosystems." In *Advances in ecological research*. Vol. 42: 139–170. Academic Press. https://doi.org/10.1016/B978-0-12-381363-3.00003-4

Riley, J. R., D. R. Reynolds, S. A. N. K. A. R. Mukhopadhyay, M. R. Ghosh, and T. K. Sarkar. 1995. "Long-Distance Migration of Aphids and Other Small Insects in Northeast India." *EJE* 92(4): 639–653. Available in https://www.eje.cz/pdfs/eje/1995/04/07.pdf

Romero, V., T. Zaviezo, R. Sánchez, and A. A. Grez. 2023. "More than Aphids in the Guts of Coccinellids Revealed by Molecular and Visual Analyses." *BioControl* 68(4): 435–446. https://doi.org/10.1007/s10526-023-10204-7

Root, R. B. 1973. "Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (Brassica oleracea)." *Ecological monographs* 43(1): 95–124. https://doi.org/10.2307/1942161

Sanders, D., E. Thébault, R. Kehoe, and F. J. Frank van Veen. 2018. "Trophic Redundancy Reduces Vulnerability to Extinction Cascades." *Proceedings of the National Academy of Sciences* 115(10): 2419–2424. https://doi.org/10.1073/pnas.1716825115

Schmidt, J. M., K. Russell, C. Bowers, A. W. Coffin, M. Thompson, E. E. Grabarczyk, . . . and D. Olson. 2024. "Resource Overlap and Infrequent Predation on Key Pests Show Vulnerability in Cotton Biological Control Services." *Agriculture, Ecosystems & Environment* 374: 109164. https://doi.org/10.1016/j.agee.2024.109164

Schoener, T. W. 1989. "Food Webs from the Small to the Large." *Ecology* 70(6): 1559–1589. Available in https://www.esa.org/wp-content/uploads/sites/94/2022/02/Schoener_TW.pdf

Shipley, B. 2009. "Confirmatory Path Analysis in a Generalized Multilevel Context." *Ecology* 90(2): 363–368. https://doi.org/10.1890/08-1034.1

Sloggett, J. J., and M. E. Majerus. 2000. "Habitat Preferences and Diet in the Predatory Coccinellidae (Coleoptera): an Evolutionary Perspective." *Biological Journal of the linnean Society* 70(1): 63–88. https://doi.org/10.1111/j.1095-8312.2000.tb00201.x

Smithson, M., and J. Verkuilen. 2006. "A Better Lemon Squeezer? Maximum-Likelihood Regression with Beta-Distributed Dependent Variables." *Psychological Methods* 11(1): 54–71. https://doi.org/10.1037/1082-989X.11.1.54

Soares, A. O., D. Haelewaters, O. M. Ameixa, I. Borges, P. M. Brown, P. Cardoso, . . . and J. E. Losey. 2023. "A Roadmap for Ladybird Conservation and Recovery." *Conservation Biology* 37(1): e13965. https://doi.org/10.1111/cobi.13965

Souza Jr, C. M., J. Z. Shimbo, M. R. Rosa, L. L. Parente, A. A. Alencar, B. F. Rudorff, . . . and T. Azevedo. 2020. "Reconstructing Three Decades of Land Use and Land Cover Changes in Brazilian Biomes with Landsat Archive and Earth Engine." *Remote Sensing* 12(17): 2735. https://doi.org/10.3390/rs12172735

Stouffer, D. B., and J. Bascompte. 2011. "Compartmentalization Increases Food-Web Persistence." *Proceedings of the National Academy of Sciences* 108(9): 3648–3652. https://doi.org/10.1073/pnas.101435310

Svanbäck, R., and D. I. Bolnick. 2007. "Intraspecific Competition Drives Increased Resource Use Diversity within a Natural Population." *Proceedings of the Royal Society B: Biological Sciences* 274(1611): 839–844. https://doi.org/10.1098/rspb.2006.0198

Taranto, L., I. Rodrigues, S. A. Santos, M. Villa, and J. A. Pereira. 2022. "Response of the Coccinellidae Community within Sustainable Vineyards to the Surrounding Landscape." *Agronomy* 12(9): 2140. https://doi.org/10.3390/agronomy12092140

Thébault, E., and C. Fontaine. 2010. "Stability of Ecological Communities and the Architecture of Mutualistic and Trophic Networks." *Science* 329(5993): 853–856. https://doi.org/10.1126/science.1188321

Togni, P. H. B., M. Venzon, A. C. G. Lagôa, and E. R. Sujii. 2019b. "Brazilian Legislation Leaning Towards Fast Registration of Biological Control Agents to Benefit Organic Agriculture." *Neotropical entomology* 48(2): 175–185. https://doi.org/10.1007/s13744-019-00675-8

Togni, P. H., M. Venzon, L. M. Souza, J. P. Santos, and E. R. Sujii. 2019a. "Biodiversity Provides Whitefly Biological Control Based on Farm Management." *Journal of Pest Science* 92(2): 393–403. https://doi.org/10.1007/s10340-018-1021-x

Traugott, M., J. R. Bell, L. Raso, D. Sint, and W. O. C. Symondson. 2012. "Generalist Predators Disrupt Parasitoid Aphid Control by Direct and Coincidental Intraguild Predation." *Bulletin of Entomological Research* 102(2): 239–247. https://doi.org/10.1017/S0007485311000551

Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies. 2005. "Landscape Perspectives on Agricultural Intensification and Biodiversity–Ecosystem Service Management." *Ecology letters* 8(8): 857–874. https://doi.org/10.1111/j.1461-0248.2005.00782.x

Tscharntke, T., J. M. Tylianakis, T. A. Rand, R. K. Didham, L. Fahrig, P. Batáry, . . . and C. Westphal. 2012. "Landscape Moderation of Biodiversity Patterns and Processes-Eight

Hypotheses." *Biological reviews* 87(3): 661–685. https://doi.org/10.1111/j.1469-185X.2011.00216.x

Tylianakis, J. M., & Morris, R. J. 2017. "Ecological networks across environmental gradients". *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 25-48. https://doi.org/10.1146/annurev-ecolsys-110316-022821

Tylianakis, J. M., E. Laliberté, A. Nielsen, and J. Bascompte. 2010. "Conservation of Species Interaction Networks." *Biological conservation* 143(10): 2270–2279. https://doi.org/10.1016/j.biocon.2009.12.0

Tylianakis, J. M., T. Tscharntke, and O. T. Lewis. 2007. "Habitat Modification Alters the Structure of Tropical Host–Parasitoid Food Webs." *Nature* 445(7124): 202–205. https://doi.org/10.1038/nature05429.

Urruty, N., D. Tailliez-Lefebvre, and C. Huyghe. 2016. "Stability, Robustness, Vulnerability and Resilience of Agricultural Systems. A Review." *Agronomy for sustainable development* 36(1): 1–15. https://dx.doi.org/10.1007/s13593-015-0347-5

Vanbergen, A. J., B. A. Woodcock, M. S. Heard, and D. S. Chapman. 2017. "Network Size, Structure and Mutualism Dependence Affect the Propensity for Plant–Pollinator Extinction Cascades." *Functional ecology* 31(6): 1285–1293. https://doi.org/10.1111/1365-2435.12823

Venables, W. N., & Ripley, B. D. (2013). *Modern applied statistics with S-PLUS*. Springer Science & Business Media. https://doi.org/10.2307/2685660

Venzon, M., D. S. S. L. Amaral, P. H. B. Togni, and J. A. M. Chiguachi. 2019. "Interactions of Natural Enemies with Non-Cultivated Plants." In *Natural Enemies of Insect Pests in Neotropical Agroecosystems*, 15–26. Springer, Cham. https://doi.org/10.1007/978-3-030-24733-1_2

Wissinger, S. A. 1997. "Cyclic Colonization in Predictably Ephemeral Habitats: A Template for Biological Control in Annual Crop Systems." *Biological control* 10(1): 4–15. https://doi.org/10.1006/bcon.1997.0543

Yang, F., B. Liu, Y. Zhu, K. A. Wyckhuys, W. van der Werf, and Y. Lu. 2021. "Species Diversity and Food Web Structure Jointly Shape Natural Biological Control in Agricultural Landscapes." *Communications Biology* 4(1): 979. https://doi.org/10.1038/s42003-021-02509-z

Yang, L., L. Xu, B. Liu, Q. Zhang, Y. Pan, Q. Li, . . . and Y. Lu. 2019. "Non-Crop Habitats Promote the Abundance of Predatory Ladybeetles in Maize Fields in the Agricultural

Landscape of Northern China." *Agriculture, Ecosystems & Environment* 277: 44–52. https://doi.org/10.1016/j.agee.2019.03.008

Yang, L., Y. Zeng, L. Xu, B. Liu, Q. Zhang, and Y. Lu. 2018. "Change in Ladybeetle Abundance and Biological Control of Wheat Aphids Over Time in Agricultural Landscape." *Agriculture, Ecosystems & Environment* 255: 102–110. https://doi.org/10.1016/j.agee.2017.12.013

Zhao, Y., T. Lou, R. Cao, L. Jiang, Q. Xu, and Q. Zhan. 2024. "Predation Efficiency and Biological Control Potential of *Micromus angulatus* Against *Aphis craccivora*." *Agronomy* 14(10). https://doi.org/10.3390/agronomy14102242

Appendix S1

Supplementary methods

Data analysis

We fitted generalized linear models (GLMs) with the *betareg* and *MASS* packages (Grün et al., 2012, Venables & Ripley 2013) to model local responses, including coccinellid abundance, richness, Shannon and Simpson diversity, aphid density, and network metrics (modularity, nestedness, vulnerability, connectance, generality, specialization) in separate models and without predictors (local models).

For each response variable, we built landscape GLMs by adding individual land cover predictors (forest, savanna, grassland, pasture, agriculture, and urban) to the respective local model. We tested each cover type in separate models. For coccinellid abundance, network modularity and nestedness, we also tested additive combinations when individual predictors showed significant effects ($p < 0.05$). The additive models were: coccinellid abundance: forest + savanna; modularity: agriculture + savanna, agriculture + savanna + urban, forest + savanna + urban and forest + urban); nestedness: agriculture + grassland, grassland + pasture . To ensure model parsimony and avoid overfitting, we restricted additive models to justified cases. For the same reason, we neither included all predictors in a single model nor tested for variable interactions, as the resulting complexity would have exceeded the predictive capacity of our analysis, increasing the chances for type I errors. After extracting the landscape composition from the most explanatory buffer size for each response variable, we proceeded by selecting only the landscape cover types that had significant effects on local variables for the subsequent analysis. We selected all final models based on the lowest AIC values (Tables S1 and S2) and validated model assumptions using residual diagnostics from the *DHARMa* package (Hartig & Lohse 2020). These objectively selected variables were then carried forward into statistical models where we tested the effects of landscape composition on the local metrics.

Supplementary results

Table S1. Model selection results for *Scalescape* models assessing the effects landscape composition on coccinellid abundance, coccinellid diversity (Simpson and Shannon indices) and aphid density. Data were collected from 28 organic brassica farms in the Brazilian Federal District between 2022 and 2024. Within these models, bold parameter values are statistically significant ($p < 0.05$).

Response	Model	Predictors	AIC	ΔAIC	Weight	Estimate	SE	t	p.value
Coccinnelid abundance	1	**Forest**	263.77	0.00	0.423	**-2.301**	**0.890**	**-2.585**	**0.010**
	2	**Savanna**	265.03	1.26	0.226	**2.513**	**1.044**	**2.407**	**0.016**
	3	Grassland	266.68	2.91	0.099	11.575	7.133	1.623	0.105
	4	Forest Savanna	266.73	2.96	0.096	-1.663 1.334	1.109 1.268	-1.500 1.052	0.134 0.293
	5	Urban	266.94	3.17	0.087	-1.540	0.817	-1.884	0.060
	6	Pasture	268.71	4.94	0.036	0.603	0.590	1.022	0.307
	7	Agriculture	268.87	5.10	0.033	-1.188	1.260	-0.943	0.346
Simpson Index	8	**Agriculture**	47.94	0.00	0.836	**2.146**	**0.708**	**3.030**	**0.006**
	9	Savanna	52.88	4.94	0.071	-1.179	0.631	-1.868	0.073
	10	Urban	54.38	6.44	0.033	-1.171	0.838	-1.397	0.174
	11	Grassland	55.06	7.12	0.024	-6.016	5.319	-1.131	0.268
	12	Forest	55.27	7.33	0.021	-9.470	9.113	-1.039	0.308
	13	Pasture	56.03	8.09	0.015	-0.450	0.757	-0.594	0.558
Shannon index	14	**Agriculture**	57.10	0.00	0.708	**2.139**	**0.824**	**2.596**	**0.015**
	15	Savanna	61.03	3.92	0.100	-1.213	0.774	-1.568	0.129
	16	Urban	61.76	4.65	0.069	-1.271	0.966	-1.315	0.200

	17	Grassland	62.44	5.34	0.049	-6.251	6.068	-1.030	0.312
	18	Forest	62.79	5.69	0.041	-8.842	10.424	-0.848	0.404
	19	Pasture	63.23	6.13	0.033	-0.437	0.786	-0.554	0.584
Aphid density	20	**Forest**	312.00	0.00	0.484	**-11.697**	**4.482**	**-2.61**	**0.009**
	21	**Pasture**	314.38	2.38	0.147	**3.852**	**2.120**	**1.817**	**0.069**
	22	**Urban**	314.69	2.69	0.126	**-1.711**	**0.850**	**-2.013**	**0.044**
	23	**Forest**	314.98	2.98	0.109	**-12.64**	**4.644**	**-2.721**	**0.007**
		Urban				1.211	1.188	1.019	0.308
	25	Grassland	316.43	4.43	0.053	5.591	4.714	1.186	0.236
	26	Savanna	316.95	4.95	0.041	0.965	0.928	1.04	0.298
	27	Agriculture	316.99	5.00	0.040	-0.795	0.782	-1.016	0.309

Table S2. Model selection results from *Scalescape* models assessing the effects of landscape composition on network modularity, nestedness, connectance, and generality. Network metrics not included here are those for which no land-use variable had a statistically significant effect. Data were collected from 28 organic brassica farms in the Brazilian Federal District between 2022 and 2024. Within these models, bold parameter values are statistically significant ($p < 0.05$).

Response	Model	Predictors	AIC	ΔAIC	Weight	Estimate	SE	z	p.value
Network modularity	1	**Agriculture**	-106.29	0.00	0.436	**4.122**	**1.286**	**3.205**	**0.001**
		Savanna				**4.236**	**1.290**	**3.283**	**0.001**
	2	**Agriculture**	-104.58	1.72	0.185	**4.442**	**1.464**	**3.034**	**0.002**
		Savanna				**4.752**	**1.650**	**2.880**	**0.004**
		Urban				1.032	1.968	0.524	0.600
	3	Forest	-103.85	2.45	0.128	-26.242	13.936	-1.883	0.060
		Savanna				2.094	1.144	1.831	0.067
		Urban				-2.302	1.499	-1.536	0.125
	4	**Savanna**	-103.77	2.52	0.124	**3.535**	**1.008**	**3.507**	**0.001**
	5	**Forest**	-102.49	3.80	0.065	**-34.461**	**12.775**	**-2.698**	**0.007**
		Urban				**-3.545**	**1.361**	**-2.605**	**0.009**
	6	**Forest**	-101.53	4.76	0.040	**-27.029**	**8.173**	**-3.307**	**0.001**
	7	**Agriculture**	-98.71	7.59	0.010	**3.460**	**1.378**	**2.511**	**0.012**
	8	**Urban**	-98.27	8.03	0.008	**-3.626**	**1.372**	**-2.643**	**0.008**
	9	**Pasture**	-96.40	9.90	0.003	**1.735**	**0.723**	**2.399**	**0.016**

	10	Grassland	-93.09	13.20	0.001	-4.953	7.214	-0.687	0.492
Network nestedness	11	**Grassland**	155.02	0.00	0.568	**-138.453**	**24.405**	**-5.673**	**< 0.0001**
	12	Agriculture	157.11	2.09	0.200	-6.073	4.241	-1.432	0.165
		Grassland				**-116.370**	**30.588**	**-3.804**	**0.001**
	13	**Grassland**	157.31	2.29	0.181	**-127.925**	**26.283**	**-4.867**	**< 0.0001**
		Pasture				12.009	9.425	1.274	0.214
	14	**Agriculture**	164.10	9.08	0.006	**-7.888**	**1.968**	**-4.009**	**0.001**
	15	**Pasture**	171.98	16.96	0.000	**28.785**	**12.004**	**2.398**	**0.024**
	16	Forest	173.34	18.32	0.000	7.273	3.531	2.059	0.050
	17	Savanna	175.96	20.94	0.000	-7.674	6.192	-1.239	0.226
	18	Urban	177.13	22.11	0.000	6.875	10.706	0.642	0.526
Network connectance	19	**Savanna**	-88.28	0.00	0.490	**-0.112**	**0.052**	**-2.164**	**0.040**
	20	Grassland	-86.32	1.96	0.184	-0.552	0.342	-1.615	0.118
	21	Urban	-85.51	2.76	0.123	0.051	0.038	1.341	0.191
	22	Forest	-84.93	3.35	0.092	0.392	0.355	1.105	0.279
	23	Pasture	-84.02	4.26	0.058	0.032	0.053	0.595	0.557
	24	Agriculture	-83.82	4.46	0.053	0.029	0.070	0.407	0.687
Network generality	25	**Grassland**	60.87	0.00	0.456	**8.040**	**3.840**	**2.094**	**0.046**
	26	Savanna	62.97	2.11	0.159	-0.913	0.618	-1.477	0.152
	27	Urban	63.43	2.57	0.126	0.999	0.762	1.312	0.201
	28	Forest	63.47	2.61	0.124	13.197	10.173	1.297	0.206
	29	Pasture	64.39	3.52	0.078	-1.129	1.270	-0.889	0.382
	30	Agriculture	65.00	4.14	0.058	-0.459	0.998	-0.460	0.650

Table S3. Model selection results for Generalized Linear Models (GLMs) assessing the effects of forest (0.11 km radius) and savanna (10.9 km radius) proportions and aphid density on coccinellid abundance. Data were collected from 28 organic brassica farms in the Brazilian Federal District between 2022 and 2024. A confidence set of models was selected using a ΔAICc < 6 threshold. Within these models, bold parameter values are statistically significant ($p < 0.05$).

Model	Predictors	estimate	logLik	AICc	ΔAICc	Relative weight	χ^2	d.f.	p.value
1	**Aphid density**	**0.007**	-123.52	256.78	0	0.58	**11.68**	**1**	**< 0.001**
	Savanna	**2.311**					**6.58**	**1**	**0.01**
2	**Aphid density**	**0.009**	-123.442	259.61	2.83	0.11	**11.74**	**1**	**0.001**
	Savanna	**2.801**					**66.14**	**1**	**0.010**
	Aphid:Savanna	-0.007					0.16	1	0.689
3	**Aphid density**	**0.007**	**-123.51**	**259.74**	**2.96**	**0.13**	**0.03**	**1**	**0.003**
	Savanna	2.186					3.41	1	0.065
	Forest	-0.173					8.69	1	0.872
4	**Aphid density**	**0.006**	-125.13	260.00	3.22	0.12	**5.99**	**1**	**0.014**
	Forest	-1.477					2.89	1	0.089
5	**Aphid density**	**0.007**	-126.52	260.03	3.25	0.11	**9.82**	**1**	**0.002**
6	**Aphid density**	**0.007**	-122.873	261.75	4.964	0.04	**10.06**	**1**	**0.002**
	Savanna	2.989					3.54	1	0.060
	Forest	2.449					0.03	1	0.868
	Forest:Savanna	-8.708					1.29	1	0.256
7	**Aphid density**	**0.005**	-124.90	262.54	5.75	0.02	**60.80**	**1**	**0.014**
	Forest	-2.460					29.41	1	0.086
	Aphid:Forest	0.018					0.46	1	0.499
8	**Forest**	**-2.301**	-127.37	262.77	5.99	0.03	**6.42**	**1**	**0.011**

Table S4. Model selection results for Generalized Linear Models (GLMs) assessing the effects of agriculture (3.6 km radius) and savanna (10.9 km radius) proportions on network modularity. Data were collected from 28 organic brassica farms in the Brazilian Federal District between 2022 and 2024. A confidence set of models was selected using a ΔAICc < 6 threshold. Within these models, bold parameter values are statistically significant ($p < 0.05$).

Model	Predictors	estimate	logLik	AICc	ΔAICc	Relative weight	χ^2	d.f.	p.value
1	**Agriculture**	**3.853**	44.72	-79.70	0	0.68	**9.86**	**1**	**0.002**
	Savanna	**3.819**					**7.80**	**1**	**0.005**
2	**Agriculture**	**2.691**	44.78	-76.83	2.87	0.16	**9.84**	**1**	**0.001**
	Savanna	**3.491**					**7.84**	**1**	**0.008**
	Agriculture:Savanna	3.772					0.04	1	0.835
3	**Agriculture**	**2.859**	41.20	-75.39	2.86	0.08	**5.99**	**1**	**0.014**
4	**Savanna**	**2.856**	40.85	-74.70	4.99	0.06	**5.12**	**1**	**0.024**

Table S5. Model selection results for Generalized Additive Models (GAM) assessing the effects of coccinellid abundance, aphid density and network metrics on Biological Control Index (BSI). Data were collected from 28 organic brassica farms in the Brazilian Federal District between 2022 and 2024. A confidence set of models was selected using a ΔAICc < 6 threshold. Within these models, bold parameter values are statistically significant ($p < 0.05$).

Model	Predictors	estimate	logLik	AICc	ΔAICc	Relative weight	χ^2	d.f.	p.value
1	**Connectance**	**-5.381**	26.07	-42.39	0.00	0.22	**6.085**	**1**	**0.014**
	Aphid density	**-0.005**					**11.382**	**1**	**0.001**
2	Connectance	-4.239	27.04	-41.36	1.04	0.13	3.319	1	0.069
	Modularity	1.308					1.791	1	0.181
	Aphid density	**-0.004**					**10.494**	**1**	**0.001**
3	**Modularity**	**1.924**	25.31	-40.88	1.51	0.10	**4.232**	**1**	**0.020**
	Aphid density	**-0.003**					**6.742**	**1**	**0.009**
4	Coccinellid abundance	-0.001	26.12	-39.52	2.88	0.05	0.182	1	0.670
	Connectance	**-5.855**					**5.727**	**1**	**0.017**
	Aphid density	**-0.005**					**9.378**	**1**	**0.002**
5	**Connectance**	**-5.084**	26.07	-39.40	2.99	0.05	**0.029**	**1**	**0.029**
	Generality	0.045					0.096	1	0.760
	Aphid density	**-0.005**					**10.824**	**1**	**0.001**
6	**Connectance**	**-5.506**	26.03	-39.33	3.06	0.05	**5.584**	**1**	**0.018**
	Nestedness	0.003					0.028	1	0.867
	Aphid density	**-0.005**					**11.086**	**1**	**0.001**
7	**Aphid density**	**-0.003**	23.15	-39.30	3.09	0.05	**4.846**	**1**	**0.028**
8	Generality	0.118	25.68	-38.62	3.77	0.03	0.709	1	0.400
	Modularity	1.808					3.643	1	0.056
	Aphid density	**-0.003**					**6.839**	**1**	**0.009**
9	**Modularity**	**1.986**	25.62	-38.52	3.87	0.03	**4.423**	**1**	**0.035**
	Nestedness	-0.015					0.626	1	0.429
	Aphid density	**-0.003**					**6.816**	**1**	**0.009**
10	Coccinellid abundance	-0.001	27.05	-38.09	4.30	0.03	0.121	1	0.728
	Connectance	-4.645					3.196	1	0.074
	Modularity	1.281					1.669	1	0.196
	Aphid density	**-0.004**					**8.969**	**1**	**0.003**
11	Connectance	-3.969	27.02	-38.04	4.35	0.02	2.593	1	0.107
	Generality	0.043					0.088	1	0.766
	Modularity	1.303					1.723	1	0.189
	Aphid density	**-0.004**					**9.958**	**1**	**0.002**
12	Coccinellid abundance	0.001	25.38	-38.02	4.37	0.02	0.194	1	0.660

	Modularity	**1.876**					**3.858**	**1**	**0.050**
	Aphid density	**-0.003**					**5.921**	**1**	**0.015**
13	Connectance	-4.096	26.98	-37.96	4.44	0.02	2.593	1	0.107
	Modularity	1.333					1.711	1	0.191
	Nestedness	-0.002					0.015	1	0.901
	Aphid density	**-0.004**					**9.865**	**1**	**0.002**
14	Generality	0.158	23.78	-37.83	4.56	0.02	1.174	1	0.279
	Aphid density	**-0.003**					**5.393**	**1**	**0.020**
15	Coccinellid abundance	0.002	23.34	-36.94	5.45	0.01	0.382	1	0.540
	Aphid density	**-0.003**					**4.747**	**1**	**0.029**
16	Nestedness	-0.012	23.32	-36.89	5.50	0.01	0.348	1	0.556
	Aphid density	**-0.003**					**4.895**	**1**	**0.027**
17	~ 1	NA	20.54	-36.61	5.79	0.01	NA	NA	NA
18	Modularity	1.571	21.71	-36.42	5.97	0.01	2.309	1	0.129
19	Coccinellid abundance	-0.002	26.20	-36.41	5.99	0.01	0.339	1	0.560
	Connectance	**-5.575**					**4.973**	**1**	**0.026**
	Generality	0.082					0.267	1	0.605
	Aphid density	**-0.004**					**8.482**	**1**	**0.004**

Table S6. Non-crop plants adjacent to brassica fields where coccinellid species were observed. Data are ordered by the total number of coccinellids counted on each plant species. Observations were made on 28 organic brassica farms in the Brazilian Federal District from 2022 to 2024. The native/exotic status and native range for each non-crop species are based on information from Plants of the World Online (Royal Botanic Gardens, Kew 2025).

Scientific name	**Family**	**Coccinellid species observed**	**Total observed**	**Native or exotic**	**Native range**
Bidens pilosa	Asteraceae	*Cycloneda sanguinea*, *Eriopis connexa*, *Harmonia axyridis*, *Hippodamia convergens*, *Hyperaspis festiva*, *Scymnus* sp.	279	Native	Tropical and Subtropical America
Sonchus oleraceus	Asteraceae	*Cycloneda sanguinea*, *Eriopis connexa*, *Harmonia axyridis*, *Hippodamia convergens*	179	Exotic	Africa, Europe
Galinsoga parviflora	Asteraceae	*Coleomegilla maculata*, *Cycloneda sanguinea*, *Eriopis connexa*, *Hippodamia convergens*	68	Native	North, Central and South America
Cyperus rotundus	Cyperaceae	*Cycloneda sanguinea*, *Eriopis connexa*, *Hippodamia convergens*	62	Exotic	Africa, Europe, Asia
Brachiaria sp.	Poaceae	*Cycloneda sanguinea*, *Eriopis connexa*, *Harmonia axyridis*, *Hippodamia convergens*	34	Exotic	Africa

Tithonia diversifolia	Asteraceae	*Cycloneda sanguinea*, *Eriopis connexa*, *Harmonia axyridis*, *Hippodamia convergens*	32	Exotic	Mexico to Central America.
Amaranthus sp.	Amaranthaceae	*Cycloneda sanguinea*, *Harmonia axyridis*, *Hippodamia convergens*	23	Native	Tropical America
Amaranthus viridis	Amaranthaceae	*Cycloneda sanguinea*, *Eriopis connexa*, *Hippodamia convergens*	21	Native	Tropical America
Unknown	Poaceae	*Cycloneda sanguinea*, *Eriopis connexa*, *Hippodamia convergens*	18	Unknown	Unknown
Oxalis latifolia	Oxlidaceae	*Eriopis connexa*, *Harmonia axyridis*, *Hippodamia convergens*	12	Native	North, Central and South America
Canavalia ensiformis	Fabaceae	*Hippodamia convergens*	4	Native	North, Central and South America
Emilia fosbergii	Asteraceae	*Hippodamia convergens*	4	Unknown	Unknown
Rumex obtusifolius	Polygonaceae	*Eriopis connexa*, *Harmonia axyridis*, *Hippodamia convergens*	3	Exotic	Europe, Africa
Solanum americanum	Solanaceae	*Eriopis connexa*, *Harmonia axyridis*	3	Native	Tropical and Subtropical America
Lepidium didymum	Brassicaceae	*Hippodamia convergens*	2	Native	South America
Nicandra physalodes	Solanaceae	*Harmonia axyridis*, *Hippodamia convergens*	2	Exotic	Peru to Northwest Argentina
Plantago major	Plantaginaceae	*Hippodamia convergens*	1	Exotic	Africa, Europe

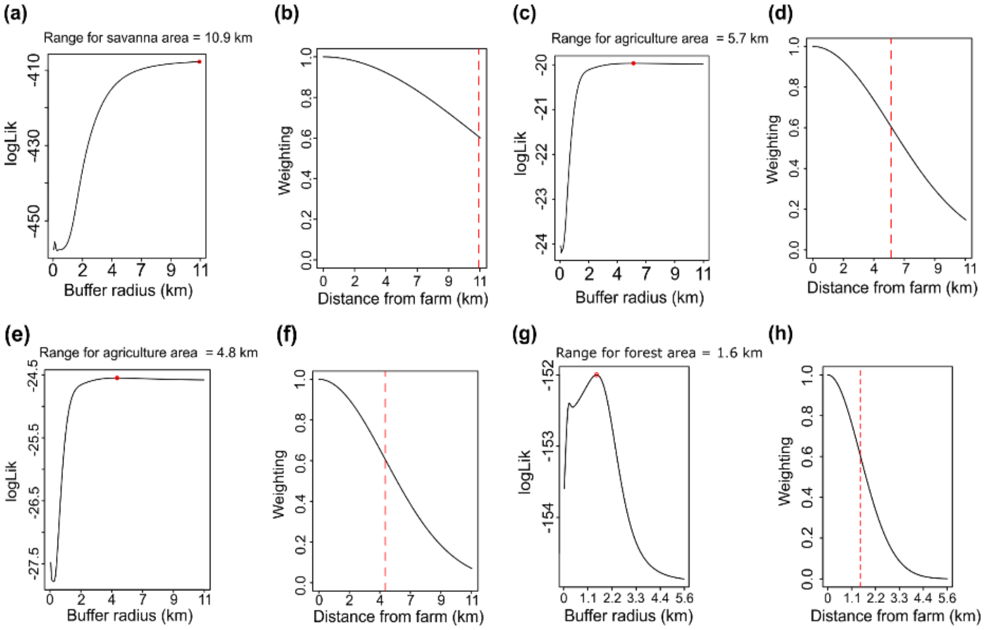

Figure S1. Effects and optimal buffer radius of landscape composition on coccinellid and aphid communities. Proportion of savanna on coccinellid abundance (a, b); agriculture on coccinellid diversity (Simpson index – c,d; Shannon index e,f); forest (g, h) and grassland (i,j) on aphid density. For each land cover type, we show: (left) the optimal buffer radius for model fit, and (right) the decay in natural area weight with distance from the farm.

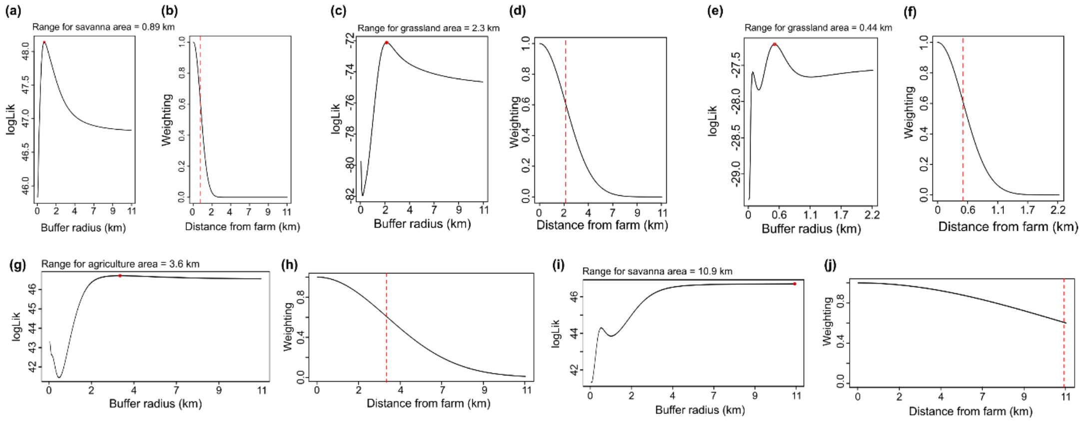

Figure S2. Effects and optimal buffer radius of landscape composition on metrics of tritrophic interaction network. Proportion of savanna on network connectance (a, b); grassland on nestedness (c, d) and on generality (e, f); agriculture (g, h) and savanna (i, j) on modularity. For each land cover type, we show: (left) the optimal buffer radius for model fit, and (right) the decay in natural area weight with distance from the farm.

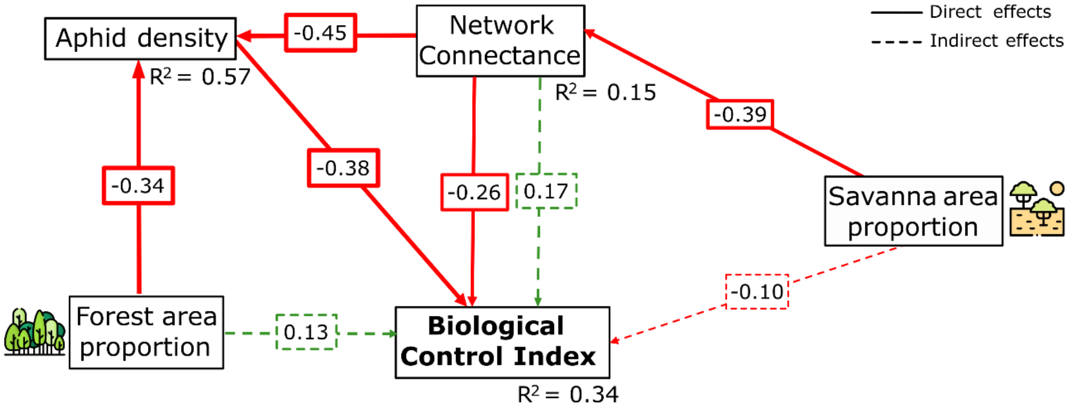

Figure S3. Path diagram of the Structural Equation Model (SEM) testing the direct and indirect pathways through which landscape composition mediated by network connectance and aphid density affects aphid biological control, quantified by the Biological Control Index (BSI). Green arrows indicate positive effects and red arrows negative effects.

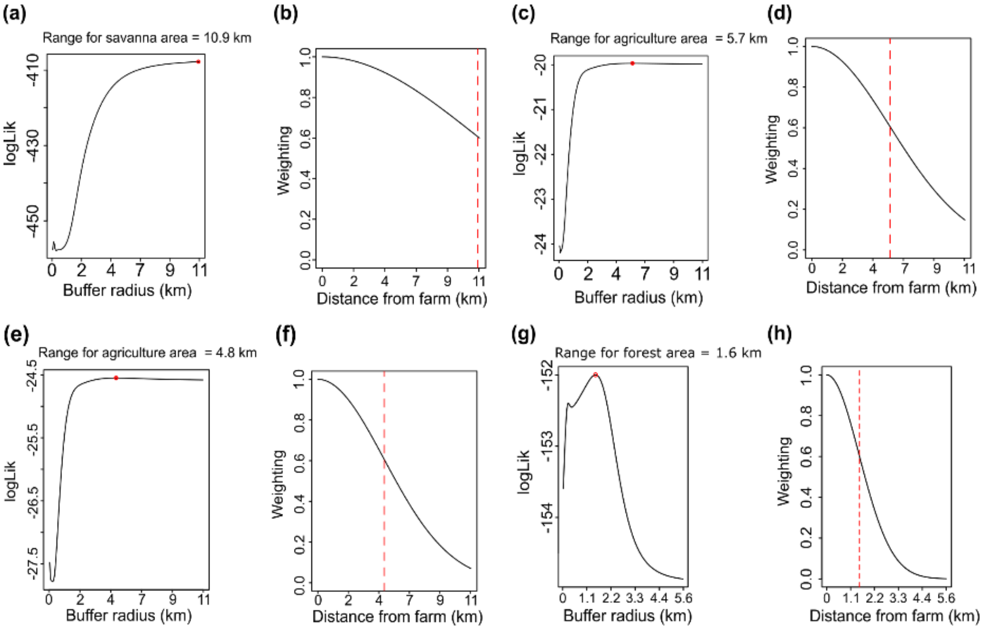

Figure S1. Effects and optimal buffer radius of landscape composition on coccinellid and aphid communities. Proportion of savanna on coccinellid abundance (a, b); agriculture on coccinellid diversity (Simpson index – c,d; Shannon index e,f); forest (g, h) and grassland (i,j) on aphid density. For each land cover type, we show: (left) the optimal buffer radius for model fit, and (right) the decay in natural area weight with distance from the farm.

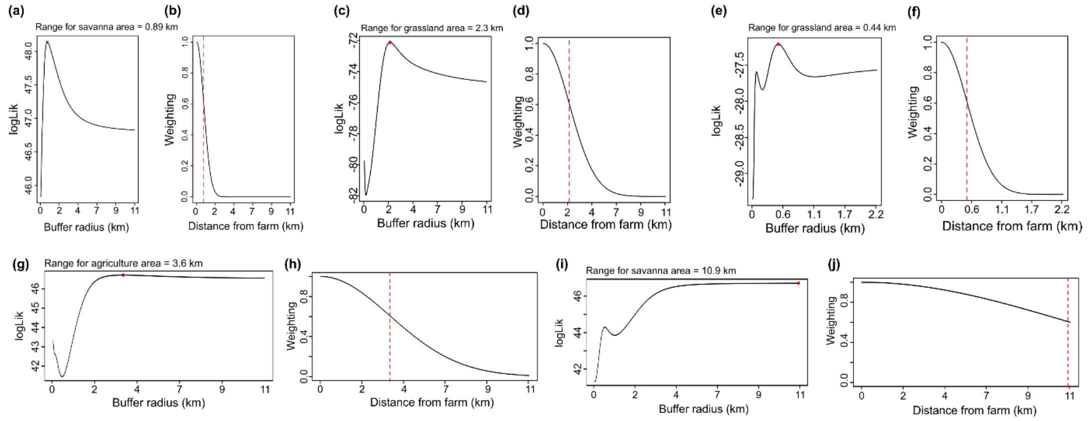

Figure S2. Effects and optimal buffer radius of landscape composition on metrics of tritrophic interaction network. Proportion of savanna on network connectance (a, b); grassland on nestedness (c, d) and on generality (e, f); agriculture (g, h) and savanna (i, j) on modularity. For each land cover type, we show: (left) the optimal buffer radius for model fit, and (right) the decay in natural area weight with distance from the farm.

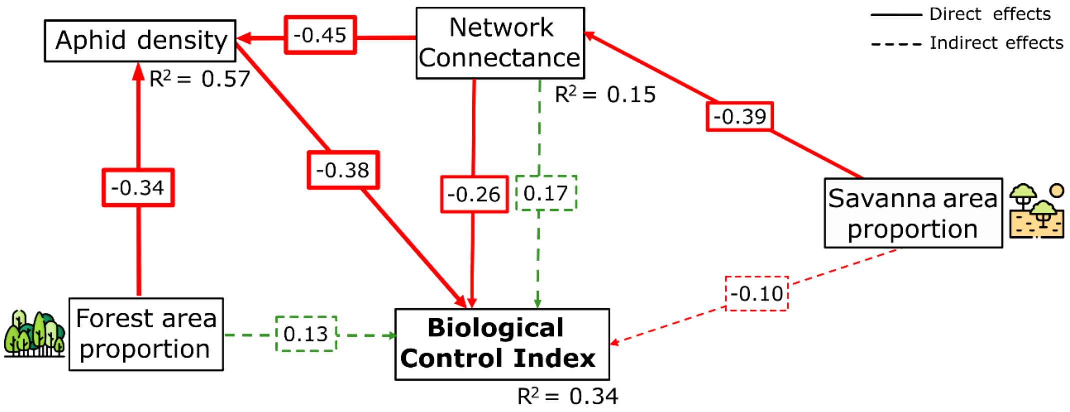

Figure S3. Path diagram of the Structural Equation Model (SEM) testing the direct and indirect pathways through which landscape composition mediated by network connectance and aphid density affects aphid biological control, quantified by the Biological Control Index (BSI). Green arrows indicate positive effects and red arrows negative effects.

GENERAL CONCLUSIONS

In this work, we unveiled some of the ecological interactions and habitat use patterns that affect the communities of important generalist predators in agroecosystems, as well as how spatial and temporal factors influence the structure and dynamics of their interactions. By investigating how different coccinellid species utilize resources spatially segregated within organic brassica crops, we demonstrated that they partition habitat and microhabitat throughout their life cycles. Furthermore, we found that behavior of species directly determined the degree of spatial niche overlap, leading us to propose a functional classification for the species studied. Also, changes in prey availability directly impact coccinellids and their trophic interactions, resulting in shifts in the species' trophic position over a relatively short period. Additionally, the structure of the coccinellid trophic interaction networks become more nested with increasing prey availability. This pattern is likely a direct result of changes in species' feeding behavior, where some become more generalist while others become more specialist, thus probably driving a nested network structure.

Finally, by evaluating the effect of the landscape not only on the abundance and diversity of the communities but also on their trophic interactions, we demonstrated that the landscape is a crucial factor for the structuring of these interactions. Moreover, we showed that interactions directly affect the biological control service, with the surrounding landscape indirectly influencing the provision of this ecosystem service. Thus, we have elucidated key processes that sustain coexistence and drive the trophic structuring of generalist predator communities, enabling their formation and establishment even in environments with constant disturbance regimes. By analyzing these mechanisms across multiple spatial scales, we connected factors at the individual and population levels (such as behavior and interspecific interactions) with ecosystem-level processes (such as energy transfer along the trophic chain).

To summarize, all those findings emphasize that the landscape is an important predictor of species diversity and abundance, where different types of natural vegetation will predict the potential colonizers of agricultural habitats. However, this thesis goes further by demonstrating that these landscape habitats influence how species interactions filtered from the regional pool are structured locally, which in turn can affect the efficiency of the biological control service. In this context, non-crop plants are important mediators of these interactions and represent an opportunity for the maintenance of species and their interactions. Specifically, coccinellid species use these plants as food, which is essential during periods of prey scarcity. Consequently, the use of non-crop plants food resources and prey density explain the trophic variations of the species throughout the season and how energy transfer occurs between trophic levels. Even so, general patterns that occur at the community level may vary among species due to differences in life histories, functional traits, and behavior.