Urban to Agro Ecosystems: Effects of Land Use on Pollinators and Ecosystem Services

by

Chatura Vaidya

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Ecology and Evolutionary Biology)
in the University of Michigan
2023

Doctoral Committee:

Professor John Vandermeer, Chair
Professor Catherine Badgley
Professor Ivette Perfecto
Professor Nathan Sanders

Chatura Vaidya

chatura@umich.edu

ORCID iD: 0000-0002-1655-9496

Dedication

To my mother and father, for all their sacrifices and undying support.

To my husband, Pranav, for his immense patience and unconditional love, in that order.

Acknowledgements

Even though on the face of this dissertation lies my name alone, it has taken a village for its completion, and I would like to take a few moments to acknowledge all the people who made it possible.

I would like to thank my committee members, Catherine Badgley and Nathan Sanders. Catherine has been on my committee since my Master's degree in EEB and her insights and feedback have been extremely valuable. Nate graciously agreed to be on my committee in my fifth year and I am grateful to him for his constant encouragement and support.

My tryst with the Perfectomeer lab started when I read the famous book –"Nature's Matrix" and soon after, I emailed Ivette Perfecto, my honorary co-advisor, to see if I could volunteer in her lab and learn the ropes of ecology. If it weren't for her, I would not be writing this today. She has provided constant feedback, encouragement and wisdom not just about my research but also about life. Finally, I am forever indebted to John Vandermeer, my advisor. He has been my mentor and my friend. He has expanded my horizons without a doubt – through conversations about science, politics and the intermingling of science and politics, and taught me to be critical about the work we do and who we do it for. He has cheered me when I do well and told me to not rest on my laurels, all in the same breath. He has encouraged me through numerous rejections and taught me to wear them proudly on my sleeve. For all these lessons and countless more, I thank him deeply.

The Perfectomeer lab community has been an important part of my growth as a scientist at the University of Michigan. Starting with Linda Marin and David Gonthier, who took me under their wings as a volunteer in Ivette's lab, their friendship and mentorship was important to me during the initial years. I continued to make more friends in the Perfectomeer lab along the way: Kaleigh Fisher, Senay Yitbarek, Kevin Li, Paul Glaum, Carolina Simao, Theresa Ong, Lauren Schmitt, Bolivar Aponte-Rolon, Aldo de la Mora, Mariana Valencia, Aaron Iverson, Beatriz Otero Jimenez, Estelí Soto-Jimenez, Kate Ennis, Kim Williams-Guillén, Jenny Flores, Jacob Longmeyer, Simone Oliphant, Nicholas Medina, Alexa White, Naim Edwards, Sophia Moon. Special thanks to my *amigos chismosos*– Kristel Sanchez, Zach Hajian-Forooshani, Iris Rivera and Jonno Morris. From our travels to faraway countries to the many countless evenings spent in your company in Ann Arbor, you made my life richer. Gordon Fitch, my friend and collaborator- we were the only ones interested in bees in a world full of ants, and knowing that we could count on each other has been tremendously reassuring for me.

I did most of my fieldwork in Finca Irlanda in Mexico, where I spent many a winters and summers in the company of Gustavo López Bautista and Gabirel Dominguez. We spent mornings drinking chai and afternoons telling each other about the most amazing things we witnessed on that day in the field. Gabriel also provided invaluable support in the field which made fieldwork even more enjoyable. Doña Cruz, Suly and Flor cooked for an army every summer and witnessed chaos at the lunch table every day. Working on the farm has been the single most exhilarating experience of my life.

My research would not have been completed without the tireless work of my research assistants – Anna Oana, Sophie Alphonso, John Gonzalez and Pia Lu. Thank you for your efforts and your dedication. I also want to thank my funding sources – Rackham Graduate School and the EEB department.

I have been lucky to be part of a wonderful and bright cohort of fellow PhD students. Thanks to Sonal Gupta, Sasha Bishop, Peter Cerda, Giorgia Auteri, and Nicholas Medina. Special thanks to Tamara Milton, we have practically written our entire dissertations together and I couldn't have asked for a better friend to go through periodic oscillations of panic and calm with.

Our tremendous EEB staff made navigating through this degree a lot easier – thanks to Cindy Carl, Nathan Sadowsky, Gail Kuhnlein, Linda Gardcia, Michael Ehnis, John Megahan, Amanda Kosnik and Jen Wolff. Mike Palmer at the Matthaei Botanical Gardens helped me take care of my coffee plants for nearly 2 years, even through the pandemic. I was one of the lucky few to see the corpse flower in bloom, thanks to him. I have been fortunate to teach with thoughtful and amazing teachers from whom I have learned a lot – Laura Eidietis, Lynn Carpenter, Cindee Giffen, Annette Ostling, Diarmaid O'Foighil and John Vandermeer.

Finally, I am grateful to my family – I learned the meaning of sincerity and dedication from my father. My mother has been fiercely fighting for my education ever since I remember. For that and everything she has sacrificed for me, I am truly grateful. My friends from back home, now dispersed all over the world - I am lucky to be able to call you whenever I want. A few days before I started my PhD, we found Pippin, a goofy, loving, and crazy strong dog. He has not left my side since then. Finally, I want to thank my husband, Pranav, for being my in-house statistician, for being the best travel partner, and above all, for being with me in this life.

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

We are now living in the Anthropocene, a new epoch characterized by unprecedented levels of anthropogenic impact on earth systems. This epoch has seen a dramatic decline in biodiversity and, consequently, poses a great challenge to humanity, as we encounter diminished provisioning of ecosystem services, vital for our survival. My dissertation examines the ecological impact of land-use change, driven by agriculture and urbanization on the provisioning of ecosystem services, mainly pollination, and the pollinators that provide this ecosystem service. I investigate these questions at the level of the landscape within both the agricultural and urban matrix, and at the level of the farm scale in the agricultural matrix.

In chapter 2, I evaluate the suitability of three habitats (organic shaded coffee farm, sun coffee farm, forest fragment) in supporting populations of *Scaptotrigona mexicana,* a stingless bee species that is native to Mexico and of great cultural significance. I find that stingless bee colony growth, survival and pollen-diet richness are comparable in the shaded coffee farm and the forest fragment. In contrast, I find that the sun coffee farm is inhospitable for stingless bee colonies likely due to use of agrochemicals and low floral resources.

In chapters 3-4, I investigate the effects of nitrogen-fixing shade tree management on the interactions of two ecosystem services – pest control and pollination – in an organic shaded coffee farm in Mexico. I examine the effects of pollinators and ants as agents of pollination and pest control on *Coffea arabica.* I find that there is no trade-off between pest control and pollination services despite the deterrence of pollinators by the dominant and aggressive ant

species, *Azteca sericeasur,* which also controls the coffee berry borer, a major pest of *Coffea arabica.* I find additive effects of pest-control and pollination on early fruit set and fruit weight, and that nitrogen-fixing shade trees only has indirect effects on pest-control via the reduction of *Azteca* ant activity on the coffee bushes.

In chapter 5, I study the pollination ecology of green cardamom, *Elleteria cardamomum.* I examine the relative contributions of common pollinator species visiting cardamom using single-visit pollen deposition and fruit set in an organic shaded coffee plantation. I find that cardamom has both legitimate visitors and nectar-robbers. However, the nectar-robbers also collect pollen from the flowers, thereby pollinating the plant despite robbing its nectar. The most effective pollinator of *E. cardamomum* in its introduced range is a native stingless nectar-robbing bee species, *Scaptotrigona mexicana*, highlighting the need to examine the activity of visitors that on the face of it appear to only "rob" plants but may turn out to be the most effective pollinators. I discuss the implications of this finding by laying out conditions under which cardamom may adapt to its most effective pollinator, if at all.

In chapter 6, I investigate the influence of roads on pollinator movement and pollination by examining patterns of pigment transfer. I find that plants across a road from a pigment-added plant receive significantly less pigment than plants on the same side of the road and this effect is mediated by the size of the pollinators. I show that roads pose substantial barriers to bee movement, reducing pollen flow between plants located across roadways from one another.

Finally in chapter 7, I discuss the implications of my studies and future directions.

Chapter 1 : Introduction

We are experiencing a dramatic loss in biodiversity around the world. Human land use, which has resulted in habitat loss and degradation, has been implicated as one of the key drivers (Butchart et al., 2010; Maxwell et al., 2016). This loss of biodiversity can severely impact ecological processes not only necessary for ecosystem function but also critical for human well-being. My research examines the ecological impact of land-use change as driven by agriculture and urbanization. Together, agriculture and urban areas make up 51% of habitable land on Earth (FAO, 2020), making it imperative to study the ecology within those lands and understand their impacts on biodiversity and ecosystem function. We can no longer afford to only focus on the ecology of “pristine” lands without any knowledge of how organisms are interacting with these new environments.

In my dissertation I evaluate the effects of land-use change on plant-insect interactions, and the ecosystem services that they provide as a result, with a particular focus on pollination. Animal pollinators are responsible for pollinating ~88% of angiosperm species, providing vital ecosystem services (ES) to wild plants and food crops worldwide (Ollerton et al., 2011). Several pollinator taxa, including bees, are in decline in many parts of the world due to multiple interacting pressures including diseases, climatic changes, pesticides and land-use change, with the latter having the most impact on pollinator declines (Potts et al., 2010). As a result, plants that depend on pollinators for reproduction are under serious threat and may face parallel declines (Biesmeijer et al., 2006; Pauw and Hawkins, 2011). Thus, understanding the magnitude of negative impacts land-use change may have on plants and pollinators alike is a matter of urgency.

My dissertation is divided into two parts, the first part focuses on the effects of different agricultural management practices on ecosystem services, mainly pollination but also pest control, and the species that provision these ecosystem services, within the agricultural matrix. The second part of my dissertation focusses on the impact of urbanization, particularly through the effect of roads on creating barriers for pollinator movement and pollen flow within the urban matrix. Below, I explore these parts in greater detail and situate my work within this framework.

Agricultural matrix

Roughly 40% of terrestrial land has been converted to agriculture (FAO, 2015). At the same time, lands already under cultivation have undergone massive intensification, characterized by habitat destruction and fragmentation. On the one hand, agricultural intensification relies heavily on external inputs such as synthetic fertilizers, pesticides and herbicides, and a land stripped of native plant and animal biodiversity to make way for crop monocultures. This ecological simplification of agroecosystems is usually driven by the sole goal of increasing yields at the cost of everything else. On the other hand, agroecological farms or diversified farming systems, largely utilize low external inputs and rely heavily on native and introduced biodiversity for ecological processes. The prevailing narrative, largely pushed by proponents of industrial agriculture, is that the only way to grow enough food to feed an increasing global population is through agricultural intensification. Still, a growing literature shows that agroecosystems that are diverse and managed less intensively, can provide ecosystem services such as pollination, pest control, nutrient cycling, water purification and soil fertility, which in turn can enhance food production (Kremen and Miles, 2012) and serve the dual role of preserving biodiversity and supporting the growing demands of food production efficiently (Chappell and LaValle, 2011). However, we still lack a full understanding of how agricultural management practices on the farm and landscape level influence the many ecological processes that contribute to the productivity of these agroecosystems.

Coffee (*Coffea spp*., Rubiaceae) agroecosystems are model systems to study the ecological impacts of different land-management regimes. Coffee is grown on 11million hectares in the tropics (FAO, 2015) and many of these coffee-growing regions happen to be the most biodiverse regions in the world. Coffee also provides livelihoods to millions of farmers, meaning that its importance, both environmentally and economically, cannot be overstated. Wild coffee is a shade-tolerant species that grows under the canopy of trees, and coffee was traditionally also cultivated under shade trees. However, a push for high-yielding varieties, along with changes in management, and expansion of the global coffee market over the past 50 years have encouraged some growers to reduce, and at times eliminate, shade trees from coffee farms (Jha et al., 2014; Perfecto et al., 1996). Coffee is now cultivated under varying degrees of management ranging from “rustic coffee”, in which coffee is grown under the canopy of wild tree species, almost resembling a forest, to “sun coffee”, which is a monoculture of coffee trees with few shade trees. (Moguel and Toledo, 1999). This makes coffee agroecosystems ideal systems to evaluate the role of management in conserving biodiversity and providing ecosystem services.

In chapter 2, I examine the effect of management practices at the landscape scale, using a gradient of agricultural intensification, on the population dynamics of a native stingless bee species, *Scaptotrigona mexicana.* Stingless bees are important for the pollination of several economically important crops (Heard, 1999; Slaa et al., 2006) and also have tremendous cultural importance. With bees declining globally (Potts et al., 2010; Powney et al., 2019; Zattara and Aizen, 2021) and a concomitant decline in wild stingless bee populations in the tropics, understanding how agricultural management practices influence stingless bee colony health is important not only for protecting ecosystem function and ecosystem services in agricultural landscapes, but also for maintaining and reinvigorating traditional cultural practices. I examine the joint influence of habitat suitability and seasonality on the fitness of stingless bees in three different habitats - 1) a certified organic shaded coffee farm, 2) a conventional sun coffee farm and 3) a forest fragment located between the two farms, in the coffee-growing region of southern Mexico.

In chapters 3-4, I shift focus from the landscape to the farm scale and examine how management practices on the organic shaded coffee plantation influence the interactions between two key ecosystem services: pest-control and pollination. While these services have received much attention separately in order to promote sustainable management practices, research needs to address how ES interact and together contribute to the productivity of the focal crops, while preserving biodiversity. I specifically examine how management of shade trees, in particular nitrogen-fixing shade trees, influence the species that provide pest control and pollination and the interactions among them? In Chapter 3, I examine how soil fertility affects the ant-hemipteran mutualism of *Azteca sericeasur- Coccus viridis,* as a proof-of-concept study to understand whether enhanced nitrogen in coffee plants via nitrogen-fixing shade trees can change the magnitude or strength of the ant-hemipteran mutualism. I investigate whether an increase in soil nutrients leads to an increase in the strength of the mutualism between *Azteca sericeasur* via and its hemipteran partner, *Coccus viridis,* the green coffee scale via increased *Azteca sericeasur* on the coffee plants. The result of the ant-hemipteran mutualism is twofold – one, it results in the control of a major coffee pest by *Azteca sericeasur,* thereby providing pest control, but two, it also results in the deterrence of the pollinators that visit the flowers of the coffee plants, thereby interfering with pollination.

In chapter 4, I further explore the interaction between the aggressive ants, *Azteca sericeasur* and the bees that pollinate the coffee plants and investigate whether management of shade trees mediates the outcome of the bee-ant interaction on the services of pollination and pest control and ultimately on the yield of coffee.

Agriculture has introduced many organisms outside of their native habitats, *Coffea spp.* being an example. Crops that rely on, or benefit from, animal pollination encounter new pollinator communities in their introduced range and may be faced with the challenge of adapting to those novel conditions. With the decline in pollinators, it is all the more essential to understand how plants might respond to shifts in pollinators. In chapter 5, I explore the pollination ecology of *Eletteria cardamomum*, the second most expensive spice in the world that is native to India but is increasingly being cultivated in Mexico and other parts of Central America. I examine the contribution of specific pollinator types to the reproductive success of *E. cardamomum* in its introduced range and lay out conditions under which it may adapt to its most effective pollinator.

Urban matrix

As with agriculture, another key driver implicated in habitat loss is urbanization. Urbanization involves conversion of large areas of natural habitat to impervious surfaces including roads, buildings, parking lots, causing habitat loss and degradation. Urbanization has been accelerating and is projected to increase by 1.2 million km^2 globally by 2050 (Dulac, 2013). For context, urban built-up land is currently occupying 1.5 million km^2 of habitable land. This rapid increase in development is also projected to largely take place in the global South, including in many regions of high biodiversity and vital ecosystem services. While there is an increasing body of literature examining the effects of urbanization on the species composition and community structure of pollinators, little much is known about the effect of roads on pollinator movement.

Road development is a global driver of environmental stress. Currently, the general road network extends to about 32 million km across the globe (IRF, 2017), and is projected to increase by 25 million km globally by 2050 (Dulac, 2013). Roads can have profound impacts on animal populations and ecological communities and there is increasing evidence that roads impact not just vertebrates but also invertebrates – particularly insects – both via mortality from vehicle collisions (Baxter-Gilbert et al., 2015) and by altering movement patterns (Andersson et al., 2017; Bhattacharya et al., 2002). As the road network expands and cuts through highly suitable habitat, roads may not only be barriers to pollinator movement, and therefore pollen movement, but also isolate plant populations genetically. Additionally, different road characteristics may pose different challenges or levels of difficulties for pollinator movement. To address these knowledge gaps, in chapter 6, I investigate the influence of roads on pollinator movement and pollination by examining patterns of pigment transfer between focal plants of two species, *Coreopsis verticillata* and *Monarda fistulosa,* native to Michigan. I evaluate whether roads reduce pigment transfer, and whether this effect is mediated by road characteristics (such as lane number, traffic volume, traffic speed), roadside habitat, or pollinator assemblage.

In the concluding chapter, I summarize the results of the preceding chapters and suggest future directions.

Bibliography

Andersson P, Koffman A, Sjödin NE, Johansson V. Roads may act as barriers to flying insects: Species composition of bees and wasps differs on two sides of a large highway. Nat Conserv 2017. https://doi.org/10.3897/natureconservation.18.12314.

Baxter-Gilbert JH, Riley JL, Neufeld CJH, Litzgus JD, Lesbarrères D. Road mortality potentially responsible for billions of pollinating insect deaths annually. J Insect Conserv 2015. https://doi.org/10.1007/s10841-015-9808-z.

Bhattacharya M, Primack RB, Gerwein J. Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? Biol Conserv 2002. https://doi.org/10.1016/S0006-3207(02)00130-1.

Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, et al. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science (80-) 2006;313:351–4. https://doi.org/10.1126/science.1127863.

Butchart SHM, Walpole M, Collen B, Van Strien A, Scharlemann JPW, Almond REA, et al. Global biodiversity: Indicators of recent declines. Science (80-) 2010. https://doi.org/10.1126/science.1187512.

Chappell MJ, LaValle LA. Food security and biodiversity: Can we have both? An agroecological analysis. Agric Human Values 2011. https://doi.org/10.1007/s10460-009-9251-4.

Dulac J. Global land transport infrastructure requirements. Iea 2013.

FAO. Food and agriculture organisation: Statistical pocketbook 2015. 2015.

Heard TA. The role of stingless bees in crop pollination. Annu Rev Entomol 1999. https://doi.org/10.1146/annurev.ento.44.1.183.

Jha S, Bacon CM, Philpott SM, MÉndez VE, LÄderach P, Rice RA. Shade coffee: Update on a disappearing refuge for biodiversity. Bioscience 2014. https://doi.org/10.1093/biosci/biu038.

Kremen C, Miles A. Ecosystem Services in Biologically Diversified versus Conventional Farming Systems : Benefits , Externalities , and Trade-Offs 2012;17.

Maxwell SL, Fuller RA, Brooks TM, Watson JEM. Biodiversity: The ravages of guns, nets and bulldozers. Nature 2016. https://doi.org/10.1038/536143a.

Moguel P, Toledo VM. Biodiversity conservation in traditional coffee systems of Mexico. Conserv Biol 1999. https://doi.org/10.1046/j.1523-1739.1999.97153.x.

Ollerton J, Winfree R, Tarrant S. How many flowering plants are pollinated by animals?

Oikos 2011;120:321–6. https://doi.org/10.1111/j.1600-0706.2010.18644.x.

Pauw A, Hawkins JA. Reconstruction of historical pollination rates reveals linked declines of pollinators and plants. Oikos 2011;120:344–9. https://doi.org/10.1111/j.1600-0706.2010.19039.x.

Perfecto I, Rice RA, Greenberg R, Van Der Voort ME. Shade coffee: A disappearing refuge for biodiversity: Shade coffee plantations can contain as much biodiversity as forest habitats. Bioscience 1996. https://doi.org/10.2307/1312989.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. Global pollinator declines: Trends, impacts and drivers. Trends Ecol Evol 2010;25:345–53. https://doi.org/10.1016/j.tree.2010.01.007.

Powney GD, Carvell C, Edwards M, Morris RKA, Roy HE, Woodcock BA, et al. Widespread losses of pollinating insects in Britain. Nat Commun 2019. https://doi.org/10.1038/s41467-019-08974-9.

Slaa EJ, Sánchez Chaves LA, Malagodi-Braga KS, Hofstede FE. Stingless bees in applied pollination: Practice and perspectives. Apidologie 2006. https://doi.org/10.1051/apido:2006022.

Zattara EE, Aizen MA. Worldwide occurrence records suggest a global decline in bee species richness. One Earth 2021. https://doi.org/10.1016/j.oneear.2020.12.005.

Chapter 2 : Management Practices and Seasonality Affects Stingless Bee Colony Growth, Foraging Activity and Pollen Diet in Coffee Agroecosystems.[1]

2.1 Abstract

Stingless bees are essential pollinators in tropical ecosystems. Yet compared to their temperate zone counterparts, we know very little about how they respond to agriculture-associated land use, or how this effect is modulated by seasonal variation. Coffee, a dominant crop across the tropics, is grown under diverse management practices, making coffee agroecosystems an important context in which to investigate effects of agricultural management on stingless bees. We evaluated the performance and pollen diet of replicated experimental colonies of the stingless bee *Scaptotrigona mexicana* in three habitats in a coffee-dominated landscape in southern Chiapas, Mexico: a conventional sun coffee farm, a shaded organic coffee farm, and a forest fragment. We asked how habitat and seasonality influenced colony weight, forager activity, and pollen diet over one year. We placed eight experimental colonies in three habitats (three each in coffee farms; two in forest) and monitored them monthly for colony weight and forager activity. We collected corbicular pollen loads from returning foragers and identified pollen morphospecies. We compared performance and diet metrics across habitats and between wet and dry seasons. Colony performance was dramatically worse in the sun coffee farm; all sun coffee colonies died during the study. Colony performance did not differ between shade coffee

---

[1] co – authors are Fitch G*., Dominguez Martinez G.H., Oana A.M., and Vandermeer J.

* co-first author

and forest. Pollen diet richness was lower in the sun coffee colonies, but all habitats shared the same core pollen morphospecies. Across habitats, colony weight and activity and pollen diet richness decreased during the wet season. Our study suggests that conventional sun coffee cannot support stingless bee colonies, likely because of combined effects of agrochemical exposure and floral resource scarcity. By contrast, shade coffee is equivalent to forests in supporting stingless bee colonies.

2.2 Introduction

Populations of bees are in decline in many parts of the world (Potts et al., 2010; Powney et al., 2019; Zattara and Aizen, 2021). This is a grave concern for the maintenance of biodiversity and agricultural yield, as bees are among the most important animal pollinators. Over 85% of angiosperm species rely on animal pollination for reproduction (Ollerton et al., 2011), and pollinator-dependent crops comprised nearly 10% of the world's agricultural production as of 2005, making crop pollination worth >$150 billion (Gallai et al., 2009). Cultivation of pollinator-dependent crops has only increased since the 2005 report (Aizen et al., 2019, 2008).

Bee declines are associated with reductions in both wild plant populations (Biesmeijer et al., 2006; Ehlers et al., 2021; Rodger et al., 2021) and crop production (Reilly et al., 2020). Multiple interacting factors threaten bee populations, foremost among which are habitat loss, pesticide exposure, climate change, and disease (Goulson et al., 2015; Potts et al., 2010). Ironically, given the importance of bee pollination to agricultural productivity, agriculture is a major driver of these threats, and has been implicated in bee declines (Grab et al., 2019). Bees require a consistent supply of floral resources during their foraging season. In intensively managed agricultural landscapes, floral resources may be scarce outside of the period of crop flowering, leading to reduced pollinator population and increasing reliance on managed pollinators, especially honey bees (Klein et al., 2012; Kremen et al., 2002). Agriculture is also a prominent source of exposure to harmful chemicals in bees. There is ample evidence that agrochemical exposure can cause a range of lethal and sub-lethal effects in bees (Dorneles et al., 2021; Straw and Brown, 2021; Wu-Smart and Spivak, 2016). Moreover, food limitation and pesticide exposure together can have additive negative effects on bees, including reduced reproduction and increased mortality (Siviter et al., 2018; Stuligross and Williams, 2021).

The bulk of research on the effects of land use on bees has taken a community-level approach, with fewer focusing on population dynamics (Dorian and Crone, 2022). A relatively small number of studies have looked at how land use influences colony success in eusocial bees; most of these have been done in the temperate zone, and have focused on either bumble bees (*Bombus* spp.) (Goulson et al., 2002; Vaidya et al., 2018) or the European honey bee (*Apis mellifera*) (Samuelson et al., 2020). In the tropics, however, stingless bees (Apidae: Meliponini) are more common and diverse than these better-studied taxa, and are important for the pollination of several economically important crops (Heard, 1999; Slaa et al., 2006). Stingless bees also have tremendous cultural significance. For example, in Mexico, where the present study took place, stingless bee honey and pollen stores are highly valued for culinary and medicinal uses, and their wax is used for handicrafts (Reyes-González et al., 2014). While there is a long history of meliponiculture (stingless bee husbandry) in Mexico, traditional meliponiculture has declined dramatically in recent decades, and experienced stingless beekeepers in the region have reported concomitant declines in wild stingless bee populations, perhaps due to habitat loss and/or human exploitation of wild colonies (Reyes-González et al., 2020; Villanueva-Gutiérrez et al., 2013). Thus, understanding how agricultural management practices influence stingless bee colony health is important not only for protecting ecosystem function and ecosystem services in agricultural landscapes, but also for maintaining and reinvigorating traditional cultural practices.

Stingless bee colonies are perennial and active year-round. Nevertheless, seasonality plays an important role in colony activity, both because precipitation and temperature directly influence foraging activity (Figueiredo-Mecca et al., 2013; Pereboom and Biesmeijer, 2003), and because of seasonality in floral resource availability. Our study region is characterized by distinct wet and dry seasons, and floral resource availability is higher in the dry season. Seasonality in floral resource availability, particularly pollen, strongly influences colony dynamics and worker life history in stingless bees, with colonies responding to low pollen availability by reducing or ceasing brood production, and worker lifespan nearly doubling during periods of pollen scarcity (Maia-Silva et al., 2016; Roubik, 1982). Most studies investigating seasonality in the regulation of the foraging activity and brood production in stingless bees have focused on colonies in a single habitat type; fewer have investigated the joint influence of habitat type and seasonality on stingless bee colony growth and survival. Yet understanding how both seasonal variation and land use/land management practices jointly shape activity and long-term fitness of these colonies is essential to maintaining stingless bee populations and the pollination services they provide.

We investigated how resource use, forager activity, and colony growth and survival of experimentally placed colonies of the stingless bee *Scaptotrigona mexicana* differed across three habitats that varied in floral resource availability and management, over the span of an entire year. We also investigated the relationships among resource use, colony activity, and colony growth and survival, to determine whether effects of habitat on growth and survival were mediated by food intake. The study took place in a region dominated by coffee agriculture in southern Mexico. Three habitats investigated were, 1) a certified organic shaded coffee farm, 2) a conventional sun coffee farm and 3) a forest fragment located between the two farms.

Coffee agriculture is ubiquitous in the tropics and is practiced using a wide range of management regimes (Moguel & Toledo 1999). One key differentiating feature is the density and diversity of shade trees. Wild coffee is an understory species, and coffee was traditionally cultivated under shade, but changes in crop breeding and management and the global coffee market over the past 50 years have encouraged some growers to reduce, and at times eliminate, shade trees from coffee fields (Jha et al., 2014; Perfecto et al., 1996). In this study, the organic shaded coffee farm has a diverse and relatively dense canopy of shade trees, containing over 100 species. In contrast, the sun coffee farm has many fewer shade trees representing just a handful of species (primarily Inga spp.) (J. Vandermeer, unpublished data). Shade trees are an important nesting resource for cavity- nesting stingless bee species, such as *S. mexicana*, along with providing nutritive (pollen and nectar) and defensive (resin) resources.

We hypothesized that the amount and diversity of pollen collected by *S. mexicana* colonies would differ across the three habitats and would track patterns of flowering plant abundance and diversity in each habitat. Thus, we expected the highest and most diverse pollen intake for colonies in the shaded organic coffee farm, since this habitat has high abundance and diversity of flowering plants (a combination of retained forest trees, planted native and exotic shade trees, planted native and exotic ornamental shrubs, and native and exotic weeds); intermediate levels in the forest fragment; and lowest levels in the conventional sun coffee farm (Fitch and Vandermeer, 2021; Jha and Vandermeer, 2010; C. Vaidya & G. Fitch personal observation). We hypothesized that colony growth rates would correspond to resource availability, and so would be highest in the shaded organic coffee farm followed by the forest fragment, and lowest in the conventional sun coffee farm. Finally, we hypothesized that colonies would grow more, and show greater foraging activity, in the dry season (when floral resource availability is high) than the wet season (when floral resources are relatively scarce) and that the effect of seasonality would be consistent across habitats.

2.3 Methods

2.3.1 Study System

Research took place in the Soconusco region of Chiapas, Mexico, in the foothills of the Sierra Madre de Chiapas (1175-1300 m.a.s.l.). This region is dominated by coffee agriculture, with management practices varying substantially among farms. In particular, farms vary in 1) their use of agrochemicals and 2) the density and diversity of canopy trees. Remnant primary and regenerating secondary forest fragments also occur in the landscape, mostly in areas where steep slopes make cultivation difficult.

Scaptotrigona mexicana (Apidae: Meliponini) is a stingless bee native to the region, with a range extending from NE Mexico to northern Central America. It is a generalist species and an important pollinator of several crops, including rambutan and coffee (Ramírez-Arriaga and Martínez-Hernndez, 2007). There is a long history of human husbandry of *S. mexicana* in parts of Mexico (Quezada-Euán et al., 2018). At the same time, its honey is highly valued for medicinal purposes, and wild colonies in the study region are frequently disrupted by people wishing to extract their honey (MA Guzmán Diaz and G Domingo Martinez, personal communication), with potential implications for the population of wild *S. mexicana* in populated areas.

Colonies of *S. mexicana* used in this study were obtained from El Colegio de la Frontera Sur – Tapachula (ECOSUR). ECOSUR is encouraging the use of *S. mexicana* colonies in pollination of rambutan (Nephelium lappaceum), and has a small program to propagate *S. mexicana* colonies. The popularity of *S. mexicana* with rambutan growers, together with the difficulty of colony propagation, meant that only a small number of colonies were available for this project. Colonies were contained in wooden boxes (interior dimensions approx. 16 cm x 12 cm x 14 cm); prior to the study, all colonies were kept on the campus of ECOSUR (120 m.a.s.l.), prior to field deployment.

2.3.2 Field data collection

On March 8, 2018, colonies were deployed at three sites that corresponded to three land use types: a ca. 300 ha certified-organic shaded coffee farm; a ca. 300 ha. conventional sun coffee farm; and a 23.9 ha. forest fragment, the latter of which includes areas of both regenerating secondary forest and primary forest. We placed three colonies each in the shaded coffee farm and the sun coffee farm, and two colonies in the forest fragment. Because *S. mexicana* colonies, including ones in human-made boxes, are frequently depredated for honey by local residents, we clustered colonies within a site in a single secure area. Within a site, colonies were separated by 10 m; distance between sites ranged from 700-950 m.

To assess colony weight change, we weighed colonies, box included, prior to placing them in the field, and weighed them once a month for the next 12 months, at the same time as forager observations and pollen collection (see below). There was no significant difference in starting weight of colonies among the three sites.

Beginning in April 2018 and continuing until March 2019, we conducted monthly observations of foraging activity and pollen collection. Observations were 10 minutes long, and were conducted under sunny or partly cloudy, calm conditions, except during the month of October 2018, when persistent precipitation meant that observations had to be conducted under light mist. We conducted three observations per colony per month, with one observation between 0800-0930, another between 1000-1130, and a third between 1200-1330, where each month's observations generally occurred on three consecutive days. We conducted observations at multiple colonies on a single day, but each colony was only observed once per day. At each observation, the observer was situated ~2 m away from the colony, facing the colony entrance. After arriving at the observation site, the observer waited for 5 minutes to allow foragers to acclimate to their presence before beginning observations. During the 10 minutes observation period, the observer recorded the total number of foragers returning to the colony (i.e., only foragers that fully entered the colony were recorded). We counted the number of returning workers that carried pollen and resin on their corbiculae separately.

Pollen collection occurred immediately after the observation period. For pollen collection, we captured 10 returning foragers that were carrying pollen on their corbiculae and placed them in individual 5 mL plastic vials. Once we had collected 10 foragers with pollen, we used forceps to remove the pollen load from the left corbicula of each bee, and placed all pollen loads from a collection bout in a 2.5 mL microcentrifuge tube filled with 1 mL 70% ethanol and these were stored for subsequent analysis. Bees were then released.

2.3.3 Laboratory data collection

In the lab, the contents of each pollen vial were transferred to a 1.5mL microcentrifuge tube, the mass of which was determined prior to adding the pollen solution. We then centrifuged the tubes for 2 minutes at 13,000 rpm, removed the supernatant, and recorded the mass of the tube. The mass of the pollen sample was determined by subtracting the mass of the empty tube from the mass of the tube with pollen.

In addition to pollen load mass, we analyzed pollen load composition from the samples collected in April, May, July, September, and December 2018 and February 2019. These months were chosen 1) to represent conditions in the early, mid and late dry (Dec, Feb, Apr) and early and mid wet (May, Jul, Sep) seasons and 2) because sampling was relatively complete across all colonies in these months (i.e., no incomplete sampling because of inclement weather). Incomplete sampling meant we were not able to analyze pollen loads from the late wet season. To prepare pollen samples for composition analysis, we first resuspended the pelleted pollen in 20 uL 95% ethanol, then pipetted 20 ul of the resulting solution onto a microscope slide. We allowed the ethanol to evaporate, then added 10 uL Caberla's solution (Kearns and Inouye, 1993) to the slide to stain the pollen grains, covered the sample with a coverslip, and sealed the coverslip to the slide with clear nail polish.

To image the samples, we used a Nikon E800 microscope. Under 10x magnification, we imaged a single horizontal transect of the sample, choosing the focal plane such that pollen was well-separated and in focus, and the total number of pollen grains in a transect exceeded 400. In cases where no single transect of the slide contained 400 pollen grains, we imaged a second transect. Images were stitched together using Nikon Elements-D software version 5.20.01. We identified distinct pollen morphotypes in the transects, and made a pollen morphotype library to facilitate recognition of pollen morphotypes across samples. We then counted the number of grains of each pollen morphotype in each transect, beginning at the left edge of the image and continuing until we reached 400 grains. We used the open-source software DotDotGoose (Ersts, 2020) to aid in counting, and a single observer (AM Oana) did all counting to ensure consistent identification of pollen morphotypes. For the most part, we were unable to determine the species identification for pollen grains, though identification of a few species was possible.

2.3.4 Data analysis

To examine the how colony weight change was affected by habitat and season, we used a generalized linear mixed-effects model with habitat and date from hive placement as fixed effects and colony as the random effect. Raw data exploration suggested a quadratic relationship between colony weights and date. Consequently, we modeled date as a second-order polynomial of days from experiment initiation. We used the 'glmer.nb()' function from `lme4`, with a negative binomial distribution. We then used Tukey Honest Significance Difference (HSD) test from the "glht" package for pairwise comparisons between the three habitats.

To understand how total forager activity varied over the year, we constructed a generalized linear mixed-effects model, with total number of foragers as the response variable, and, habitat, a second order polynomial of days from hive placement and weather conditions during observations as fixed effects, and colony and time of observation as random effects. We did not include observations on days when it rained or when the conditions were misty since no foraging occurred on those days. For this model we used the 'glmer.nb()' function from `lme4`, with a negative binomial distribution. We used Tukey HSD test from the "emmeans" package for pairwise comparisons. To understand how pollen forager activity and resin forager activity varied over the year, we constructed a generalized linear mixed-effects model with a zero-inflated negative binomial distribution using the package “glmmTMB”, with number of resin/pollen foragers as the response variable, with log(total number of foragers) included as an offset. Predictor variables were habitat, date since hive placement and climatic conditions during observations as fixed effects and colony as random effect and zero-inflation formula equal to 1, to denote equal probability of producing zeros over all observations. Observations on rainy and misty days were excluded (see above). Tukey HSD test (“emmeans” package) was then used for pairwise comparisons.

We assessed the amount of pollen being brought into each colony in two ways. For pollen mass, we took the total mass of pollen collected from the corbiculae of 10 returning foragers. For pollen intake, we calculated per-forager pollen load by dividing pollen mass by 10, then, for each observation, multiplied this value by the number of pollen foragers observed returning to the colony, giving us the amount of pollen brought into the colony per 10 min period. To evaluate the effect of date and habitat on the mass of collected pollen and pollen intake, we constructed a linear mixed-effects model with days from the initiation of the experiment and habitat as fixed effects, and colony as a random effect, using the ‘lmer()’ function from `lmerTest`. Because inspection of plots of raw data suggested a quadratic relationship between pollen mass (and richness) and date, we compared models that included and omitted a second-order polynomial of days from experiment initiation, using AIC. We similarly compared the goodness of fit for a model including a habitat × date interaction and one with no interaction using AIC, to see if the effect of habitat differed over the course of the year. We constructed another model with season (wet/dry) as a fixed effect and colony nested within habitat as random effects to assess the effect of season on pollen mass. We constructed parallel models assessing the effect of location, date, and season on pollen morphotype richness using the subset of samples analyzed for pollen composition; for these models we used the ‘glmer()’ function from `lme4`, with a Poisson error distribution. We checked for model compliance with assumptions using the `DHARMa` package.

To assess whether pollen morphotype richness differed across habitats and seasons, we first constructed sample-level species accumulation curves using the ‘specaccum()’ function from the `vegan` package. Habitats varied in the number of samples analyzed, and morphotype counts for all habitats and both seasons did not fully reach an asymptote. Therefore, we estimated the size of the pollen morphotype pool for each habitat and season using the ‘specpool()’ function in `vegan`. We investigated whether pollen composition differed across habitats and seasons using PERMANOVA, as implemented in the `vegan` function ‘adonis2()’, stratifying our data by colony. Given the unequal number of samples across habitats and seasons, we evaluated multivariate dispersion across habitats and seasons using the `vegan` function ‘betadisper()’. To visualize differences in pollen composition across habitats and seasons, we used nonparametric multidimensional scaling (NMDS), implemented with ‘metaMDS()’ from `vegan`.

All analyses were performed in R version 4.1.2 (Team, 2021).

2.4 Results

2.4.1 Colony weight change and survival

Mean colony weights significantly differed among habitats ($\chi2 = 32.4$, d.f. = 2, $p < 0.001$; Table 2.1) and were significantly lower in the sun coffee farm than the organic shade coffee farm (Tukey test, $ß = -0.29 \pm 0.07$, $z = 3.7$, $p<0.001$) and the forest fragment (Tukey test, $ß = -0.48$, ± 0.08, $z = 5.6$, $p<0.001$), but the difference between shade coffee farm and forest fragment was not statistically significant. None of the three colonies in the sun coffee farm survived for the entire duration of the study; the first colony died in August 2018, the second in November 2018 and the third in December 2018. On the other hand, all colonies in both the organic shaded coffee farm and the forest fragment survived for the duration of the study. Date since initiation of experiment also had a significant effect on colony weights ($\chi2 = 37.7$, d.f. = 2, $p < 0.001$; Fig 1A). Colony weights increased marginally during the wet season (linear term: $ß = 0.08 \pm 0.25$, $z = .34$, $p = 0.73$), followed by a sharp and significant increase in the dry season (quadratic term: $ß = 1.47 \pm 0.22$, df = 116, $t = 4.7$, $p < 0.001$).

2.4.2 Forager activity (total, pollen, and resin)

Forager activity tracked colony weights (Fig 2.1B). Date since initiation had a significant effect on forager activity ($\chi2 = 6.3$, d.f. = 2, $p = 0.04$), decreasing marginally during the wet season (linear term: $ß = -1.6 \pm 0.9$, $z = -1.6$, $p = 0.11$), with a subsequent significant increase in the dry season (quadratic term: $ß = 2.1 \pm 0.9$, $z = 2.3$, $p = 0.02$). Forager activity was significantly lower during cloudy days than on sunny and partly cloudy days habitats ($\chi2 = 23.2$, d.f. = 2, $p < 0.001$). Habitat had a significant effect on total forager activity ($\chi2 = 6.1$, d.f. = 2, $p = 0.04$, Table 2.1); activity trended lower in the sun coffee farm than the other two habitats, though this difference was not significant. While trends in the number of pollen and resin foragers generally followed total forager numbers, the proportion of foragers of each type varied somewhat over the period of the experiment and among habitats. Habitat had a significant effect on the proportion of foragers collecting pollen ($\chi2 = 9.4$, d.f. = 2, $p < 0.01$, Fig 2.2B). The sun coffee farm had a significantly lower proportion of pollen foragers compared to the organic shade coffee farm (Tukey test, $ß = -0.36 \pm 0.1$, df= 187, $t = -2.9$, $p=0.01$) and the forest fragment (Tukey test, ß = -

0.37 ± 0.1, p=0.01), with no difference between the shade coffee farm and forest fragment (Tukey test, ß = -0.009 ±0.1, df= 187, t= -0.09, p=0.99). Proportion of foragers collecting pollen were consistent in each habitat over the course of the study. As with total foraging activity, pollen foraging was significantly lower on cloudy and partly cloudy days compared to sunny days ($\chi2$ = 6.8, d.f. = 2, p = 0.03). The proportion of foragers collecting resin was highest in the forest fragment and decreased significantly over the course of the study ($\chi2$ = 24.3, d.f. = 1, p < 0.001); this decrease was driven largely by the colonies in both the coffee farms (Figure A1). Post-hoc Tukey tests revealed a significant difference in the proportion of resin foragers between the sun coffee farm and the forest fragment (Tukey test, ß = -2.1 ±0.7, df= 187, t= -2.7, p=0.01); differences between the sun and shade coffee farms, and between the forest fragment and shade coffee farm, were not statistically significant (p = 0.3 in both cases). Climatic conditions did not have any significant effects on resin foraging ($\chi2$ = 2.4, d.f. = 2, p=0.3).

2.4.3 Pollen collection – mass and intake

Collected pollen mass (the total mass of corbicular pollen loads from 10 returning foragers) did not differ among habitats ($\chi2$ = 2.5, d.f. = 2, p = 0.3; Fig 2.2A). However, there was a clear seasonal trend in pollen mass. Pollen mass was lowest in Sep, in the latter half of the wet season, then gradually increased to a peak in the latter half of the dry season (Feb-Mar), before declining (Fig 2.2). This was reflected in the quadratic relationship between pollen mass and days since colony placement that was retained in the best model (linear term: ß = -21.1±4.8, df = 116, t = -4.4, p < 0.001; quadratic term: ß = 22.03±4.7, d.f. = 116, t = 4.7, p < 0.001; for model including only linear term, ΔAIC = 18.0), and in the significant difference in pollen mass between wet and dry seasons ($\chi2$ = 10.3, d.f. = 1, p = 0.001). This seasonal pattern was consistent across habitats, with no significant habitat × date interaction (ΔAIC = -4.8 for model omitting interaction).

Results for pollen intake (the total amount of pollen brought into the colony per 10 min observation period) were very similar. As with pollen mass, pollen intake did not differ among habitats ($\chi 2 = 1.2$, d.f. = 2, p = 0.6; Figure A2), but showed strong seasonality, with an increase in pollen intake in the dry season ($\chi 2 = 7.9$, d.f. = 1, p = 0.005; Fig 2.2B).

2.4.4 Pollen collection – morphospecies composition

We analyzed pollen composition from 59 samples, including eight from colonies in the conventional sun coffee farm, 24 from the organic shade coffee farm, and 27 from the forest fragment, with 29 of these samples collected in the dry season and 30 in the wet season. We encountered a total of 23 pollen morphospecies across all samples. Of these, eight morphospecies were found in samples from all habitats, 10 were found only in the forest fragment or shade coffee, one was found only in the shade and sun coffee farms, three were found only in the forest fragment, and one was found only in the shade coffee. No morphospecies was detected only in samples from the sun coffee. Estimated pollen morphospecies richness was similar between colonies in the organic shaded coffee farm and the forest fragment but was substantially lower for colonies in the conventional sun coffee farm, regardless of estimator used (Fig 2.3, Figure A3). Estimated pollen richness was significantly higher during the dry season, irrespective of habitat or of estimator (Fig 2.3, Figure A3). The composition of pollen loads differed significantly between wet and dry seasons but was similar across habitats (season: $R^2 = 0.13$, F = 8,34, d.f. = 1, p = 0.005; habitat: $R^2 = 0.01$, F = 0.28, d.f. = 2, p = 0.5). Multivariate dispersion was homogeneous across habitats (F = 1.34, d.f. = 2, p =

0.3), but differed significantly between seasons, with greater dispersion in the dry season (average distance to median in dry season = 0.54; in wet season = 0.43; F = 7.31, d.f. = 1, p = 0.009) Consistent with the PERMANOVA results, NMDS plots indicate near-total overlap in composition of pollen loads across habitats, with partial overlap in pollen load composition between seasons (Figure 2.3).

2.5 Discussion

Our study demonstrates that a certified-organic shade coffee farm was as good at supporting *Scaptotrigona mexicana* colonies as a nearby forest fragment, since the growth and survival of experimental colonies in these two habitats was nearly identical. In contrast, colonies of *S. mexicana* experienced reduced growth rates and high mortality in an adjacent conventional sun coffee farm. Additionally, total foraging activity was similar in the organic shade coffee farm and the forest fragment, but trended lower in the sun coffee farm. This is unsurprising, since colony weight is strongly coupled with forager abundance and forager activity levels: more foragers both contribute their body mass to the weight of the colony and increase resource intake and therefore further growth. These results are broadly consistent with our hypotheses, although we did not anticipate that all colonies in the sun coffee farm would die before the conclusion of the study. Coffee agriculture is ubiquitous in the tropics and the potential of coffee farms to support high levels of biodiversity, and the crucial importance of farm management practices in determining the biodiversity-conserving potential of coffee agroecosystems, are well-documented (De Beenhouwer et al., 2013; Jha et al., 2014; Perfecto et al., 1996). However, ours is the first study to compare colony dynamics of a bee species, especially stingless bee species, between these habitats and show that organic shaded coffee farms are equivalent to forest fragments in supporting stingless bee colonies, while more intensified coffee production strongly negatively influences colony performance and survival.

We found that seasonality plays a significant role in all aspects of colony growth and survival. Except in the sun coffee farm, colonies increased in weight during the dry season, while, across all habitats, colony weight declined during the early wet season before stabilizing and remaining unchanged through the remainder of the wet season. Because all colonies in the sun coffee farm had died by the onset of the dry season, we cannot be sure that these colonies would have experienced weight gain over the dry season had they survived. Total forager activity tracked the seasonal trends in colony weights, with higher foraging activity across habitats in the dry season compared to the wet season. Our results are in line with previous studies that have found similar effects of seasonality, from higher reproduction in the dry season than the wet season in the dry lowlands of Costa Rica (Slaa, 2006) to a significant reduction in foraging activities in the wet season compared to the dry season in northeast Brazil (Do Nascimento and Nascimento, 2012). We note that our measure of colony weights included the wooden box which housed the colony. Given the high humidity in the wet season, it is likely that weight of the boxes alone was somewhat higher in the wet compared to the dry season. This suggests that our estimates of the effect of seasonality on colony weight change are conservative.

As expected, both the amount and morphotype richness of pollen collected by foragers showed strong seasonality, with more and more diverse pollen collected per foraging trip in the dry season. This parallels the greater availability of floral resources in the dry season and is consistent with Aleixo et al. (2017) and Figueiredo-Mecca et al. (2013), both of which found that peaks in pollen collection by stingless bees corresponded with high floral resource availability. Interestingly, pollen intake, which we calculated for each observation by multiplying the number of pollen foragers returning to the nest by the average mass of pollen loads we collected, was largely consistent from the initiation of the study (late-dry season) until the beginning of the dry season the following year, despite modest declines in per-forager pollen loads over this same period. This suggests that colonies compensate to some extent for reduced pollen availability by increasing the number of pollen foragers during periods of floral resource scarcity. This seems to contradict our finding that the proportion of pollen foragers did not differ between wet and dry season, but close inspection of the relationship between proportion of pollen foragers and date (Fig. 2B) suggests that the proportion of pollen foragers is high in the mid wet season (~150-225 days since colony placement) but is low in the early and late wet season. This variation is masked by considering the wet season as a whole, but the mid wet season peak in allocation of foragers to pollen collection corresponds to the nadir in pollen forager efficiency, consistent with the idea of compensatory allocation to pollen foraging during times of pollen scarcity. It would be interesting to know whether this corresponds to a dip in colony-level nectar intake, as workers are allocated to pollen foraging, or whether pollen and nectar availability are not so tightly coupled, such that the apparent mid-wet season low point in pollen availability does not correspond to the time of greatest nectar scarcity.

We had expected pollen collection, and particularly the composition of collected pollen, to differ across habitats, given the substantial differences in plant community composition and floral resource availability (Kaluza et al., 2017). However, we found no difference in the mass of corbicular pollen loads or total pollen intake among habitats, and small difference in pollen morphospecies composition (Figures 2A & 3C). Given the smaller proportion of pollen foragers in colonies in the sun coffee farm, it is somewhat surprising that pollen intake was not significantly lower for those colonies; this likely reflects the rather high variability in per-forager pollen loads across observations within a colony, leading to wide variance in pollen intake from one observation to the next. Pollen richness, on the other hand, was substantially reduced for colonies located in the sun coffee farm. That is, colonies in the sun coffee farm appear to focus pollen collection on a reduced number of species that were also core to the pollen diets of colonies located in other habitats, with colonies in the shade coffee farm and forest fragment additionally collecting pollen from other species.

There are two non-exclusive explanations for the surprising similarity in pollen diets across habitats, despite substantial differences in overall plant community composition. First, stingless bees sometimes focus pollen collection on a relatively small number of species (Aleixo et al., 2017; Machado et al., 2020; Wilson et al., 2021). It may be that many of these species happened to occur across all three habitats; anecdotally, we know this to be the case for several plant species where we have observed pollen collection by *S. mexicana* (e.g., *Conostegia xalapensis, Inga* spp.; G. Fitch personal observation). Second, it may be that foragers whose colonies were located in one habitat foraged for pollen in another habitat. Evidence for this comes from the observation that pollen loads from bees in the forest fragment contained substantial coffee (*Coffea arabica*) pollen, even though very few coffee plants are found in the forest. Moreover, recent evidence from an agriculture-dominated region in Indonesia suggests that stingless bee colonies located within crop fields often forage well beyond the boundaries of the farm (Carneiro de Melo Moura et al., 2022). We suspect both putative mechanisms played a role in generating the overall similarity in pollen diets across habitats, though further identification of the specific plant species involved, and an assessment of their abundance across the three habitats, would be necessary to evaluate the relative importance of each mechanism. Either way, these findings point to the importance of retaining plant species in the landscape that provide pollen sources for stingless bees, whether as a component of the cultivated area (e.g., as shade trees for coffee, as in the case of *Conostegia* and *Inga*), or within forest fragments.

It is also worth noting that, because all colonies in the sun coffee farm died before the end of the study, we do not have a complete picture of the pollen diet in this habitat over the span of the year. In particular, we lack information on the amount and composition of pollen diet in the early-mid dry season, a time when pollen intake peaked for other colonies. This may explain the reduced richness of the pollen diet of colonies in the sun coffee farm; the additional species found in the diet of colonies in the shaded coffee farm and forest fragment may bloom primarily in the early-mid dry season.

Only a small fraction of returning foragers carried resin. Even so, there was a significantly lower proportion of foragers collecting resin in the sun coffee farm than the forest fragment. Resin use by stingless bees is important and multifunctional: resin is used in nest construction, to preserve food stores, and as a primary line of defense against predators and invaders; it also influences the microbial communities associated with both the colony and individual bees (reviewed in Shanahan and Spivak, 2021). As defense, resins from different plant species are effective against different organisms (Drescher et al., 2014), suggesting that access to diverse resin sources is important for colony health in stingless bees. It may be that the plants relied on by *S. mexicana* for resin collection were scarce in the sun coffee farm. Alternatively, the relative scarcity of sources of pollen and nectar in the immediate surrounds may have forced foragers to focus efforts on collecting nutritive (pollen and nectar) rather than protective (resin) plant resources. More research is needed on the foraging choices of stingless bees for different plant resources (i.e., nectar, pollen, and resin) along environmental gradients.

Multiple factors may have contributed to the poor performance of colonies in the sun coffee farm compared to colonies in the other two habitats, including floral resource availability, increased parasitism, and agrochemical exposure. Below, we discuss each factor in turn; while the available evidence does not allow us to definitively determine the contribution of each factor, as we discuss below, it seems likely that agrochemical exposure played a key role.

We did not collect floral resource data in the three habitats. However, as already noted, the sun coffee farm had, anecdotally, fewer bee-attractive flowering plants than the other habitats, particularly the shaded coffee farm and especially in the dry season. While the distances between habitats are small enough that foragers from colonies in one habitat could make foraging trips to the other habitats, the relative lack of floral resources close to the nest, and the longer travel distances for individual foragers, likely increased stress on individual foragers and may have decreased per-forager food intake. Previous research has found significant costs to reproduction in solitary bees that forage longer distances in search of resources (Zurbuchen et al., 2010). That said, given the proximity of the three habitats, and the evidence for pollen diet similarity across all colonies, it seems likely that colonies in the sun coffee farm had access to sufficient floral resources to maintain colony growth. That is, while low floral resource availability in the sun coffee farm may have limited growth of those colonies somewhat, it is unlikely to explain the weight loss and eventual death of these colonies. This is consistent with other studies of the effects of floral resource availability on eusocial bee colony growth, which have found that patch-scale resource availability does not predict colony growth (Goulson et al., 2002), but landscape-scale resource availability does (Crone and Williams, 2016; Williams et al., 2012), and also influences colony reproduction (Crone and Williams, 2016; Kaluza et al., 2018). As further evidence for the secondary importance of within-habitat floral resource availability in determining colony growth in our study system, the forest fragment had significantly lower floral resource availability than the shade coffee farm (Fitch and Vandermeer, 2021), but growth of colonies in the forest fragment was not significantly different from that of colonies in the shade coffee farm. Future research into the environmental determinants of eusocial bee colony dynamics would benefit from recording the abundance and diversity of floral resources at relevant scales and across the year.

Predation and parasitism are well-documented factors contributing to poor colony performance and reduced survival in eusocial bees (Schweiger et al., 2022; Vaidya et al., 2018). As noted above, plant resins are an important component of defense in stingless bees. The low level of resin collection by colonies in the sun coffee farm may have increased their susceptibility to predation and/or parasitism. Because we did not check colonies for evidence of parasitism and predation, we cannot confirm whether colonies from different habitats differed in the degree of parasitism or depredation.

The conventional sun coffee farm in our study routinely uses agrochemicals, including fungicides, herbicides and insecticides, and it is likely that agrochemical exposure negatively affected colonies in this habitat. While we were unable to ascertain all agrochemicals used, or the timing and precise location of application, one chemical that we know was used during the study is copper sulfate, deployed to control the coffee leaf rust fungus (*Hemileia vastatrix*) (Z. Hajian-Forooshani, personal communication). Copper sulfate is known to adversely affect the foraging behavior and survival of stingless bees (Bernardes et al., 2022; Rodrigues et al., 2016). Agrochemicals are also used on the farm to control the coffee berry borer beetle (*Hypothenemus hampei*) and weeds (G. Lopez Bautista, personal communication), and products commonly used for these purposes have known negative effects on stingless bees (Tomé et al., 2017). While we tried to shield colonies from direct exposure to agrochemicals, it is highly likely that foraging bees came into contact with one or more chemicals, and it is also possible that chemicals came into contact with colony boxes through drifting. Exposure to agrochemicals close to the onset of the experiment may explain why colonies in the sun coffee farm experienced rapid weight loss in the first months of the experiment, during a time of relative floral abundance (late dry season), when the weights of colonies in other habitats increased.

While the available evidence implicates agrochemical exposure as a cause of poor colony performance in the sun coffee farm, evidence from other studies suggests that multiple factors may have played a role. For example, higher pollen diet quality can reduce toxicity of agrochemicals (Barascou et al., 2021); reduced pollen diet diversity in colonies in the sun coffee farm may therefore have exacerbated the adverse effects of agrochemical exposure. Future research that disentangles the relative importance of agrochemical exposure, predation and parasitism pressure, and food availability in determining stingless bee colony performance across habitats, for example through manipulation of one of these factors (reviewed in (Siviter et al., 2021)), would do a great deal to advance our understanding of the needs and sensitivities of these ecologically and economically significant pollinators.

We found substantial variation in colony performance within the same habitat, particularly in the shade coffee farm. Given the close proximity of all colonies within a habitat, this suggests that other factors unrelated to habitat caused this variation. We did not screen the colonies for pathogens, so we don’t know whether any of our colonies were impacted. While the prevalence of protozoan parasites that infect honeybees and bumblebees is very low in stingless bees (Nunes-Silva et al., 2016), recent evidence suggests that *S. mexicana* in Mexico is now susceptible to honey bee viruses such as deformed wing virus and black queen cell virus, both of which can impact colony performance (Guzman-Novoa et al., 2015). The organic shaded coffee farm in our study has steadily increased the number of honeybee apiaries in recent years, potentially increasing the probability of contact between honey bees and other bee species either directly or via floral visits. Thus, it is possible that these viruses were transmitted to the stingless bees in our study from managed or feral honeybees. Genetic differences among colonies may also contribute to differential performance (Büchler et al., 2014; Costa et al., 2012). Whatever the cause of within-habitat variation in colony performance, these findings highlight the importance of including replicate colonies within a site when attempting to study the effects of environmental conditions on social bees. Our study, constrained by access to *S. mexicana* colonies, was limited to single examples of each of the three representative habitats in close proximity, so our ability to extrapolate from this study may be limited. Future research would do well to include multiple examples of each habitat type over a wider geographic area. Nevertheless, there are several management implications from our study that may have wider application. First, it is clear that the wet season is a stressful period for bees, likely because of floral resource scarcity. Land managers and those interested in stingless bee husbandry would therefore do well to avoid compounding this stress with other perturbations (e.g., agrochemical application, tree clearing, flowering weed clearing). Similarly, increasing the abundance and diversity of flowering resources can provide the bees with enough resources to survive the wet season. Additionally, shade trees provide both floral and nesting resources for stingless bees and therefore are an important resource both for their nutritive diet as well as for defense. Thus, managing farms to include a diversity of shade trees can also help stingless bees weather bad seasons. But our results also highlight the inadequacy of thinking only at the scale of the individual farm or land parcel. Our findings are in accordance with other studies showing that stingless bee foraging is not limited to the habitat in which their colony is located (e.g., Carneiro de Melo Moura et al., 2022). Indeed, it has been shown that bees tend to forage over longer distances during seasons when the area immediately surrounding the nest has few floral resources (Pope and Jha, 2018). This reality necessitates taking a landscape-scale approach to thinking about pro-pollinator management interventions (Kennedy et al., 2013), which involves collaboration and partnership among landowners and users with potentially conflicting aims and priorities. Such an approach reaps substantial benefits not only for bees and pollination, but also for other organisms and ecosystem services of conservation concern (Rusch et al., 2016; Tscharntke et al., 2021). In the Neotropics, efforts to revive traditional practices of meliponiculture may provide a fruitful avenue towards encouraging this type of landscape-scale thinking and acting.

2.6 Acknowledgements

We are grateful to Peter Walters and Bernardo Walters, owners of Finca Irlanda, and T. Edelmann, owner of Finca Hamburgo, for allowing us to conduct research on their farms. Miguel Ángel Guzmán generously provided us with Scaptotrigona mexicana colonies, Gustavo Lopez Bautista assisted with logistics in the field, and Kaleigh Fisher conducted a pilot project that informed our study design. Thanks to Tamara Milton for helpful comments on earlier versions of the manuscript. Funding was provided by the Rackham Graduate School and the Department of Ecology and Evolutionary Biology, University of Michigan.

2.7 References

Aizen MA, Aguiar S, Biesmeijer JC, Garibaldi LA, Inouye DW, Jung C, et al. Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. Glob Chang Biol 2019. https://doi.org/10.1111/gcb.14736.

Aizen MA, Garibaldi LA, Cunningham SA, Klein AM. Long-Term Global Trends in Crop Yield and Production Reveal No Current Pollination Shortage but Increasing Pollinator Dependency. Curr Biol 2008. https://doi.org/10.1016/j.cub.2008.08.066.

Aleixo KP, Menezes C, Imperatriz Fonseca VL, da Silva CI. Seasonal availability of floral resources and ambient temperature shape stingless bee foraging behavior (Scaptotrigona aff. depilis). Apidologie 2017. https://doi.org/10.1007/s13592-016-0456-4.

Barascou L, Sene D, Barraud A, Michez D, Lefebvre V, Medrzycki P, et al. Pollen nutrition fosters honeybee tolerance to pesticides. R Soc Open Sci 2021. https://doi.org/10.1098/rsos.210818.

De Beenhouwer M, Aerts R, Honnay O. A global meta-analysis of the biodiversity and ecosystem service benefits of coffee and cacao agroforestry. Agric Ecosyst Environ 2013. https://doi.org/10.1016/j.agee.2013.05.003.

Bernardes RC, Fernandes KM, Bastos DSS, Freire AFPA, Lopes MP, de Oliveira LL, et al. Impact of copper sulfate on survival, behavior, midgut morphology, and antioxidant activity of Partamona helleri (Apidae: Meliponini). Environ Sci Pollut Res 2022. https://doi.org/10.1007/s11356-021-16109-1.

Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, et al. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science (80-) 2006;313:351–4. https://doi.org/10.1126/science.1127863.

Büchler R, Costa C, Hatjina F, Andonov S, Meixner MD, Le Conte Y, et al. The influence of genetic origin and its interaction with environmental effects on the survival of Apis Mellifera L. Colonies in Europe. J Apic Res 2014. https://doi.org/10.3896/IBRA.1.53.2.03.

Costa C, Lodesani M, Bienefeld K. Differences in colony phenotypes across different origins and locations: Evidence for genotype by environment interactions in the Italian honeybee (Apis mellifera ligustica)? Apidologie 2012. https://doi.org/10.1007/s13592-012-0138-9.

Crone EE, Williams NM. Bumble bee colony dynamics: Quantifying the importance of land use and floral resources for colony growth and queen production. Ecol Lett 2016. https://doi.org/10.1111/ele.12581.

Dorneles AL, Rosa-Fontana A de S, dos Santos CF, Blochtein B. Larvae of stingless bee Scaptotrigona bipunctata exposed to organophosphorus pesticide develop into lighter, smaller and deformed adult workers. Environ Pollut 2021. https://doi.org/10.1016/j.envpol.2020.116414.

Drescher N, Wallace HM, Katouli M, Massaro CF, Leonhardt SD. Diversity matters: how bees benefit from different resin sources. Oecologia 2014. https://doi.org/10.1007/s00442-014-3070-z.

Ehlers BK, Bataillon T, Damgaard CF. Ongoing decline in insect-pollinated plants across Danish grasslands. Biol Lett 2021. https://doi.org/10.1098/rsbl.2021.0493.

Figueiredo-Mecca G De, Bego LR, Nascimento FS do. Foraging behavior of Scaptotrigona depilis (Hymenoptera, Apidae, Meliponini) and its relationship with temporal and abiotic factors. Sociobiology 2013. https://doi.org/10.13102/sociobiology.v60i3.267-282.

Fitch G, Vandermeer J. Changes in partner traits drive variation in plant–nectar robber interactions across habitats. Basic Appl Ecol 2021. https://doi.org/10.1016/j.baae.2021.02.013.

Gallai N, Salles JM, Settele J, Vaissière BE. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 2009. https://doi.org/10.1016/j.ecolecon.2008.06.014.

Goulson D, Hughes WOH, Derwent LC, Stout JC. Colony growth of the bumblebee, Bombus terrestris, in improved and conventional agricultural and suburban habitats. Oecologia 2002. https://doi.org/10.1007/s004420100803.

Goulson D, Nicholls E, Botías C, Rotheray EL. Bee declines driven by combined Stress from parasites, pesticides, and lack of flowers. Science (80-) 2015. https://doi.org/10.1126/science.1255957.

Grab H, Branstetter MG, Amon N, Urban-Mead KR, Park MG, Gibbs J, et al. Agriculturally dominated landscapes reduce bee phylogenetic diversity and pollination services. Science (80-) 2019;363:282–4. https://doi.org/10.1126/science.aat6016.

Guzman-Novoa E, Md Hamiduzzaman M, Anguiano-Baez R, Correa-Benítez A, Castañeda-Cervantes E, Arnold NI. First detection of honey bee viruses in stingless bees in North America. J Apic Res 2015. https://doi.org/10.1080/00218839.2015.1100154.

Heard TA. The role of stingless bees in crop pollination. Annu Rev Entomol 1999. https://doi.org/10.1146/annurev.ento.44.1.183.

Jha S, Bacon CM, Philpott SM, MÉndez VE, LÄderach P, Rice RA. Shade coffee: Update on a disappearing refuge for biodiversity. Bioscience 2014. https://doi.org/10.1093/biosci/biu038.

Jha S, Vandermeer JH. Impacts of coffee agroforestry management on tropical bee communities. Biol Conserv 2010;143:1423–31. https://doi.org/10.1016/j.biocon.2010.03.017.

Kaluza BF, Wallace H, Keller A, Heard TA, Jeffers B, Drescher N, et al. Generalist social bees maximize diversity intake in plant species-rich and resource-abundant environments. Ecosphere 2017. https://doi.org/10.1002/ecs2.1758.

Kaluza BF, Wallace HM, Heard TA, Minden V, Klein A, Leonhardt SD. Social bees are fitter in more biodiverse environments. Sci Rep 2018. https://doi.org/10.1038/s41598-018-30126-0.

Kennedy CM, Lonsdorf E, Neel MC, Williams NM, Ricketts TH, Winfree R, et al. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecol Lett 2013. https://doi.org/10.1111/ele.12082.

Klein AM, Brittain C, Hendrix SD, Thorp R, Williams N, Kremen C. Wild pollination services to California almond rely on semi-natural habitat. J Appl Ecol 2012. https://doi.org/10.1111/j.1365-2664.2012.02144.x.

Kremen C, Williams NM, Thorp RW. Crop pollination from native bees at risk from agricultural intensification. Proc Natl Acad Sci U S A 2002. https://doi.org/10.1073/pnas.262413599.

Machado T, Viana BF, da Silva CI, Boscolo D. How landscape composition affects pollen collection by stingless bees? Landsc Ecol 2020. https://doi.org/10.1007/s10980-020-00977-y.

Maia-Silva C, Hrncir M, Imperatriz-Fonseca VL, Schorkopf DLP. Stingless bees (Melipona subnitida) adjust brood production rather than foraging activity in response to changes in pollen stores. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol 2016. https://doi.org/10.1007/s00359-016-1095-y.

Do Nascimento DL, Nascimento FS. Extreme effects of season on the foraging activities and colony productivity of a stingless bee (Melipona asilvai Moure, 1971) in northeast Brazil. Psyche (London) 2012. https://doi.org/10.1155/2012/267361.

Nunes-Silva P, Piot N, Meeus I, Blochtein B, Smagghe G. Absence of Leishmaniinae and Nosematidae in stingless bees. Sci Rep 2016. https://doi.org/10.1038/srep32547.

Ollerton J, Winfree R, Tarrant S. How many flowering plants are pollinated by animals? Oikos 2011;120:321–6. https://doi.org/10.1111/j.1600-0706.2010.18644.x.

Pereboom JJM, Biesmeijer JC. Thermal constraints for stingless bee foragers: The importance of body size and coloration. Oecologia 2003. https://doi.org/10.1007/s00442-003-1324-2.

Perfecto I, Rice RA, Greenberg R, Van Der Voort ME. Shade coffee: A disappearing refuge for biodiversity: Shade coffee plantations can contain as much biodiversity as forest habitats. Bioscience 1996. https://doi.org/10.2307/1312989.

Pope NS, Jha S. Seasonal food scarcity prompts long-distance foraging by a wild social bee. Am Nat 2018. https://doi.org/10.1086/694843.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. Global pollinator declines: Trends, impacts and drivers. Trends Ecol Evol 2010;25:345–53. https://doi.org/10.1016/j.tree.2010.01.007.

Powney GD, Carvell C, Edwards M, Morris RKA, Roy HE, Woodcock BA, et al. Widespread losses of pollinating insects in Britain. Nat Commun 2019. https://doi.org/10.1038/s41467-019-08974-9.

Quezada-Euán JJG, Nates-Parra G, Maués MM, Imperatriz-Fonseca VL, Roubik DW. Economic and cultural values of stingless bees (hymenoptera: Meliponini) among ethnic groups of tropical America. Sociobiology 2018. https://doi.org/10.13102/sociobiology.v65i4.3447.

Ramírez-Arriaga E, Martínez-Hernndez E. Melitopalynological characterization of scaptotrigona mexicana Gurin (Apidae: Meliponini) and Apis mellifera L. (Apidae: Apini) honey samples in Northern Puebla State, Mexico. J Kansas Entomol Soc 2007. https://doi.org/10.2317/0022-8567(2007)80[377:MCOSMG]2.0.CO;2.

Reilly JR, Artz DR, Biddinger D, Bobiwash K, Boyle NK, Brittain C, et al. Crop production in the USA is frequently limited by a lack of pollinators. Proc R Soc B Biol Sci 2020. https://doi.org/10.1098/rspb.2020.0922.

Reyes-González A, Camou-Guerrero A, del-Val E, Ramírez MI, Porter-Bolland L. Biocultural Diversity Loss: the Decline of Native Stingless Bees (Apidae: Meliponini) and Local Ecological Knowledge in Michoacán, Western México. Hum Ecol 2020. https://doi.org/10.1007/s10745-020-00167-z.

Reyes-González A, Camou-Guerrero A, Reyes-Salas O, Argueta A, Casas A. Diversity, local knowledge and use of stingless bees (Apidae: Meliponini) in the municipality of Nocupétaro, Michoacan, Mexico. J Ethnobiol Ethnomed 2014. https://doi.org/10.1186/1746-4269-10-47.

Rodger JG, Bennett JM, Razanajatovo M, Knight TM, van Kleunen M, Ashman TL, et al. Widespread vulnerability of flowering plant seed production to pollinator declines. Sci Adv 2021. https://doi.org/10.1126/sciadv.abd3524.

Rodrigues CG, Krüger AP, Barbosa WF, Guedes RNC. Leaf fertilizers affect survival and behavior of the neotropical stingless bee friesella schrottkyi (Meliponini: Apidae: Hymenoptera). J Econ Entomol 2016. https://doi.org/10.1093/jee/tow044.

Roubik DW. The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. Ecology 1982. https://doi.org/10.2307/1938953.

Rusch A, Chaplin-Kramer R, Gardiner MM, Hawro V, Holland J, Landis D, et al. Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. Agric Ecosyst Environ 2016. https://doi.org/10.1016/j.agee.2016.01.039.

Samuelson AE, Gill RJ, Leadbeater E. Urbanisation is associated with reduced Nosema sp. infection, higher colony strength and higher richness of foraged pollen in honeybees. Apidologie 2020. https://doi.org/10.1007/s13592-020-00758-1.

Schweiger SE, Beyer N, Hass AL, Westphal C. Pollen and landscape diversity as well as wax moth depredation determine reproductive success of bumblebees in agricultural landscapes. Agric Ecosyst Environ 2022. https://doi.org/10.1016/j.agee.2021.107788.

Shanahan M, Spivak M. Resin use by stingless bees: A review. Insects 2021. https://doi.org/10.3390/insects12080719.

Siviter H, Bailes EJ, Martin CD, Oliver TR, Koricheva J, Leadbeater E, et al. Agrochemicals interact synergistically to increase bee mortality. Nature 2021. https://doi.org/10.1038/s41586-021-03787-7.

Siviter H, Brown MJF, Leadbeater E. Sulfoxaflor exposure reduces bumblebee reproductive success. Nature 2018. https://doi.org/10.1038/s41586-018-0430-6.

Slaa EJ. Population dynamics of a stingless bee community in the seasonal dry lowlands of Costa Rica. Insectes Soc 2006. https://doi.org/10.1007/s00040-005-0837-6.

Slaa EJ, Sánchez Chaves LA, Malagodi-Braga KS, Hofstede FE. Stingless bees in applied pollination: Practice and perspectives. Apidologie 2006. https://doi.org/10.1051/apido:2006022.

Straw EA, Brown MJF. Co-formulant in a commercial fungicide product causes lethal and sub-lethal effects in bumble bees. Sci Rep 2021. https://doi.org/10.1038/s41598-021-00919-x.

Stuligross C, Williams NM. Past insecticide exposure reduces bee reproduction and population growth rate. Proc Natl Acad Sci U S A 2021. https://doi.org/10.1073/pnas.2109909118.

Team RC. R: A Language and Environment for Statistical Computing. R Found Stat Comput 2021.

Tomé HVV, Ramos GS, Araújo MF, Santana WC, Santos GR, Guedes RNC, et al. Agrochemical synergism imposes higher risk to neotropical bees than to honeybees. R Soc Open Sci 2017. https://doi.org/10.1098/rsos.160866.

Tscharntke T, Grass I, Wanger TC, Westphal C, Batáry P. Beyond organic farming – harnessing biodiversity-friendly landscapes. Trends Ecol Evol 2021. https://doi.org/10.1016/j.tree.2021.06.010.

Vaidya C, Fisher K, Vandermeer J. Colony development and reproductive success of bumblebees in an urban gradient. Sustain 2018. https://doi.org/10.3390/su10061936.

Villanueva-Gutiérrez R, Roubik DW, Colli-Ucán W, Güemez-Ricalde FJ, Buchmann SL. A Critical View of Colony Losses in Managed Mayan Honey-Making Bees (Apidae: Meliponini) in the Heart of Zona Maya. J Kansas Entomol Soc 2013. https://doi.org/10.2317/JKES130131.1.

Williams NM, Regetz J, Kremen C. Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. Ecology 2012. https://doi.org/10.1890/11-1006.1.

Wilson RS, Keller A, Shapcott A, Leonhardt SD, Sickel W, Hardwick JL, et al. Many small rather than few large sources identified in long-term bee pollen diets in agroecosystems. Agric Ecosyst Environ 2021. https://doi.org/10.1016/j.agee.2020.107296.

Wu-Smart J, Spivak M. Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. Sci Rep 2016. https://doi.org/10.1038/srep32108.

Zattara EE, Aizen MA. Worldwide occurrence records suggest a global decline in bee species richness. One Earth 2021. https://doi.org/10.1016/j.oneear.2020.12.005.

Zurbuchen A, Cheesman S, Klaiber J, Müller A, Hein S, Dorn S. Long foraging distances impose high costs on offspring production in solitary bees. J Anim Ecol 2010. https://doi.org/10.1111/j.1365-2656.2010.01675.x.

2.8 Tables and Figures

Table 2.1. Mean ± std dev of colony weights, total number of foragers, and pollen and resin foragers across habitats, averaged over the duration of the study.

	Sun coffee farm	**Shade coffee farm**	**Forest fragment**
Colony weight	2241 ± 401 g	3010 ± 702 g	3631 ± 550 g
Total # foragers	62.3 ± 73.2	168 ± 145	182 ± 112
Pollen foragers	11.2 ± 15.5	53.8 ± 69.7	56 ± 51.6
Resin foragers	0.19 ± 0.57	1.8 ± 3.83	2.87 ± 3.61

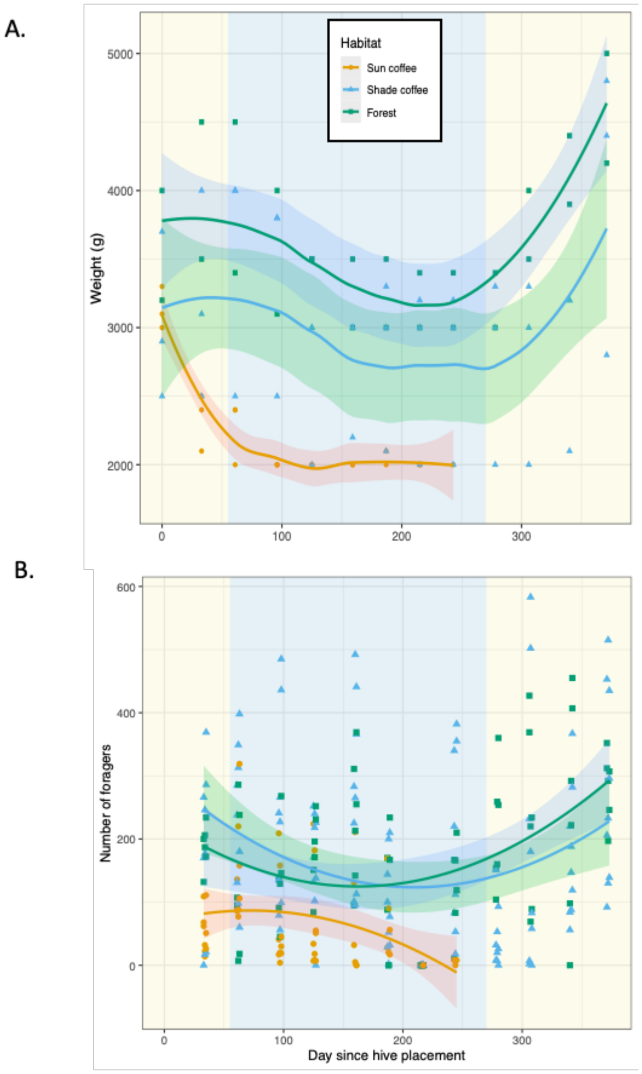

Figure 2.1. A) Weight of the colonies in the three habitats over the course of the and B) total number of foragers in the three habitats over the course of the study. In A, points represent weights recorded from each observation; solid line represents best-fit line, with shading indicating 95% CI, fit is with LOESS with span = 1. In B, points represent number of foragers recorded from each observation; solid line represents best-fit line from a second-order polynomial solid line represents best-fit line, with shading indicating 95% CI. Yellow-shaded region indicates dry season; blue-shaded region indicates wet season.

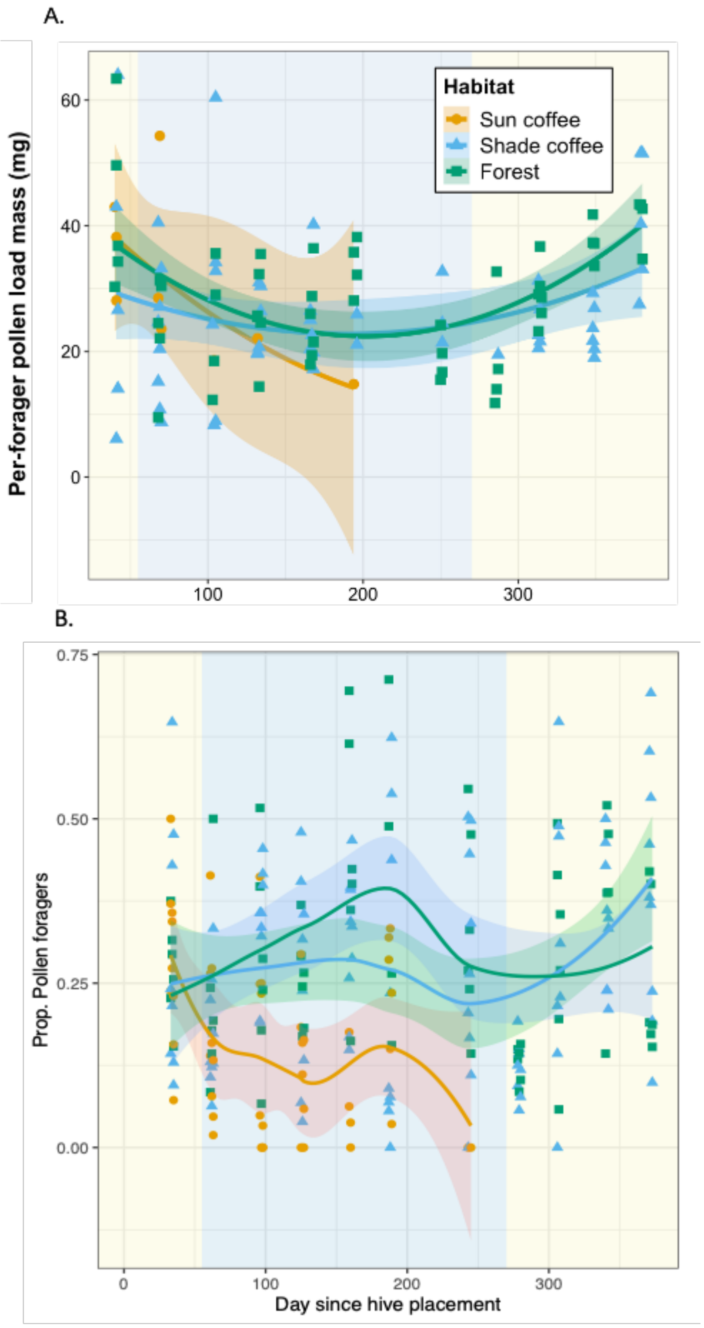

Figure 2.2. A) Mass of per-forager corbicular pollen load from returning foragers and B) proportion of total returning foragers carrying pollen over the course of the study. In A, points represent mean pollen mass recorded from each observation; solid line represents best-fit line from a second-order polynomial, with shading indicating 95% CI. In B, points represent proportion of foragers carrying pollen recorded from each observation; solid line represents best-fit line, with shading indicating 95% CI, fit is with LOESS with span = 1. Yellow-shaded region indicates dry season; blue-shaded region indicated wet season.

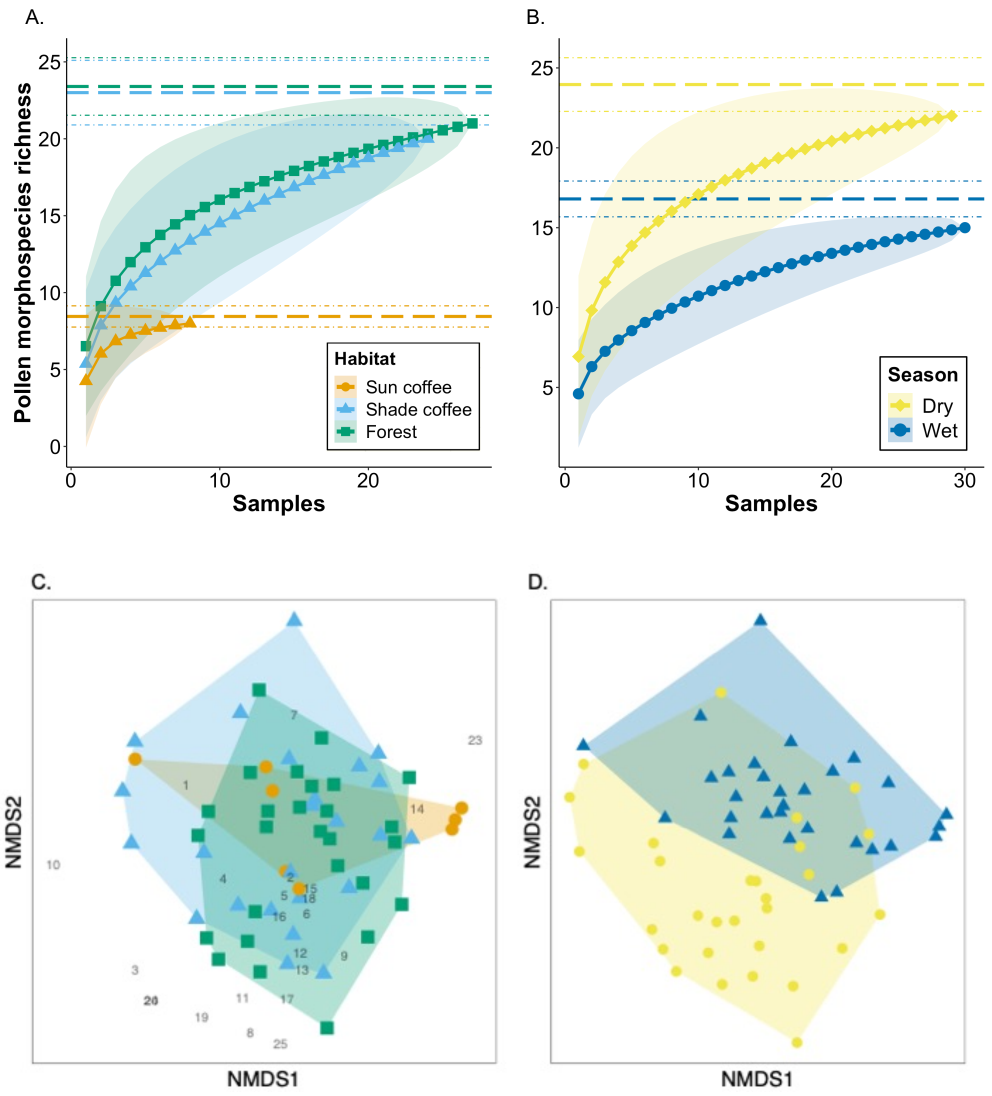

Figure 2.3. Pollen diet richness and composition. A-B) Pollen morphospecies accumulation curves and bootstrapped richness estimates for S. mexicana corbicular pollen loads. B-C) NMDS plots illustrating pollen composition. In A and C, samples are divided by habitat, in B and D by season. In A-B, shaded areas indicate 95% CI; thick dashed lines indicate total morphospecies richness determined by bootstrap estimation; fine dashed lines indicate 95% CI for these estimates. See supplemental material for richness estimates using alternative estimators. In C-D, points represent pollen samples from individual observations (i.e., pooled pollen loads from 10 foragers). In C, numbers indicate location of pollen morphospecies in community space.

Chapter 3 : Ant's Choice: The Effect of Nutrients on a Key Ant-Hemipteran Mutualism[2]

3.1 Abstract

Soil fertility is understood to act in many indirect ways, in addition to directly providing nutrients to plants. Given that higher order indirect interactions frequently are key to understanding community structure, it would be natural to expect that indirect mutualisms should be as important as other interactions in generating such structure. Although mutualisms are ubiquitous in nature, exploring the myriad ways in which they interconnect with other elements of a system is less common. That soil fertility has an indirect effect on community structure is well known, suggests that soil fertility may be important in determining the effect of mutualisms on the structure of the ecological communities in which they are embedded. Here we report on a laboratory study that specifically examines the direct connection between the behavior of a mutualist and nutritional properties of the soil. We tested the effects of soil fertilization (high, medium and low levels) on the *Azteca seriseasur – Coccus viridis* mutualism on coffee plants in Mexico. We found that ants foraged significantly more on plants that were infected with *C. viridis* over control plants. Moreover, ants preferred to tend *C. viridis* on high fertilized over medium and low fertilized plants. This study highlights the importance of the links between soil

---

[2] This paper was published as: Vaidya, C., & Vandermeer, J. (2021). Ant's choice: The effect of nutrients on a key ant–hemipteran mutualism. *Arthropod-Plant Interactions*, *15*(4), 545-550.

nutrient properties and their effects on a key ant-hemipteran mutualism, illustrating the cascading effects on other interactions involving this keystone ant species.

3.2 Introduction

Community ecology generally deals with the way in which species are connected with one another, usually through trophic connections such as predator and prey, while ecosystems ecology generally deals with the way in which energy and materials move through trophic connections. The evident connection between these two fields is generally acknowledged -- the "pipes" through which energy and materials get from predator to prey are also the pipes that symbolize the connection between them. Yet recent complications in the work of community ecology challenge this evident convergence. Community structure is now acknowledged to contain, sometimes driven by, many indirect effects, changes in not only the biomass of state variables but in the interactions those variables have with one another, known by various names such as trait-mediated indirect interactions (TMII) or non-consumptive effects, among others (Abrams 1995, Vandermeer et al. 2010, Werner & Peacor 2003, Bairey et al. 2016). This focus challenges the framework of a direct relationship between energy and material flows from prey to predator (or, more generally, resource to consumer) and the population dynamics implied by only the connection itself.

Precisely how the energy and material flowing through the system should be incorporated into this brand of interaction is not immediately evident (but see Breviglieri et al. 2017). The question has been posed before in a variety of particular interactions. For example, changes in soil fertility alters the way plants compete with one another (Brown et al. 2019, Buckland & Grime 2000; Fraser & Grime 1999), how predator and prey interact (Hohberg and Traunspurger

2005, Janssen et al. 1997), or how herbivores consume plants (Meyer 2000, Nowak & Komor 2010). Yet, to our knowledge, the potential of soil fertility to alter a TMII, has yet to be explored. Here, we pursue this question for a particular model system, the well-known relationship between the ant *Azteca seriseasur* and the green coffee scale insect *Coccus viridis*. To our knowledge ours is the first study to ask the question in the context of a mutualism.

We illustrate the logic of our study in Figure 1. Materials (illustrated by N for nitrogen in Figure 1 flow from the soil to the plant (coffee) into the hemipteran and finally, by way of the honeydew produced by the hemipteran, into the ant, a flow illustrated by the thick blue line surrounding the solid red arrows. The mutualism is thus effected as a classical TMII (equivalently, a higher order effect) illustrated in figure 1 by the dashed lines with small circles (indicating negative effect) connecting the ants with the effect of the predators and other herbivores. As is well documented (Vandermeer & Perfecto 2006; Jiménez-Soto & Morris 2019;Rivera-Salinas et al. 2018), the ant negatively affects both the predators of the hemipteran and the "other herbivores" that attack the plant. The question we pose is, do the nutrients supplied to the plant have an effect on the indirect effect, which, simply put, do the nutrients, in addition to supplying the plant, hemipteran, and ant with sustenance, affect the TMII. This hypothesis is symbolized by the thin dotted lines with arrowheads connecting N with the dashed lines that indicate the ant's TMII on the predators and other herbivores. We take the measure of ant activity on the hemipterans as a direct measure of this effect. That is, to the extent that the ants increase their foraging activity on the hemipterans feeding on plants subjected to higher soil fertility, the TMII effects (dashed lines from the ants in figure 1) will be increased. Thus, scoring ant activity can be taken as scoring the TMII.

In the coffee agroecosystems of MesoAmerica, a keystone mutualism is evident, involving the ant, *Azteca sericeasur* and the hemipteran pest, green coffee scale insect, *Coccus viridis*. These ants defend the coffee plants from several herbivores while tending the scale insects, most notably controlling the coffee berry borer, *Hypothenemus hampei*, which can have a devastating effect on coffee yields (Damon 2000). While the general system has been extensively studied (Vandermeer et al. 2010, 2019), the ant-hemipteran mutualism's potential as a link between soil properties and pest control is rarely suggested and never experimentally studied (but see Gonthier et al. 2013). And although most studies have investigated the effect of soil fertility on herbivore preference and growth (Jansson & Smilowitz 1986, Kaakeh et al.1992, Nowak & Komor 2010) and how ant mutualism changes the concentration and composition of honeydew amino acids (Yao & Akimoto 2001, 2002), most of these studies have not looked directly at ant preference for tending hemiptera on nutrient rich over nutrient poor plants.

Here we report on a laboratory study that specifically examines the direct connection on the interplay between the behavior of a mutualist (the ant) and nutritional properties of the soil via the feeding of the ant on honeydew produced by the hempiteran insects tended by these ants, We hypothesize that high-fertilized plants infected with green coffee scale insects would be preferentially tended by ants over low fertilized plants, leading to a nutrient modification of this TMII.

3.3 Methods

3.3.1 Study System

All work was done on Finca Irlanda, a shaded coffee plantation in the Socunusco region of Chiapas, Mexico on the cusp of the rainy season (May through July) of 2017. Coffee plants have numerous pests, two of which are generally considered to cause the most damage – the coffee berry borer (*Hypothenemus hampei*) and the green coffee scale (*Coccus viridis*). The green coffee scale is a phloem feeder and forms mutualistic interactions with several ant species, sometimes reaching high densities and causing significant production loss at a local level. On Finca Irlanda, the green coffee scale forms its most prominent interactions with the keystone ant species - *Azteca sericeasur* (Liere & Perfecto 2014). These ants defend the green coffee scale insects from other herbivores that may compete with them, thus indirectly providing protection to the coffee bushes from herbivores other than the green coffee scale. *Azteca sericeasur* is an arboreal nesting species and makes large carton nests in shade trees. It routinely nests in *Inga micheliana* (~45% of all nests) – a nitrogen fixing shade tree which is heavily planted in the coffee plantations of this region. *Coccus viridis* reaches highest densities on coffee bushes closest to shade trees that house nests of *A. sericeasur* ants and reduce the attack of the coffee berry borer significantly (Vandermeer et al. 2010), an effective compensation for the energy taken from the coffee by the scale insects.

3.3.2 Experimental setup

We acquired 18 four-month-old coffee seedlings from the farm's nursery, produced in a growth medium composed of half organic compost and half soil until they were acquired for the experiment. Since the root ball is fragile, we used the same growth medium the seedlings came with and supplemented them with additional fertilization depending on which treatments they belonged to. We used 3 levels of fertilization - low, medium and high. All fertilization treatments took place between May 25- July 15. We prepared a compost "tea" (stewed compost) using the organic compost (vermiculture of coffee parchment, chicken manure and calcium carbonate) prepared in-house on Finca Irlanda's composting facility. Plants assigned high fertilization levels were fertilized weekly with 100 ml of compost tea, medium fertilization levels received 50 ml compost tea and 50 ml water and the low fertilization levels received only 100 ml water. The seedlings were kept in insect enclosures to deter herbivory. Twelve seedlings (each fertilization level X 4) were infected with *C. viridis* (treatments) and 6 seedlings were kept as control (no scale insect infection). It took roughly 3 weeks for the scale insects to reach the density of an average of 30 insects per seedling. Before each trial, all treatment seedlings had the same number of scale insects (30 insects on an average) to ensure that ants were not responding to the number of scale insects (which presumably would be more on high fertilized seedlings over medium and low), but were responding to the quality of their mutualistic partners, i.e. the quality of the honeydew. *Azteca sericeasur* ants make large carton nests on shade trees. We obtained one such carton nest from the field and kept it alive in the lab by providing it with honey and insects. We then removed a piece of carton consisting of approximately 150-200 ants from the main carton nest. Each trial consisted of 6 seedlings (3 treatment and 3 control seedlings) kept in a large container with an *A. sericeasur* nest in the center such that all seedlings were equidistant from the nest (Fig 3.2). We applied Fluon on the rim of the container to ensure that ants did not dessert the nest and escape. Ant abundance on all seedlings was measured for a period of 5 minutes every 2-3 hours for 24 hours resulting in 9 observations per trial. Each trial was replicated 4 times and subsequently repeated using another piece of the carton nest with approximately the same number of ants in the piece.

At the end of the 50-day period, we cut the aerial portions from the soil surface and belowground portions of all 18 plants and recorded dry weights for biomass measurements.

3.3.3 Statistical Analysis

We performed all analyses using R v.3.6.3 (R Core Team, 2020). We tested the difference between treatment (presence of scale insects) and control with a generalized linear mixed-effects model with log link function and poisson distribution using mean number of ants (432 observations: 6 plants/trial* 9 observations/trial*4 trials/nest*2 nests) as the response variable, presence of scale insects, effect of time (9 time points) and nest as the fixed effects and plant and trials as the random effects to account for intra-plant correlation within and between trials. We then fit another generalized linear mixed-effects model to test ant preference within fertilization groups infected with scale insects. The response variable for this model was number of ants per trial (216 observations: 3 treatment plants/trial* 9 observations/trial*4 trials/nest*2 nests) and the fixed effects for this model included effect of time, treatment and nest and plant and trials as the random effects. The models were fit using the glmer() function from the lme4 package of R (Bates et al. 2015). To test significance between the three treatment groups we used generalized linear hypothesis testing on the generalized linear mixed-effects model using a Tukey Honest Significance Difference (Tukey HSD) test. This was fit using the “Tukey” test in the glht() mode from the multcomp package of R.

Associations between plant growth variables (roots and shoots) and predictors such as presence of scale insects and three fertilization groups were assessed using a linear regression model.

3.4 Results

Ants foraged significantly (Table 3.1, $p<0.001$) more on plants that were infected with scale insects (Mean ants ± S.E: 6.95 ± 0.24) over control plants (Mean ants ± S.E: 1.32 ± 0.11) and this result was consistent regardless of which ant nests were used (n_{plant}=18, n_{trial}=8, n=432 observations, Table 3.1). Within treatment groups, ants preferred plants with high fertilization over medium (p<0.001, Table 3.2) and low fertilization (n_{plant}=12, n_{tria}l=8, n=216 observations, p<0.001, Table 3.2). Post hoc TukeyHSD test revealed that there was a significant difference between high and low fertilization treatment plants (Table 3.2 p<0.001) and a difference between medium and high fertilization treatments (Table 3.2 p<0.001). There was no difference between the medium and low fertilization treatments (Fig 3.3 and Fig 3.4). Again, these results were consistent across both pieces of ant nests. Biomass measurements did not reveal statistically significant results between treatments for either scale insects or fertilization levels suggesting that the difference in ant behavior was not simply a result of healthier plants.

3.5 Discussion

Although symbioses and mutualisms are ubiquitous in nature, exploring the myriad ways in which they interconnect with other elements of a system is less common than, for example, the effect of predation on competition (see review by Chase et al. 2002), despite the popularity of analyzing networks of mutualisms themselves (Bascompte & Jordano 2007). The fact that there is general acknowledgment that nonlinear indirect interactions (e.g. trait mediated or non-consumptive effects) frequently are key to understanding community structure (Levine et al. 2017), it would be natural to expect that mutualisms should be as important as other interactions in generating such structure. This study suggests that mutualisms can play a key role as a link between bottom-up and top-down effects.

We found, as expected, ants foraged more on plants with green coffee scale insects (treatment) than on control plants, indicating that ants prefer foraging on plants with green coffee scale insects to ones without. Within treatments, ant activity on plants with high fertilization was higher than on those with both medium and low fertilization, and marginal/no significant difference between activity on high versus medium fertilization treatments. Though we did not use a continuous scale for fertilization, these results nevertheless suggest that there could be a non-linear relationship between plant fertilization and ant preference; there is perhaps a threshold amount of nutrient enrichment necessary for the ants to discern a detectable difference in the honeydew produced by the green coffee scale insects.

In the field, scale insects must reach a critical density to be "discovered" by the ants so as to begin tending and thus protecting them against *Azya orbigera* (Vandermeer & Perfecto 2019), its main predator (Liere & Perfecto 2008). With ant protection, the population density of the scale insects increases dramatically. The detail of how the ants discover the scale insects is yet not completely clear. Is it a discovery made by chance when they happen to encounter a sufficiently large local concentration of scale insects or are other factors involved? The results from this experiment suggest that there might be a link between bottom-up effects of soil fertilization on the nutritional quality of plants and hence the nutritional quality of the honeydew produced by the scale insects on these plants. Might it be the case that ants not only respond to a critical population density of scale insects to initiate tending activity, but are also attracted to a high nutritional quality of the insects.

Theoretically we postulate that soil fertility impacts this keystone mutualism, ants and scale insects, dually. First, soil fertility increases the nutritional quality of the sap of the plants, increasing the scale insect local population growth rate of the green coffee scale insects, resulting in reaching the critical population density of these insects faster than on a low nutrition plant (Jansson & Smilowitz, Nowak & Komor 2010). Hence, if ants find scales randomly, the chances they will find scale densities sufficiently large to initiate their tending should be higher on plants that have higher nutrient availability. Second, and perhaps more likely, ants find scale insects randomly but the scale density critical to initiate tending is lower for honeydew of a higher quality. This is perhaps useful to the ants when their food resources are at low density since the higher quality honeydew can sustain the ant colony better until the population density of the scale insects starts increasing (Fig 1). Indeed this is commonly seen in wild bees, especially social bees, where the bees tend to be more selective for "high quality" floral patches as resources decline at the landscape level (Pope & Jha 2018).

It is clear that the TMII effect of soil quality on mutualisms and the associated non-linear interactions may have the ability to modify well-known direct interactions. These in turn, can and do cascade up to influence other interactions within the system. In our study system, whether or not ants that nest in nitrogen fixing shade trees forage more on the scale insects (*Octolecanium sp.*) of the shade tree itself or on green coffee scale insects on the nearby coffee bushes (Livingston et al. 2008), there is bound to be a modification to their interaction/mutualism with the green coffee scale insect, wherein it will either lower the interaction strength of this mutualism or strengthen it. Whatever the outcome, this modification will have cascading TMII effects on all other interactions within the system depicted by Fig.1 leading to either an increase or a decrease in the other pests of the coffee agroecosystems. To the best of our knowledge, this is the first study to test the links between soil nutrients and their effects on a key ant-hemipteran mutualism, illustrating the potential cascading effects on other interactions.

3.6 Acknowledgments

We are grateful to Peter Walters and Finca Irlanda for allowing us to conduct research on the farm as well as provide us with the coffee seedlings from the nursery. We would like to thank P. Yajnik for help with statistical analysis and K. Sanchez, Z. Hajian-Forooshani, I. Saraeny Rivera Salinas and N. Medina and two anonymous reviewers for helpful comments on earlier versions of the manuscript.

3.7 References

Abrams, P.A., 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. The American Naturalist, 146(1), pp.112-134.

Bairey, E., Kelsic, E.D. and Kishony, R., 2016. High-order species interactions shape ecosystem diversity. Nature communications, 7(1), pp.1-7.

Bascompte, J. and Jordano, P., 2007. Plant-animal mutualistic networks: the architecture of biodiversity. - Annual Review of Ecology, Evolution & Systematics 38: 567-593.

Bates, D. et al. 2015. Package ‘lme4’. - Convergence. 12:2.

Brown, C. et al. 2019. Species-specific size vulnerabilities in a competitive arena: Nutrient heterogeneity and soil fertility alter plant competitive size asymmetries. - Functional Ecology. 33: 1491-1503.

Breviglieri, C.P.B., Oliveira, P.S. and Romero, G.Q., 2017. Fear mediates trophic cascades: nonconsumptive effects of predators drive aquatic ecosystem function. The American Naturalist, 189(5), pp.490-500.

Buckland, S.M. and Grime, J.P., 2000. The effects of trophic structure and soil fertility on the assembly of plant communities: a microcosm experiment. - Oikos, 91: 336-352.

Chase, J. M. et al. 2002. The interaction between predation and competition: a review and synthesis. Ecology Letters. 5: 302–315.

Damon, A. 2000. A review of the biology and control of the coffee berry borer, Hypothenemus hampei (Coleoptera: Scolytidae).- Bulletin of Entomological Research. 90: 453-465.

Ehrlich, P.R. and Birch, L.C.1967. The" balance of nature" and" population control". -American Naturalist. 101: 97-107.

Fraser, L.H. and Grime, J.P. 1999. Interacting effects of herbivory and fertility on a synthesized plant community. –Journal of Ecology 87: 514-525.

Forkner, R.E. and Hunter, M.D. 2000. What goes up must come down? Nutrient addition and predation pressure on oak herbivores. -Ecology, 81:1588-1600.

Gonthier, D.J.et al. 2013. Bottom-up effects of soil quality on a coffee arthropod interaction web. -Ecosphere, 4:1-15.

Hairston, N.G. et al. 1960. Community structure, population control, and competition.- American Naturalist. 94: 421-425.

Hohberg, K. and Traunspurger, W. 2005. Predator–prey interaction in soil food web: functional response, size-dependent foraging efficiency, and the influence of soil texture. –Biology and fertility of soils, 41: 419-427.

Hunter, M.D. and Price, P.W. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. -Ecology, 73:724-732.

Janssen, A., Bruin, J., Jacobs, G., Schraag, R. and Sabelis, M.W., 1997. Predators use volatiles to avoid prey patches with conspecifics. Journal of Animal Ecology, pp.223-232.

Jansson, R.K. and Smilowitz, Z. 1986. Influence of nitrogen on population parameters of potato insects: abundance, population growth, and within-plant distribution of the green peach aphid, Myzus persicae (Homoptera: Aphididae).- Environmental Entomolgy.15: 49-55.

Jimenez-Soto, E., Morris, J.R., Letourneau, D.K. and Philpott, S.M., 2019. Vegetation connectivity increases ant activity and potential for ant-provided biocontrol services in a tropical agroforest. Biotropica, 51(1), pp.50-61.

Kaakeh, W. et al. 1992. Combined effects of spirea aphid (Homoptera: Aphididae) and nitrogen fertilization on net photosynthesis, total chlorophyll content, and greenness of apple leaves. – Journal of Economic Entomology. 85:939-946.

Levine, J.M. et al. 2017. Beyond pairwise mechanisms of species coexistence in complex communities.- Nature, 546: 56-64.

Liere, H. and Perfecto, I., 2014. Cheating on a mutualism: indirect benefits of ant attendance to a coccidophagous coccinellid. – Environmental Entomolgy. 37: 143-149.

Livingston, G.F. et al. 2014. Indirect interactions between ant-tended hemipterans, a dominant ant Azteca instabilis (Hymenoptera: Formicidae), and shade trees in a tropical agroecosystem. - Environmental Entomolgy. 37: 734-740.

Meyer, G.A. 2000. Interactive effects of soil fertility and herbivory on Brassica nigra. -Oikos, 88:433-441.

Nakagawa S, Johnson P, Schielzeth H (2017) The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models revisted and expanded. J. R. Soc. Interface 14. doi: 10.1098/rsif.2017.0213

Nowak, H. and Komor, E. 2010. How aphids decide what is good for them: experiments to test aphid feeding behaviour on Tanacetum vulgare (L.) using different nitrogen regimes. - Oecologia, 163:973-984.

Pope, N.S. and Jha, S. 2018. Seasonal food scarcity prompts long-distance foraging by a wild social bee. - American Naturalist .191: 45-57.

R Core Team. (2020). R: a language and environment for statistical computing (3.6.3) [Computer software].

Rivera-Salinas, I.S., Hajian-Forooshani, Z., Jiménez-Soto, E., Cruz-Rodríguez, J.A. and Philpott, S.M., 2018. High intermediary mutualist density provides consistent biological control in a tripartite mutualism. Biological Control, 118, pp.26-31.

Vandermeer, J. et al. 2010. Ecological complexity and pest control in organic coffee production: uncovering an autonomous ecosystem service. - BioScience, 60:527-537.

Vandermeer, J. et al. 2019. The Community Ecology of Herbivore Regulation in an Agroecosystem: Lessons from Complex Systems. -BioScience, 69: 974-996.

Vandermeer, J. and Perfecto, I. 2019. Hysteresis and critical transitions in a coffee agroecosystem. –PNAS. 116: 15074-15079.

Werner, E.E. and Peacor, S.D., 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology, 84(5), pp.1083-1100.

Yao, I. and Akimoto, S.I. 2001. Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid Tuberculatus quercicola.- Oecologia, 128: 36-43.

Yao, I. and Akimoto, S.I. 2002. Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid Tuberculatus quercicola. Ecological Entomology 27:745-752.

3.8 Tables and Figures

Table 3.1. Results from genralized linear mixed-effect model testing the difference between treatment (presence of scale insects) and control with number of ants as a response variable, presence of green coffee scale insects, time and nest as fixed effects, and plant and trial as random effect. Marginal and conditional R^2 values were calculated using Nakagawa et al. 2017

	Ants		
Predictors	Estimate	S.E.	p
(Intercept)	0.28	0.19	0.134
Scale [yes]	1.63	0.19	**<0.001**
Nest [B]	-0.05	0.13	0.715
Time	-0.01	0.01	0.538
Random Effects			
σ^2	0.24		
$\tau_{00\ \mathrm{Plant}}$	0.13		
$\tau_{00\ \mathrm{Trial}}$	0.03		
N_{Plant}	18		
N_{Trial}	8		
Observations	432		
Marginal R^2 / Conditional R^2	0.626 / 0.777		

Table 3.2. Results from generalized linear mixed effects model to test ant preference between fertilized (infected with green coffee scale insects) treatment plants. The fixed effects for this model included treatment, effect of time (9 levels) and nest as predictors and plant and trial as

the random effects. Marginal and conditional R^2 values were calculated using Nakagawa et al. 2017

	Ants		
Predictors	*Estimate*	*SE*	*p*
(Intercept)	1.96	0.18	**<0.001**
Treatment [LO]	-0.59	0.12	**<0.001**
Treatment [MED]	-0.47	0.12	**<0.001**
nest [B]	0.02	0.19	0.906
Time [2]	0.05	0.12	0.669
Time [3]	0.32	0.11	**0.006**
Time [4]	0.28	0.12	**0.014**
Time [5]	0.33	0.11	**0.004**
Time [6]	0.38	0.11	**0.001**
Time [7]	0.18	0.12	0.125
Time [8]	0.28	0.12	**0.016**

Time [9]	0.29	0.12	**0.012**
Random Effects			
σ^2	0.14		
$\tau_{00\ \mathrm{Plant}}$	0.02		
$\tau_{00\ \mathrm{Trial}}$	0.07		
N_{Plant}	12		
N_{Trial}	8		
Observations	216		
Marginal R^2 / Conditional R^2	0.257 / 0.540		

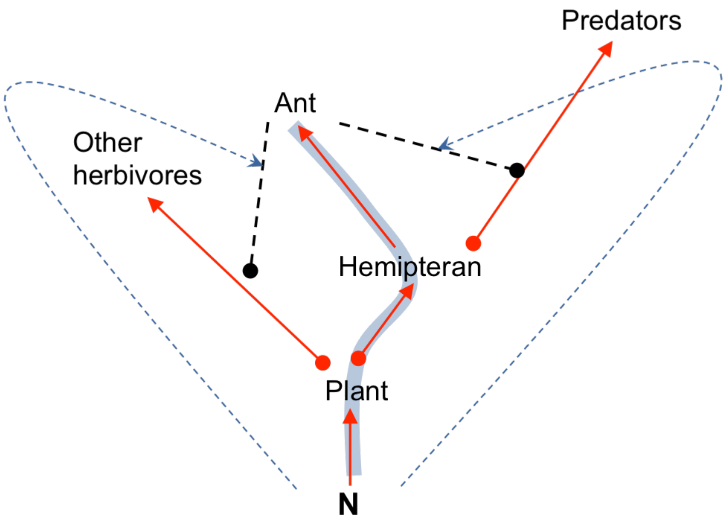

Figure 3.1. Conceptual diagram of the system. Each red line represents energy transfer at that level (transferring to an element, and thus positive, illustrated with an arrowhead, transferring

from an element, and thus negative, illustrated with a circle). Transparent blue shading indicates the materials (illustrated by N for nitrogen) flowing from the soil to the plant (coffee) into the hemipteran and finally, by way of the honeydew produced by the hemipteran, into the ant

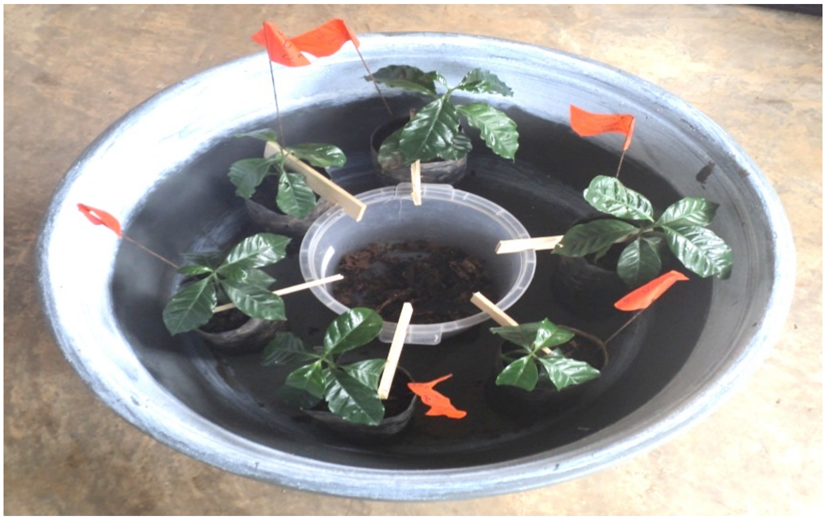

Figure 3.2 . Experimental setup – Large container with 3 control and 3 treatment coffee seedlings. All seedlings were equidistant from a smaller container that held ant nests, kept within the large container.

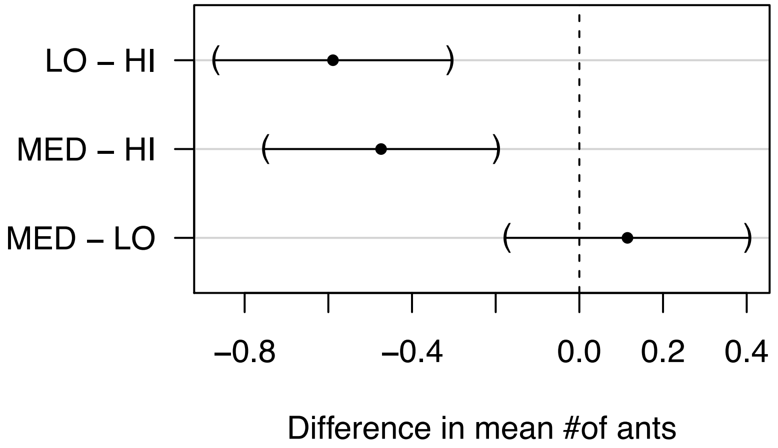

Figure 3.3. Plot of pair-wise comparisons from Tukey's HSD showing the confidence intervals for the difference in the means for each comparison. Any confidence intervals that do not contain 0 provide evidence of a difference in the fertilization groups. T stands for treatment plants (infected with scale insects) and HI, MED and LO stands for high, medium and low fertilization groups.

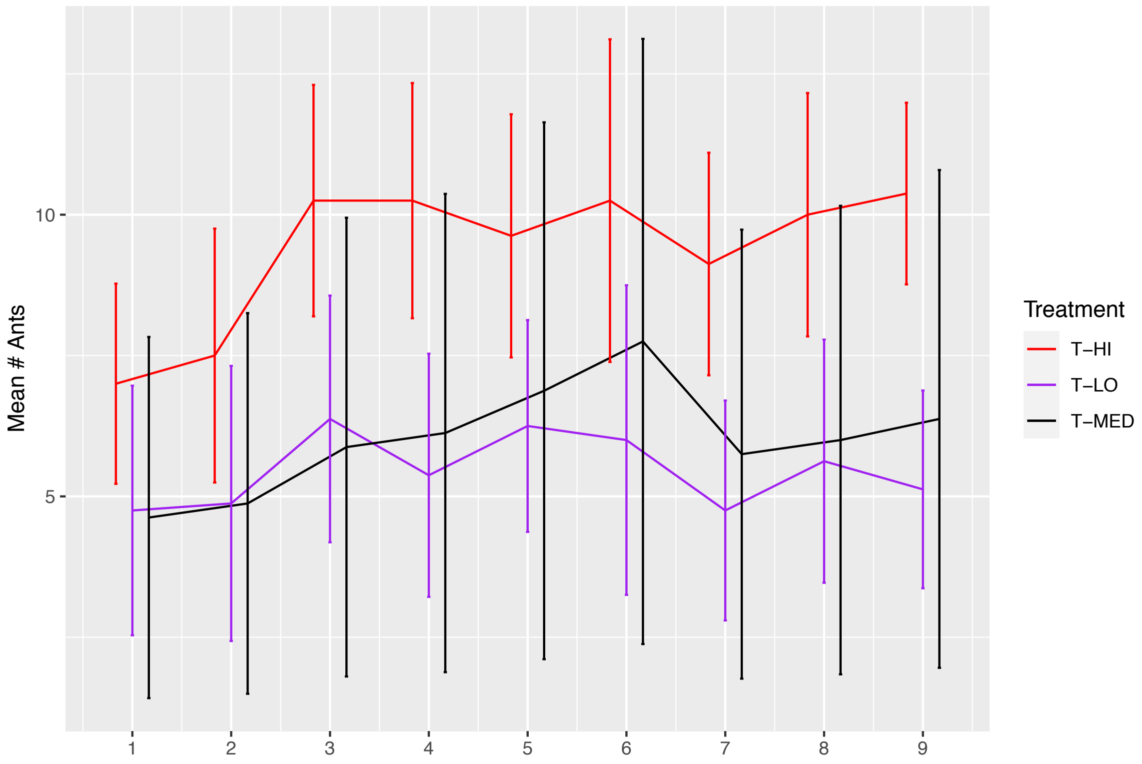

Figure 3.4. Mean ants with 95% CI at each time interval for each fertilization group on plants infected with scale (T).

Chapter 4 : Complex Interspecific Interactions Influence the Interactions between Pest Control and Pollination in Coffee Agroecosystems[3].

4.1 Abstract

Ecosystem services mediated by biodiversity are essential for the well-being of human beings. While there is ample research on individual ecosystem services (such as pollination, nutrient cycling), there is now growing recognition to examine the interactions between multiple ecosystem services and their contribution to productivity in order to manage agroecosystems sustainably. In this study, we examined the interactions between pollination and pest control in coffee agroecosystems in Chiapas, Mexico. We tested how management of shade trees, particularly of nitrogen-fixing shade trees, at the farm scale mediated the outcome of the interactions between two ES. We found that there was no trade-off between pest control and pollination services despite the deterrence of pollinators by the dominant and aggressive ant species, *Azteca sericeasur,* which also controls the coffee berry borer, a major pest of *Coffea arabica.* We found additive effects of pest-control and pollination on early fruit set and fruit weight of coffee plants. Proximity to nitrogen-fixing shade trees had indirect effects on pest-control via the reduction of *Azteca sericeasur* activity on the coffee bushes. These findings highlight that ecosystem services are a result of complex interspecific interactions and that biodiversity-friendly management practices can promote favorable outcomes of these interactions on coffee yield.

---

[3] Co-authors are : Dominguez Martinez GH., and Vandermeer J.

4.2 Introduction

Provisioning services such as food, timber, and fiber are essential to human well-being, and much research has focused on maximizing their availability by improving the management of ecosystems. Management practices can also influence the ecosystem services (hereafter, ES) that support these provisioning services, including pest control, pollination, regulation of soil fertility and nutrient cycling. Not surprisingly, a majority of the research has considered each of these ES in isolation, perhaps necessary so as to navigate around the complexity that arises, focusing on a single ES such as pest control or pollination. There is now a growing recognition that multiple ES interact with one other and that such complexity must be addressed in search of sustainable ecosystem management and the promotion of biodiversity(Chain-guadarrama et al., 2019).

Animals, including many insect species, are important pollinators for about 88% of angiosperm species, providing vital ecosystem services for wild plants and food crops worldwide (Ollerton et al., 2011). Similarly, pest control, another animal-mediated ES increases crop productivity by reducing pest damage to crops (Gutierrez-Arellano and Mulligan, 2018). Both these services have significant economic importance and therefore have received considerable attention. In the past decade, a literature has evolved focusing on the interactions between pollination and pest-control in different agroecosystems. Most of these studies have found varying effects of the interactions between these two ES on crop yields (Garibaldi et al., 2018). While some studies have found synergistic effects (the gain in crop yields when pest control and pollination interact is higher than the sum of the gain when these two ES act separately) for example in red clover and oilseed rape (Albrecht and Sutter, 2016; Lundin et al., 2012), others have found that they only act independently (no interaction) for example in cucumber crops (Barber et al., 2012), one study has found a weak negative interaction on oil seed rape yields (Bartomeus et al., 2015). Thus, even though the interactions between these two ES have variable outcomes, there is evidence that these services complement or augment one another.

Farmers worldwide, augment soil fertility by adding fertilizers – organic or synthetic, or by using cover crops or nitrogen fixing shade trees with the direct goal of supplying limiting nutrients to the plants. Nutrient availability not only affects plant productivity but can also have bottom-up effects and potentially influence a variety of interactions between trophic levels. Nitrogen (N) can influence plant-pollinator interactions through altered floral phenology (Cleland et al., 2006; Hoover et al., 2012), changes in floral production (Burkle and Irwin, 2010) and floral rewards such as nectar volume, nectar composition and pollen composition (Ceulemans et al., 2017; Gardener and Gillman, 2001). Similarly, N may also limit herbivorous insects and N availability can have direct significant effects on herbivore development, growth and reproduction and potentially increase mutualistic interactions between honeydew-producing insects and ants (Gonthier et al., 2013; Vaidya and Vandermeer, 2021). One way that soil fertility is enhanced in tropical agroecosystems is by incorporating N-fixing trees. Several studies have reported an increase in N in crops when associated with N-fixing leguminous trees, either through direct N transfer via mycelial networks or tree root exudate absorption by crops or through prunings via leaf litter mulching (reviewed in Nygren et al., 2012).

Coffee agroecosystems are excellent model systems for the study of interactions between different ES, since coffee is grown on 11million hectares of land in the tropics by millions of farmers worldwide (FAO, 2015), its importance both environmentally and economically is large. It is currently grown under a variety of management conditions from “rustic coffee” to “sun coffee" (Moguel and Toledo, 1999), and farms that grow coffee under the canopy of shade trees have been shown to support higher associated biodiversity and the provisioning of multiple ES such as pollination, pest control, carbon sequestration, and enhanced nutrient cycling (Greenberg et al., 1997a, 1997b; Jha et al., 2014; Perfecto et al., 1996; Perfecto and Vandermeer, 2008; Philpott et al., 2006; Tscharntke et al., 2011), thus making coffee agroecosystems ideal for studying the interactions among ES. Interactions between ES can either be direct – via the interactions of the species that provide the different ES, or indirect – via changes in a shared driver (Bennett et al., 2009). In the coffee plantations of southern Mexico, farmers regularly grow coffee plants under the canopy of nitrogen-fixing shade trees from the genus *Inga* (Perfecto and Vandermeer, 2002) and there is some evidence of N transfer to coffee plants from the *Inga* trees (Grossman et al., 2006; Roskoski, 1982). Thus, inquiring if this shared driver, nutrient availability, modifies the interaction outcome between pest control and pollination via indirect effects is an important piece of the puzzle.

Most research on the interactions between pest-control and pollination in coffee agroecosystems has focused on pest-control by birds and has found that these interactions have variable outcomes, from synergistic (Martínez-Salinas et al., 2022) to complementary(Classen et al., 2014). But there are several other kinds of organisms that provide pest control and among them are ants (Anjos et al., 2022). Ants provide pest-control in coffee agroecosystems (Jha and Vandermeer, 2010) and the mechanisms through which they control pests are many. For example, ant-hemipteran mutualistic interactions are abundant in nature (Delabie, 2001) in which ants protect their mutualistic partners from their predators and in doing so, also protect plants from other potentially more damaging pests (Floate and Whitham, 1994; Perfecto and Vandermeer, 2006). However, another consequence of the ant-hemipteran mutualism, especially in aggressive ants, is that ants also seem to "defend" plants from all plant visitors, including pollinators. Ants can chase away pollinators (Philpott et al., 2006; Vannette et al., 2017) and indirectly deter pollinator visitation by stealing nectar from the flowers (Ghazoul, 2001; Levan and Holway, 2015) and leaving pheromones on flowers that pollinators then tend to avoid (Lach, 2007; LeVan et al., 2014; Sidhu and Rankin, 2016). Thus, ant-hemipteran mutualisms may benefit the plant from defense against other herbivores yet may simultaneously reduce the effectiveness of the plant-pollinator mutualism thus, resulting in an apparent tradeoff (Fig 1).

In this study, we tested the interactions between two key ES - pest control and pollination via the interactions between ants and pollinators of *C. arabica.* Specifically, we investigated 1) whether the interactions between pest control and pollination are synergistic or concessionary (i.e., represent a trade-off) and, 2) whether management factors, particularly the association with nitrogen-fixing shade trees mediates the outcome of these interactions on coffee yield (Fig 4.1)

4.3 Methods

4.3.1 Study System

Coffea arabica is self-compatible but can benefit from animal pollination via increased fruit quantity and quality (Klein et al., 2003a; Philpott et al., 2006; Ricketts et al., 2004). Coffee (*Coffea arabica*) flowers in the dry season between February-May, the flowers remaining open for ~2 days or, if unpollinated, for as much as 5 days. Its berries are harvested typically between September-December. if unpollinated, can remain open for ~5 days. Coffee is frequently planted under the canopy of shade tree species. On Finca Irlanda, the site of our study, shade trees of approximately 90 different species are planted, with the most common ones fixing nitrogen and belonging to the genus *Inga* (Schmitt and Perfecto, 2021). The second most common shade tree is

Alchornea latifolia. Since the farm is an organic plantation, no pesticides or herbicides are used, and manual weeding is carried out using machetes. *Coffea arabica* has numerous pests, but the coffee berry borer (CBB, *Hypothenemus hampei*) and the green coffee scale (GCS, *Coccus viridis*) are considered to be the most prominent pest species. The CBB female bores and lays eggs inside the coffee berries and the larvae develop by eating the pulp of the berry, causing extensive damage to it. The GCS is a phloem feeder and forms mutualistic interactions with several ant species. In our study site, their most prominent interactions are with the dominant ant species, *Azteca sericeasur* (Perfecto and Vandermeer, 2006). These ants defend the GCS aggressively from its predators and other competing herbivores thus indirectly controlling the coffee plants from CBB infestation (Morris et al., 2015; Vandermeer et al., 2010). *Azteca sericeasur* does not discriminate between the natural enemies of GCS and other organisms and is known to be aggressive towards any organism that visits the coffee plants, including pollinators and other ant species (Philpott et al., 2006; Vandermeer et al., 2010; Vannette et al., 2017). Additionally, *A. sericeasur* nest arboreally nesting inside the tree trunks and occasionally making large carton nests on the trunk surface. They forage extensively on neighboring coffee bushes, mostly via tending the GCS on these bushes. The GCS reach their highest densities on coffee bushes closest (within 5 m) to those shade trees that house nests of *A. sericeasur* ants (Perfecto and Vandermeer, 2006; 2019).

4.3.2 Study Design

This study was conducted in Finca Irlanda from February 2018 to December 2019. Finca Irlanda is a 300 ha organic shaded coffee plantation in Chiapas, Mexico (92°20′29″ W and 15°10′6″ N) at an elevation range between 900 and 1200 m a.s.l, with mean annual rainfall of approximately 4500 mm (Schmitt and Perfecto, 2021).

We selected coffee bushes within 2m of shade trees; shade trees were either nitrogen-fixing shade trees from the genus *Inga* (n=33 in 2018 & n=23 in 2019) or non-nitrogen fixing shade trees (non- *Inga spp.,* multiple species- most commonly *Alchornea latifolia*, n=34 in 2018 & n=28 in 2019). Roughly half of the shade trees had *A. sericeasur* (hereafter, Azteca ants) nests on them (Fig 4.2) and foraging on coffee bushes near the shade trees, while the other half did not. Thus we had a paired design – coffee bushes paired with *Inga* spp. with Azteca ants and without ants, coffee bushes paired with non-*Inga* spp. with Azteca ants and without ants.

On each coffee bush, we selected 3 branches, two of them were kept open to animal pollinators (open pollination) and one branch was bagged (0.8-1mm mesh size) to exclude animal pollinators (bagged pollination). On each coffee bush, before coffee started flowering, we counted the total number of buds on all three branches in February 2018 and 2019. On coffee bushes that did not have Azteca ants, we applied tanglefoot at the base of the branch to exclude other ant species from all three branches (ant exclosure), while on bushes that had Azteca ants, tanglefoot was applied only on the bagged branch. Bags were removed at the end of the flowering period (end of April). Thus, we had three factors in total – pollination (open and bagged), ants (with Azteca ants and without ants) and Shade treatment (*Inga spp.* and non-*Inga spp.*) We measured Azteca ant activity on the coffee bushes by selecting a random point on the plant and counting the total number of ants passing the point for a period of one minute. Azteca ant activity ranged from 5 – 20 ants per minute.

Pollinator visitation:

When coffee plants started flowering, we counted the number of open flowers on the open pollination branches on each bush and during 10 min timed observations recorded the species of the visiting pollinators, the total number of flowers visited by the pollinators and their visit duration. We pooled total number of flowers and visits from the two open branches for each coffee bush and calculated visitation rates by dividing the number of visits by the total number of flowers.

Fruit quality and quantity

We counted the total number of fruits at two time points – once in May-June, accounting for initial pollination, and during harvest, accounting for late pollination. We counted initial and late fruit set by dividing the total number of flower buds by the total number of berries during each count respectively for all branches (open and bagged). For branches that were open to pollination, we averaged fruit set across the two branches. We harvested all available berries on the three branches of each bush and measured the weight and counted the number of beans in each harvested berry. Since the presence of one bean in a berry or fruit (peaberry) is a sign of unsuccessful pollination, this measure was useful to determine pollination success rates among the different control and treatment groups. Fruit set on bagged branches was only counted in 2019, but fruit weight and the number of beans in each fruit was measured in both years.

Coffee berry borer control

We recorded the presence/absence of the CBB in each harvested berry from all the branches that were open to pollination. Since the bagged branches on coffee bushes with Azteca ants had tanglefoot applied on them, we only compared branches open to pollination for the control of CBB.

Effect of shade trees

Along with total number of flowers on the three branches, we also measured the corolla diameter of five flowers on one branch of all coffee bushes to understand the effect of N availability on floral traits. We sampled five leaves each from the shade trees (8 *Inga spp.* and 8 non-*Inga spp.*) and their paired coffee bushes (nine of each). We ground dried samples using a coffee grinder and used a representative sub-sample to analyze % C and % N using a LECO Trumac CN combustion analyzer (LECO Corporation, St. Joseph, MI). We used the total C and total N data to calculate the carbon to nitrogen ratio (C:N).

4.4 Statistical Analysis

To understand if pollinator visitation differed between the treatments, we constructed a generalized linear mixed-effects model with a zero-inflated negative binomial distribution using the package "glmmTMB", with number of visits by pollinators as the response variable and log(number of flowers) included as an offset. Predictor variables were the ants and shade tree treatments as well as year to account for interannual variation in pollinator visits as fixed effects and tree and site as random effects. We set the zero-inflation formula equal to 1, to denote equal probability of producing zeros over all observations. To determine whether the presence of the green coffee scale insects influenced ant visitation to coffee flowers, we performed a Fisher's exact test.

We analyzed the effect of the three factors on both the initial fruitset and fruitset at the time of harvest by constructing linear mixed effects models using the 'lmer()' function from `lmerTest`. We constructed models with all three treatments- pollination, Azteca ants and shade trees- including two-way and three-way interactions and year as fixed effects. In both fruitset (initial and at the time of harvest) models, plant and site were added as random effects. Models with interactions were compared with those without interactions using likelihood ratio tests and AIC criteria using the “anova” function and the model with the lowest AIC score was chosen. To meet conditions of normality, fruitset at the time of harvest was arcsine square root transformed. We checked for model compliance with assumptions using the `DHARMa` package.

To determine the effect of all treatments and their interactions on the weight of the coffee fruits we constructed linear mixed effects models; year was also added as a fixed effect. Branch, plant and site were added as random effects. Models with interactions were compared with those without interactions using likelihood ratio tests and AIC criteria using the “anova” function and the model with the lowest AIC score was chosen. To evaluate the incidence of peaberry formation, we constructed a generalized linear mixed effects model with a binomial error distribution with all three treatments and their interactions and year as fixed effects and plant as random effect. We constructed models with and without interactions and checked goodness of fit using AIC information.

We observed a decrease in ant activity in 2019 from the previous year, therefore we tested if this difference was statistically significant by fitting a generalized linear mixed effects model with poisson log link function. We added shade trees, year and their interactions as fixed effects and plant and site as random effects for this model. To account for the inter-annual variation in ant activity, we tested the effect of treatments on CBB status within the coffee berries (presence, absence) for the two years separately. We constructed generalized linear mixed effects models with a binomial error distribution and modeled the log odds ratio of CBB presence in the berry, CBB absence in the berry as the response variable and shade tree treatment, ants treatment and their interaction as fixed effects; plant and site were added as random effects. Models with and without interactions were tested for goodness of fit using AIC information.

For all the mixed effect models described above, we performed Type II Wald chisquare tests on the selected model to determine which effects were significant. We then used post-hoc tests to estimate marginal means and contrasts to make pairwise comparisons between the treatment levels if they were significant using the “emmeans function” in the “emmeans” package.

To test the effect of shade trees on the number of flowers, we constructed a generalized linear mixed-effects model with poisson log-link function. Shade trees and year were added as fixed effects and plant and site as random effects. To test the effect of shade trees on the size of the flowers, we constructed a linear regression model with shade trees and year as the fixed effects and checked if the model satisfied conditions of normality by performing a Shapiro-Wilk test on the residuals.

To test whether there were any differences in %N and C:N ratios between *Inga spp.* and non- *Inga spp* shade trees and between the paired coffee bushes, we conducted a Wilcoxon test. All data was analyzed using R version 4.1.2.

4.5 Results

Pollinator visitation

There were a total of 1149 visits to coffee flowers in both years by pollinators; 87% of the visits were made by the introduced *Apis mellifera*, 12.7% of visits were made by native stingless bee species *Scaptotrigona mexicana, Trigona fuliventris* and *Trigona nigerrima* and the remaining visits were by the bee species *Agapostemon sp*.

Bee visits to coffee flowers on bushes with Azteca ants were significantly lower (3.8 ± 8.1; mean ± std. dev.) than on those with ant exclosures (15.6 ± 15.4, $\chi2 = 21.5$, d.f. = 1, $p < 0.001$, Fig 4.3A) and were higher in 2019 ($\chi2 = 12.6$, d.f. = 1, $p < 0.001$). We did not find significant effects of shade treatment on bee visitation. Additionally, bee visit duration was strongly reduced on coffee bushes with Azteca ants than without ants (Fig 4.3B).

Additionally, we found that Azteca ants tended to forage in the coffee flowers significantly more in the absence of the GCS ($p=0.02$).

Fruit set

Early fruit set significantly increased from 0.45 (bagged pollination) to 0.65 (open pollination) with pollinator activity ($\chi2 = 27$, d.f. = 1, $p < 0.001$ Fig 4.4). Additionally, early fruit set also increased significantly from 0.5 to 0.6 when Azteca ants were present on coffee bushes ($\chi2 = 9.9$, d.f. = 1, $p= 0.002$). The selected model with lowest AIC score did not have three-way or two-way interactions as fixed effects and shade tree treatment was not significant. Fruit set was also significantly higher in 2019 than in 2018 ($\chi2 = 9.6$, d.f. = 1, $p =0.002$).

For fruit set at the time of harvest, the model with only pollination and year was the best fit model ($\Delta AIC = -5.1$ for model omitting two-way and three-way interactions and all other fixed effects). Fruit set at the time of harvest significantly increased from 0.37 ± 0.05 to 0.61 ± 0.03 when pollinators were allowed access ($\chi2 = 27.6$, d.f. = 1, $p < 0.001$). There was no significant difference between the two years.

Fruit weight

The best model for fruit weight was the one without two-way and three-way interactions. Fruit weight increased significantly from 1.56 g ± 0.04 to 1.74 g ± 0.03 (estimated mean ± std error) with pollinator activity alone representing an 11.5% increase ($\chi^2 = 29.07$, d.f. = 1, $p < 0.001$, Fig 4.5) and by 0.07g and in the presence of Azteca ant activity ($\chi^2 = 4.23$, d.f. = 1, $p = 0.039$) representing a 4.3% increase in fruit weight.

Probability of peaberry formation was significantly lower when pollinators were allowed access than on those with pollinator exclosures ($Z = -2.199$, $p = 0.028$). The model with only pollinator exclosure as the fixed effect was the best fit model ($\Delta AIC = -5.1$ for model omitting two-way and three-way interactions and all other fixed effects).

Pest control of CBB

We found that ant activity was significantly reduced in 2019 ($\chi^2 = 58.2$, d.f. = 1, $p < 0.001$). On coffee bushes paired with non- *Inga* spp*,* Azteca ant activity was significantly higher in 2018 than 2019 (Tukey test, ß = 1.28±0.13, z = 9.53, $p < 0.0001$). We therefore decided to look at CBB control for the two years separately.

For the year 2018, the interaction effect of the shade treatment and pest-control treatment was the only significant effect ($\chi^2 = 4.2$, d.f. = 1, $p = 0.04$). Probability of the presence of CBB in coffee berries was significantly lower in bushes with Azteca ants paired with non-*Inga* spp. than without Azteca ants. There was no effect of Azteca ants on the probability of the presence of CBB in coffee berries on bushes paired with *Inga* spp (Fig 4.6A).

For the year 2019, effect of Azteca ants alone was significant ($\chi^2 = 5.1$, d.f. = 1, $p = 0.02$), with the probability of the finding CBB in coffee berries significantly lower on bushes with Azteca ants than on bushes with ant exclosures. Shade and ant treatments interaction did not have a significant effect on CBB control (Fig 4.6B).

4.6 Discussion

Our findings show that there is no trade-off between pest control and pollination services despite the deterrence of pollinators by the dominant and aggressive ant species, *Azteca sericeasur,* that also controls the coffee berry borer, a major pest of *Coffea arabica.* Indeed, we found that there is an additive effect of pest-control and pollination on early fruit set and fruit weight. Proximity to nitrogen-fixing shade trees had indirect effects on pest-control via the reduction in Azteca ant activity on the coffee bushes, the reasons for which we discuss below.

Early and late fruitset both increased significantly when pollinators were allowed access to the plants (Fig 4.4). This finding is in line with several studies that have found that although coffee is self-compatible, it benefits from animal pollination (Klein et al., 2003; Ricketts et al., 2004; Martínez-Salinas et al., 2022). Azteca ants increased the benefit of animal pollination in early fruitset, which is surprising given that Azteca ants reduced both the number of visits and the visit duration of the pollinators visiting coffee flowers. Bees generally avoided visiting flowers on plants with Azteca ants to avoid aggressive interactions with the ants. Indeed, in some instances we noticed that Azteca ants would chase away bees attempting to visit coffee flowers. This phenomenon is common in interactions between aggressive ants and pollinators (LeVan et al., 2014; Sidhu and Rankin, 2016). While ants usually display this kind of aggressive behavior to protect their hemipteran partners, we found this behavior to be present even when the green coffee scale insects were absent on the coffee bushes. Bees likely also reduced their visits or visit duration due to resource competition with Azteca ants. We found that Azteca ants were foraging on coffee flowers, especially in the absence of the GCS, their hemipteran partners. This is contrary to past research that showed that ant-aphid mutualism increased ant floral visitation, reducing pollinator visitation and seed set(Levan and Holway, 2015). It is likely that they were foraging for nectar in coffee flowers to maintain their colonies in the absence of GCS, their major carbohydrate source. Thus, Azteca ants may have increased the initial fruit set of coffee via 2 non-exclusive mechanisms. First, since initial fruit set was significantly higher on both bagged branches and those open to animal pollinators, it is possible that Azteca ants were controlling floral and other herbivores which may have prevented resources being allocated from reproductive organs to vegetative tissues. Secondly, by foraging for nectar, ants may have increased self-fertilization in flowers in the open pollination treatments and may have also increased the pollination effectiveness of bees by reducing their visit duration to the flowers (Fig 4.2B) and thereby facilitating outcrossing. The majority of the visits to coffee flowers were made by *Apis mellifera sculleta*, and previous studies have shown that interspecific competition for resources alters the behavior of honeybees and increases their pollination effectiveness by increasing the proportion of movement between trees (Brittain et al., 2013). Both these mechanisms seem likely and may have acted in concert to increase the initial fruit set. To disentangle these mechanisms, future research should compare the levels of floral and foliar herbivory on plants with and without Azteca ants and record fruit set. Observations of the movement of honeybees to plants with Azteca ants and the ones adjacent to them should either be made directly (Brittain et al., 2013; Greenleaf and Kremen, 2006) or by using fluorescent dye to detect pollen flow between them (Fitch and Vaidya, 2021). Additionally, flowers visited exclusively by Azteca ants should be marked to evaluate the overall effectiveness of ants as pollinators of coffee plants. Ants have been shown to be effective pollinators in certain plant species (Gómez, 2000); therefore their ability to pollinate coffee cannot be entirely ruled out. The effect of ants on the fruit set at the time of harvest was no longer significant (Fig 4.4B), and it is possible that plants aborted the fruits that were pollinated by Azteca ants (Rostás and Tautz, 2010). Nonetheless, fruit set was still higher on plants with Azteca ants suggesting that neither of these mechanisms can be discounted.

Fruit weight, like initial fruit set, also benefitted from pollinator and ant activity and increased by 15% in the presence of pollinators and Azteca ants. Other studies have found that pollinators, mainly bees, contribute between a 7-27% increase in fruit weights (Classen et al., 2014). Pollinators contribute to higher fruit weights by way of cross pollination which can decrease the likelihood of misshapen fruits, or in the case of coffee, peaberry (fruits with only one bean) formation (Boreux et al., 2013; Krishnan et al., 2012; Ricketts et al., 2004). In our study, access to pollinators reduced the probability of peaberries in coffee plants. Fruit weight increases with the number of beans by ~0.6g (Supplementary info, Table 1), suggesting that peaberries reduce weights of the fruit by 0.6g, which is a substantial reduction in weight. Azteca ants likely contributed to the increase in fruit weight by 1) promoting movement of bees between plants and facilitating cross pollination as discussed above and 2) by controlling the CBB (Philpott et al., 2006) (Fig 4.6), which can significantly reduce fruit weights by 7.5% (Supplementary info, Table 1).

Ants controlled the CBB differentially in the two years. In 2018, we found that the interaction between Azteca ants and shade treatment alone was significant; no other effects were significant. The probability of finding CBB in coffee fruits was significantly lower only on plants paired with non-*Inga* spp. compared to the plants with ant exclosures (Fig 4.6A). In 2018, mean ant activity on plants paired with non-*Inga spp.* was higher than those paired with *Inga* spp. (mean ± std.dev, Non- *Inga spp.*17.1± 4.7; *Inga spp.*13.5± 5.8). *Inga* spp. trees usually have their own scale insects (*Octolecanium sp.*) that Azteca ants prefer to tend over the GCS. It is hypothesized that owing to the nitrogen fixation of *Inga* spp., honeydew quality or scale insect density is higher on Inga than the ones on coffee plants (Livingston et al., 2008; Vaidya and Vandermeer, 2021). Therefore, ant activity can be lower on coffee plants closer to *Inga* spp. as seen in our data as well (Figure B1). Additionally, CBB infestation was lower in 2018 than 2019 (Figure B2) therefore it is possible that only on those plants with high ant activity, ants could locate and regulate the CBB in coffee fruits. In 2019, on the other hand, incidence of CBB in coffee fruits was significantly lower on plants with Azteca ants than on those with ant exclosures, irrespective of the shade tree treatment (Figure 4.6B). As stated earlier, CBB infestation was higher in 2019. When the density of CBB is high, the probability that ants will locate the berry borer is higher. Thus, even with lower ant activity in 2019 (ant activity was significantly lower only on plants paired with non- *Inga* spp.), ants were probably able to find and control the CBB more easily because they were present in higher densities.

We found no effects of proximity to *Inga* spp. on coffee floral traits and C:N or %N in coffee plants (Figure B3 and Figure B4). This is likely because all shade trees are pruned regularly and the leaf litter from both the N-fixing and non N- fixing shade trees gets mixed together on the ground. Additionally, coffee plants are also fertilized at the end of the flowering period using organic compost made on the farm. Thus, the mixing of the leaf litter of both N-fixing and non N-fixing shade trees along with fertilization using compost possibly masked the effects of enhanced N or other nutrients on coffee bushes paired with *Inga* spp. shade trees. While the direct effects of N-fixing shade trees were not apparent on plant traits or on resource availability, there was an indirect effect on ant activity which resulted in a difference in fruit weights and on the control of a major pest of *Coffea arabica*.

There was significant interannual variation in fruit quantity and the control of the coffee berry borer. Coffee plants had a significantly higher number of flowers in the second year

(Figure B5), as well as higher number of visits by pollinators which probably resulted in a higher fruit set in the second year. Flowering in coffee plants is closely related to rainfall conditions preceded by a period of dry conditions (Peters and Carroll, 2012). Although we did not record abiotic conditions, it is possible that this change in flowering was probably due to interannual changes in rainfall in Chiapas, Mexico where the study took place. Finca Irlanda has also steadily increased the number of honeybee hives on the farm. Along with the simple increase in their abundance, mass flowering crops such as *Coffea arabica,* are more attractive to honeybees over other flowering resources (Bänsch et al., 2020) and together, this may have increased the number of visits in the second year compared to the first. The density of CBB increased in the second year while ant activity decreased, and these changes may be due to the natural oscillations that take place in predator-prey systems. Our study therefore emphasizes the need to study interactions among ES over a longer temporal period since interannual variation in the population sizes of organisms and interspecific interactions can alter the outcomes of ES interactions.

Our study highlights that ecosystem services are a result of a set of complex interactions and that management factors can have significant effects on both the provisioning of ecosystem services as well as on the interactions among them. It is therefore important to understand the conditions under which there might be synergies or trade-offs among ecosystem services if we want to manage agroecosystems both effectively and sustainably. Our study also highlights that shade trees are essential to the existence of *Azteca sericeasur,* and the service of pest control. While the majority of the visits to coffee flowers were made by africanized honeybees, the most abundant bees in the region are all cavity nesting bees and shade trees are therefore essential for the service of pollination.

4.7 References

Albrecht M, Sutter L. Synergistic interactions of pollination and pest control services Crop yield Do multiple ecosystem services interact ? How do these interactions matter ? 2016:1–11.

Anjos D V., Tena A, Viana-Junior AB, Carvalho RL, Torezan-Silingardi H, Del-Claro K, et al. The effects of ants on pest control: a meta-analysis. Proc R Soc B Biol Sci 2022;289. https://doi.org/10.1098/RSPB.2022.1316.

Bänsch S, Tscharntke T, Ratnieks FLW, Härtel S, Westphal C. Foraging of honey bees in agricultural landscapes with changing patterns of flower resources. Agric Ecosyst Environ 2020. https://doi.org/10.1016/j.agee.2019.106792.

Barber NA, Adler LS, Theis N, Hazzard R V., Kiers ET. Herbivory reduces plant interactions with above- and belowground antagonists and mutualists. Ecology 2012. https://doi.org/10.1890/11-1691.1.

Bartomeus I, Gagic V, Bommarco R. Pollinators, pests and soil properties interactively shape oilseed rape yield. Basic Appl Ecol 2015. https://doi.org/10.1016/j.baae.2015.07.004.

Bennett EM, Peterson GD, Gordon LJ. Understanding relationships among multiple ecosystem services. Ecol Lett 2009. https://doi.org/10.1111/j.1461-0248.2009.01387.x.

Boreux V, Kushalappa CG, Vaast P, Ghazoul J. Interactive effects among ecosystem services and management practices on crop production: Pollination in coffee agroforestry systems. Proc Natl Acad Sci 2013;110:8387–92. https://doi.org/10.1073/pnas.1210590110.

Brittain C, Williams N, Kremen C, Klein AM. Synergistic effects of non-Apis bees and honey bees for pollination services. Proc R Soc B Biol Sci 2013. https://doi.org/10.1098/rspb.2012.2767.

Burkle LA, Irwin RE. Beyond biomass: Measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction. J Ecol 2010;98:705–17. https://doi.org/10.1111/j.1365-2745.2010.01648.x.

Ceulemans T, Hulsmans E, Vanden Ende W, Honnay O. Nutrient enrichment is associated with altered nectar and pollen chemical composition in Succisa pratensis Moench and increased larval mortality of its pollinator Bombus terrestris L. PLoS One 2017. https://doi.org/10.1371/journal.pone.0175160.

Chain-guadarrama A, Martínez-salinas A, Aristizábal N. Ecosystem services by birds and bees to coffee in a changing climate : A review of coffee berry borer control and pollination. Agric Ecosyst Environ 2019;280:53–67. https://doi.org/10.1016/j.agee.2019.04.011.

Classen A, Peters MK, Ferger SW, Helbig-Bonitz M, Schmack JM, Maassen G, et al. Complementary ecosystem services provided by pest predators and pollinators increase quantity and quality of coffee yields. Proc R Soc B Biol Sci 2014;281. https://doi.org/10.1098/rspb.2013.3148.

Cleland EE, Chiariello NR, Loarie SR, Mooney HA, Field CB. Diverse responses of phenology to global changes in a grassland ecosystem. Proc Natl Acad Sci U S A 2006.

https://doi.org/10.1073/pnas.0600815103.

Delabie JHC. Trophobiosis between formicidae and hemiptera (sternorrhyncha and auchenorrhyncha): An overview. Neotrop Entomol 2001. https://doi.org/10.1590/s1519-566x2001000400001.

FAO. Food and agriculture organisation: Statistical pocketbook 2015. 2015.

Fitch G, Vaidya C. Roads pose a significant barrier to bee movement, mediated by road size, traffic and bee identity. J Appl Ecol 2021;58:1177–86. https://doi.org/10.1111/1365-2664.13884.

Floate KD, Whitham TG. Aphid-ant interaction reduces chrysomelid herbivory in a cottonwood hybrid zone. Oecologia 1994. https://doi.org/10.1007/BF00323152.

Gardener MC, Gillman MP. The effects of soil fertilizer on amino acids in the floral nectar of corncockle, Agrostemma githago (Caryophyllaceae). Oikos 2001. https://doi.org/10.1034/j.1600-0706.2001.920112.x.

Garibaldi LA, Kleijn D, Rollin O, Pérez-Méndez N, Fijen TPM, Requier F, et al. Complementarity and synergisms among ecosystem services supporting crop yield. Glob Food Sec 2018;17:38–47. https://doi.org/10.1016/j.gfs.2018.03.006.

Ghazoul J. Can floral repellents pre-empt potential ant-plant conflicts? Ecol Lett 2001. https://doi.org/10.1046/j.1461-0248.2001.00229.x.

Gómez JM. Effectiveness of ants as pollinators of Lobularia maritima: Effects on main sequential fitness components of the host plant. Oecologia 2000. https://doi.org/10.1007/PL00008840.

Gonthier DJ, Dominguez GM, Witter JD, Spongberg AL, Philpott SM. Bottom-up effects of soil quality on a coffee arthropod interaction web. Ecosphere 2013. https://doi.org/10.1890/ES13-00072.1.

Greenberg R, Bichier P, Angon AC, Reitsma R. Bird populations in shade and sun coffee plantations in central Guatemala. Conserv Biol 1997a. https://doi.org/10.1046/j.1523-1739.1997.95464.x.

Greenberg R, Bichier P, Sterling J. Bird populations in rustic and planted shade coffee plantations of eastern Chiapas, México. Biotropica 1997b. https://doi.org/10.1111/j.1744-7429.1997.tb00044.x.

Greenleaf SS, Kremen C. Wild bees enhance honey bees' pollination of hybrid sunflower. Proc Natl Acad Sci U S A 2006. https://doi.org/10.1073/pnas.0600929103.

Grossman JM, Sheaffer C, Wyse D, Bucciarelli B, Vance C, Graham PH. An assessment of nodulation and nitrogen fixation in inoculated Inga oerstediana, a nitrogen-fixing tree shading organically grown coffee in Chiapas, Mexico. Soil Biol Biochem 2006. https://doi.org/10.1016/j.soilbio.2005.07.009.

Gutierrez-Arellano C, Mulligan M. A review of regulation ecosystem services and disservices from faunal populations and potential impacts of agriculturalisation on their provision, globally. Nat Conserv 2018. https://doi.org/10.3897/natureconservation.30.26989.

Hoover SER, Ladley JJ, Shchepetkina AA, Tisch M, Gieseg SP, Tylianakis JM. Warming, CO2, and nitrogen deposition interactively affect a plant-pollinator mutualism. Ecol Lett 2012;15:227–

34. https://doi.org/10.1111/j.1461-0248.2011.01729.x.

Jha S, Bacon CM, Philpott SM, MÉndez VE, LÄderach P, Rice RA. Shade coffee: Update on a disappearing refuge for biodiversity. Bioscience 2014. https://doi.org/10.1093/biosci/biu038.

Jha S, Vandermeer JH. Impacts of coffee agroforestry management on tropical bee communities. Biol Conserv 2010;143:1423–31. https://doi.org/10.1016/j.biocon.2010.03.017.

Klein AM, Steffan-Dewenter I, Tscharntke T. Pollination of Coffea canephora in relation to local and regional agroforestry management. J Appl Ecol 2003a;40:837–45. https://doi.org/10.1046/j.1365-2664.2003.00847.x.

Klein AM, Steffan-Dewenter I, Tscharntke T. Bee pollination and fruit set of Coffea arabica and C. canephora (Rubiaceae). Am J Bot 2003b. https://doi.org/10.3732/ajb.90.1.153.

Krishnan S, Kushalappa CG, Shaanker RU, Ghazoul J. Status of pollinators and their efficiency in coffee fruit set in a fragmented landscape mosaic in South India. Basic Appl Ecol 2012;13:277–85. https://doi.org/10.1016/j.baae.2012.03.007.

Lach L. A mutualism with a native membracid facilitates pollinator displacement by Argentine ants. Ecology 2007;88:1994–2004. https://doi.org/10.1890/06-1767.1.

Levan KE, Holway DA. Ant-aphid interactions increase ant floral visitation and reduce plant reproduction via decreased pollinator visitation. Ecology 2015;96:1620–30. https://doi.org/10.1890/14-0058.1.

LeVan KE, Hung KLJ, McCann KR, Ludka JT, Holway DA. Floral visitation by the Argentine ant reduces pollinator visitation and seed set in the coast barrel cactus, Ferocactus viridescens. Oecologia 2014. https://doi.org/10.1007/s00442-013-2739-z.

Livingston GF, White AM, Kratz CJ. Indirect interactions between ant-tended hemipterans, a dominant ant azteca instabilis (Hymenoptera: Formicidae), and shade trees in a tropical agroecosystem. Environ Entomol 2008. https://doi.org/10.1603/0046-225X(2008)37[734:IIBAHA]2.0.CO;2.

Lundin O, Smith HG, Rundlöf M, Bommarco R. When ecosystem services interact: crop pollination benefits. Proc R Soc … 2012:1–6.

Martínez-Salinas A, Chain-Guadarrama A, Aristizábal N, Vilchez-Mendoza S, Cerda R, Ricketts TH. Interacting pest control and pollination services in coffee systems. Proc Natl Acad Sci U S A 2022;119. https://doi.org/10.1073/pnas.2119959119.

Moguel P, Toledo VM. Biodiversity conservation in traditional coffee systems of Mexico. Conserv Biol 1999. https://doi.org/10.1046/j.1523-1739.1999.97153.x.

Morris JR, Vandermeer J, Perfecto I. A keystone ant species provides robust biological control of the coffee berry borer under varying pest densities. PLoS One 2015;10. https://doi.org/10.1371/journal.pone.0142850.

Nygren P, Fernández MP, Harmand JM, Leblanc HA. Symbiotic dinitrogen fixation by trees: An underestimated resource in agroforestry systems? Nutr Cycl Agroecosystems 2012. https://doi.org/10.1007/s10705-012-9542-9.

Ollerton J, Winfree R, Tarrant S. How many flowering plants are pollinated by animals? Oikos 2011;120:321–6. https://doi.org/10.1111/j.1600-0706.2010.18644.x.

Perfecto I, Rice RA, Greenberg R, Van Der Voort ME. Shade coffee: A disappearing refuge for biodiversity: Shade coffee plantations can contain as much biodiversity as forest habitats. Bioscience 1996. https://doi.org/10.2307/1312989.

Perfecto I, Vandermeer J. Biodiversity conservation in tropical agroecosystems: A new conservation paradigm. Ann N Y Acad Sci 2008. https://doi.org/10.1196/annals.1439.011.

Perfecto I, Vandermeer J. The effect of an ant-hemipteran mutualism on the coffee berry borer (Hypothenemus hampei) in southern Mexico. Agric Ecosyst Environ 2006;117:218–21. https://doi.org/10.1016/j.agee.2006.04.007.

Perfecto I, Vandermeer J. Quality of agroecological matrix in a tropical montane landscape: Ants in coffee plantations in Southern Mexico. Conserv Biol 2002. https://doi.org/10.1046/j.1523-1739.2002.99536.x.

Peters VE, Carroll CR. Temporal variation in coffee flowering may influence the effects of bee species richness and abundance on coffee production. Agrofor Syst 2012. https://doi.org/10.1007/s10457-011-9476-2.

Philpott SM, Uno S, Maldonado J. The importance of ants and high-shade management to coffee pollination and fruit weight in Chiapas, Mexico. Biodivers Conserv 2006;15:487–501. https://doi.org/10.1007/s10531-005-0602-1.

Ricketts TH, Daily GC, Ehrlich PR, Michener CD. Economic value of tropical forest to coffee production. Proc Natl Acad Sci U S A 2004. https://doi.org/10.1073/pnas.0405147101.

Roskoski JP. Nitrogen fixation in a Mexican coffee plantation. Plant Soil 1982. https://doi.org/10.1007/BF02182775.

Rostás M, Tautz Jü. Ants as Pollinators of Plants and the Role of Floral Scents, 2010. https://doi.org/10.1007/978-90-481-9316-5_6.

Schmitt L, Perfecto I. Coffee leaf litter decomposition: Short term home-field advantage in shaded coffee agro-ecosystems. Appl Soil Ecol 2021. https://doi.org/10.1016/j.apsoil.2020.103854.

Sidhu SC, Rankin EEW. Behavior Honey Bees Avoiding Ant Harassment at Flowers Using Scent Cues 2016:1–7. https://doi.org/10.1093/ee/nvv230.

Tscharntke T, Clough Y, Bhagwat SA, Buchori D, Faust H, Hertel D, et al. Multifunctional shade-tree management in tropical agroforestry landscapes - A review. J Appl Ecol 2011;48:619–29. https://doi.org/10.1111/j.1365-2664.2010.01939.x.

Vaidya C, Vandermeer J. Ant's choice: The effect of nutrients on a key ant–hemipteran mutualism. Arthropod Plant Interact 2021. https://doi.org/10.1007/s11829-021-09833-5.

Vandermeer J, Perfecto I, Philpott S. Ecological complexity and pest control in organic coffee production: Uncovering an autonomous ecosystem service. Bioscience 2010;60:527–37. https://doi.org/10.1525/bio.2010.60.7.8.

Vannette RL, Bichier P, Philpott SM. The presence of aggressive ants is associated with fewer insect visits to and altered microbe communities in coffee flowers. Basic Appl Ecol 2017;20:62–74. https://doi.org/10.1016/j.baae.2017.02.002.

4.8 Tables and Figures

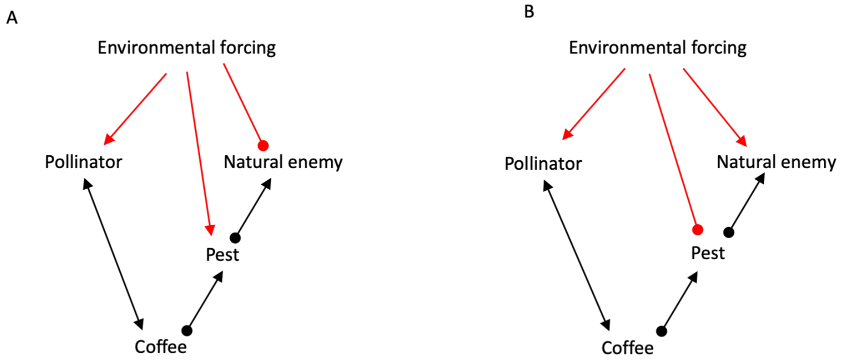

Figure 4.1. Conceptual framework showing two scenarios. Arrows show a positive effect and filled circles show a negative effect. A) depicts a trade-off between pollination and pest control when environmental conditions have a positive effect on pollinators and pests but have a negative effect on the natural enemies of the pest. B) depicts a potential positive or no interaction between pollination and pest control when environmental conditions have a negative effect on the pest and a positive effect on pollinators and the natural enemies of the pest. There can be several such permutations that may give rise to trade-offs or synergistic interactions between pest control and pollination services.

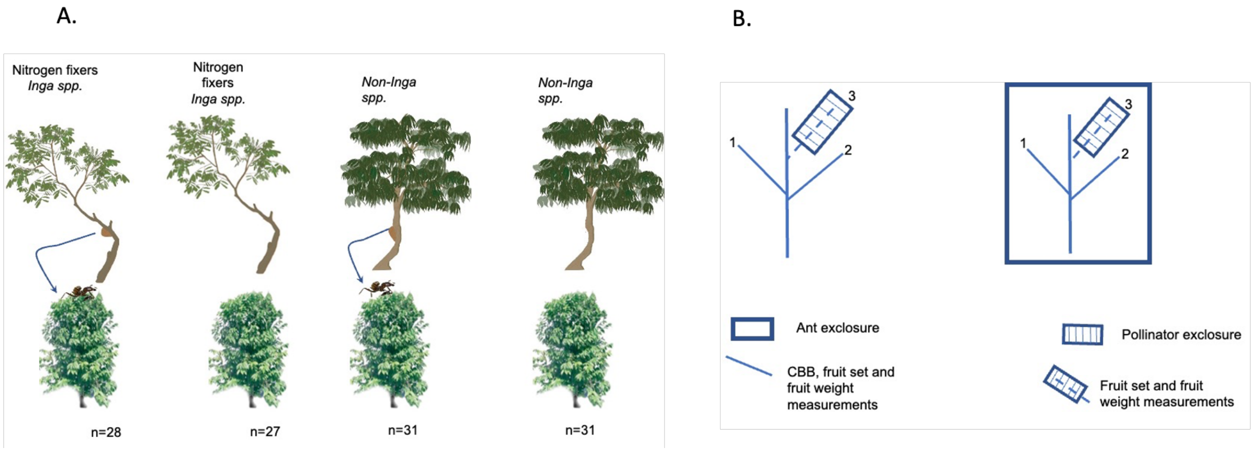

Figure 4.2. Experimental design. A) Full-factorial design of shade tree (N fixation), ant exclosure and bee exclosure treatments. We selected coffee bushes paired with Inga spp. with Azteca seriseasur and without any ants and those paired with non- Inga spp.(non-N fixers) with Azteca seriseasur and without any ants. B) On the plant level, two branches were kept open to pollination and one branch was excluded from ants and both pollinators (bagged). We assesed fruit set and fruit weight on all three branches, while CBB presence/absence in fruits was only assesed on branches that were open to pollinators (1,2).

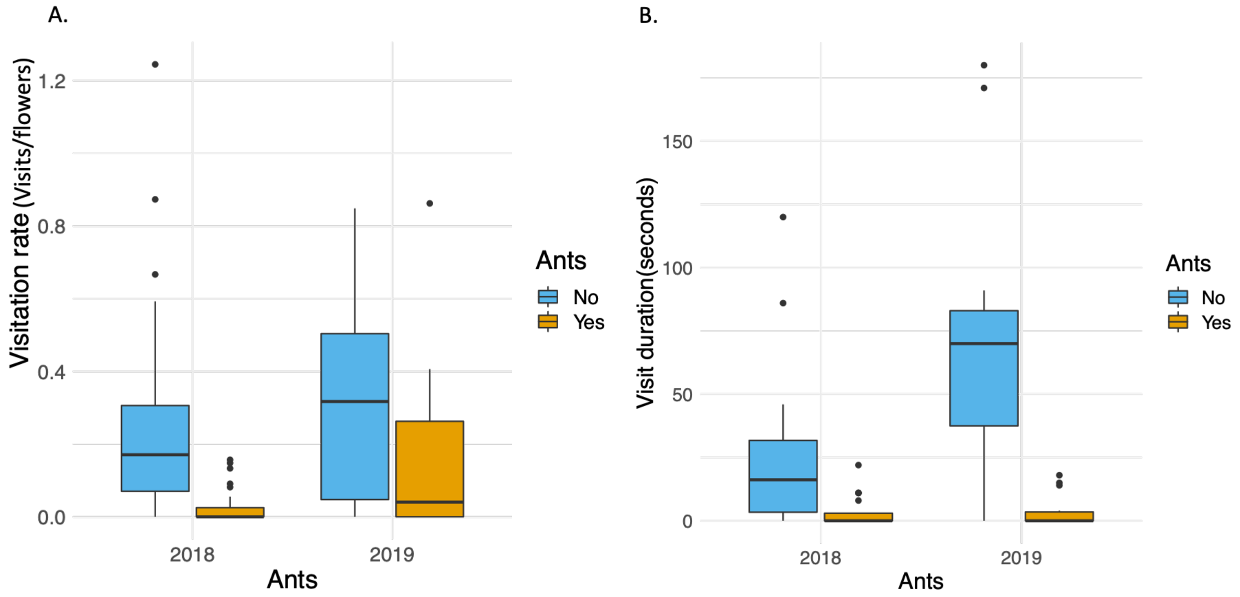

Figure 4.3. Visitation rate (A) and visit duration (B) of pollinators was significantly lower in the presence of Azteca ants. Both visitation rate and visit duration was higher in 2019.

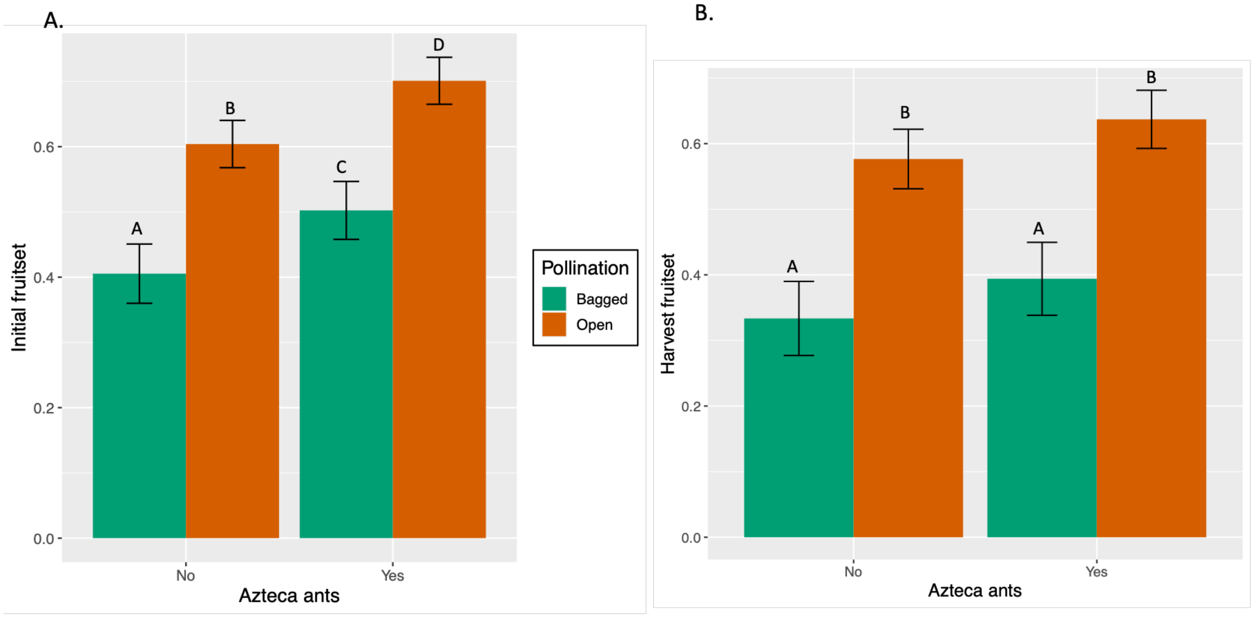

Figure 4.4. Effects of pollination and ant treatments on the intiial fruit set and fruitset at the time of harvest. Estimated marginal means with SE of the A) initial fruitset and B) fruitset at the time of harvest. Green and brown bars denote bagged and open pollination respectively. Different letters (A, B, C and D) denote statistically significant differences.

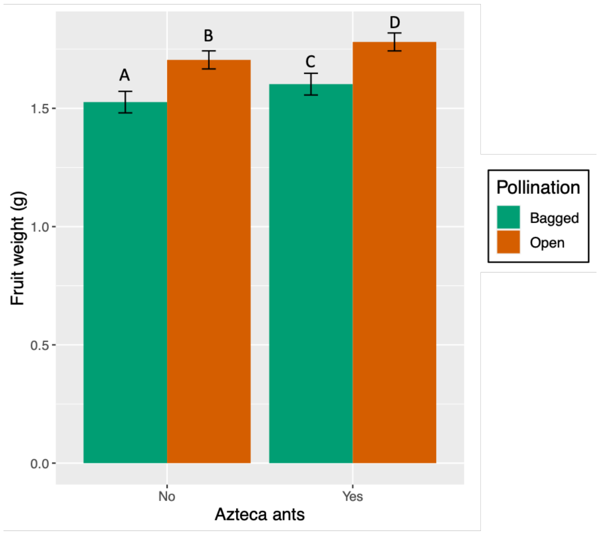

Figure 4.5. Effects of pollination and ant treatments on the weight of the coffee fruits. Estimated marginal means with SE of the bagged and open pollination treatments are denoted by green and brown bars respectively. Different letters denote statistically significant differences.

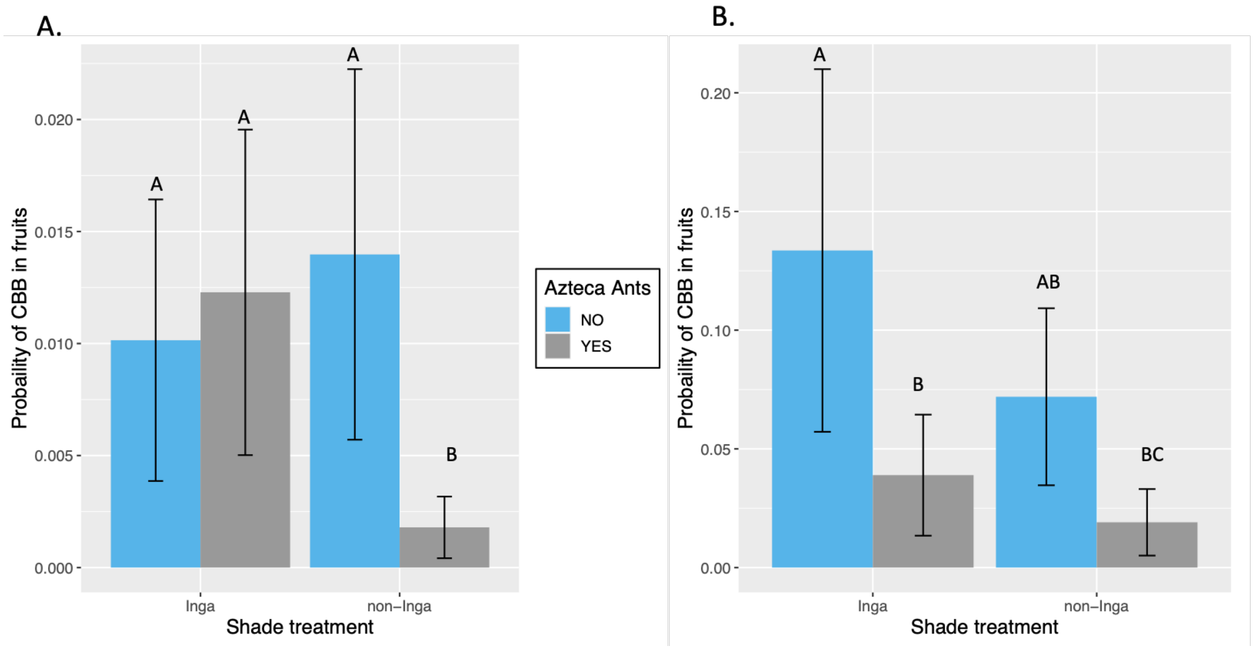

Figure 4.6. Estimated log odds ratio (probability) with SE of finding berries with CBB in them A) with and without Azteca ants on coffee bushes paired with *Inga* spp. and non-*Inga* spp in 2018, B) with and without Azteca ants on coffee bushes paired with Inga *spp*. and non-Inga *spp* in 2019. Different letters denote statistically significant differences.

Chapter 5 : Consequences of Pollinator Shifts on Plant-Pollinator interactions: A Study of the Pollination Ecology of *Eletteria cardamomum* in its Introduced Range in Mexico[4].

5.1 Abstract

Global change factors such as agriculture and climate change have introduced plants and pollinators to new environments. This introduction leads to novel interaction networks for the introduced plant or pollinator species and may have important implications for their reproductive success and survival. Understanding the conditions under which plants and pollinators may adapt to these novel environments to ensure persistence in their new habitats is of increasing importance. We studied the pollination ecology of *Eletteria cardamomum*, a plant native to India and introduced for cultivation in Mexico, and quantified the pollination effectiveness of the most common visitors of *E. cardamomum*. We found that *E. cardamomum* received maximum visitation from bee species and that these visits included both legitimate and nectar robbing visits. Native stingless bee species, the most abundant group of visitors to *E. cardamomum*, displayed both robbing and pollen collecting behavior. The most effective pollinator *of E. cardamomum* in its introduced range was the native stingless nectar robbing bee species, *Scaptotrigona mexicana*, highlighting the need to examine the activity of visitors that on the face of it appear to "rob" plants but may turn out to be the most effective pollinators. Our study is also

---

[4] Co-authors are: Dominguez Martinez GH., and Vandermeer J.

the first step in determining whether *E. cardamomum* will adapt to its most effective pollinator, even though *S. mexicana* is not morphologically adapted to pollinate it.

5.2 Introduction

Angiosperms have a diverse array of flowers varying in size, shape, colour and nectar composition to attract an equally diverse array of pollinators. Pollinators are important agents of selection of floral traits over long time periods. Introduction of plant and pollinator species in novel habitats, both intentionally (through agriculture) and unintentionally (through range shifts) can disrupt established plant-pollinator interaction networks in those areas (González-Varo and Vilà, 2017; Goulson, 2003; Grass et al., 2013) and may have significant implications for the reproductive success of plants. This can eventually lead to selection on mating strategies or floral traits to adapt to the changing pollinator communities.

Several studies have evaluated plants' response to novel pollinators following range shifts. Plants have employed different strategies, ranging from a switch from insect to wind /bird pollination on the Juan Fernandez Islands (Bernardello et al., 2001); reducing floral size to match halictid bees and partially changing mating strategies on Izu Islands as compared to the mainland (Inoue et al. 1986); changes in the length and size of the corolla in *Erythranthae lutea* to adapt to the newly introduced short-tongued pollinator species, *Bombus terrestris* (Medel et al., 2018) and also in the corolla tubes of *Digitalis purpurea* populations introduced in the Neotropics to match the new hummingbird pollinators (Mackin et al., 2021). This ability of plants to modify their floral traits can aid in rapid adaptation to new environments and assure reproductive success.

In this study, we explored the consequences of pollinator shifts, particularly via novel pollinators on *Elettaria cardamomum*. *Elettaria cardamomum* (hereafter cardamom), commonly referred to as green cardamom, is a perennial species of the Zingiberaceae family and is the second most expensive spice in the world, after saffron, making this an economically important crop. It is native to the Western Ghats in southwestern India however, cultivation of cardamom is now rapidly taking place in several countries in Central America with similar weather patterns and growing conditions, including in Mexico, resulting in changes in the pollinator communities from its native range. Cardamom was introduced in Guatemala in the 1930s and Guatemala is now the leading producer and exporter of cardamom (Nair, 2006). Thus, it is important to study the interactions of cardamom with a novel pollinator community in its introduced range. This is especially important given the decline in pollinators in many parts of the world due to several factors such as diseases, pesticides, climate and land-use, with land-use change having the most impact on pollinator declines (Potts et al., 2010).

Cardamom flowers are white and bilaterally symmetrical with a modified stamen (called labellum) that serves as a landing platform for pollinators (Fig 5.1). The labellum has violet markings that act as nectar guides leading to the mouth of the corolla tube. The anthers and stigma are situated on an erect column above the corolla tube. Pollen is produced from the paired anthers at the front of this column and the stigma contains a “pollen cup” partially covered by a lid, which consists of stiff hairs. In order to reach the nectar in the corolla tube, a pollinator is required to squeeze below the anther and the hairs then “comb” the pollen from the head and thorax of the visiting pollinator. While retrieving from the corolla tube, the pollinator’s head and thorax make contact with the anthers and pollen grains are carried with it. The average length of the corolla tube in cardamom flowers is long at 23± 2.08 mm (Belavadi et al., 1997), and long-tongued pollinators have adapted to these plants in its native range (Kuriakose et al., 2009). But short-tongued pollinators are unable to reach the nectaries in the long corolla tubes of these plants. Preliminary observations of cardamom-pollinator interactions in Mexico revealed that several bee species with short tongues rob the nectar from the flowers by biting holes at the base of the corolla, without making contact with the reproductive organs. Nectar robbing is usually viewed as detrimental to the plants (Maloof and Inouye, 2000), but often times nectar robbers also end up pollinating the plants (Navarro, 2000) or have no effect on the plant's reproductive success (Burkle et al., 2007). Many of the short-tongued bee species that rob the nectar from cardamom flowers also collect pollen from the anthers. Stingless bee species, in particular, are among the most frequent visitors of cardamom, begging the question of whether they are simply pollen thieves or effective pollinators of cardamom.

It has been suggested that specializations (trait matching) should take place only when pollinators are abundant and when the abundant pollinators are also the most effective for plant fitness (Stebbins, 1970). Further, in order for specialization to be promoted the pollinator populations should have very little temporal variation in their visitation rates (Waser et al., 1996) and there needs to be an adaptive trade-off, whereby the less effective pollinators are sacrificed in order to adapt to the most effective pollinators (Aigner, 2001). Thus, it is important to understand the effect of specific pollinator types (robbers and legitimate visitors) on plant fitness in cardamom to be able to predict its response to changes in pollinator availability.

Most research on the pollination ecology of cardamom has taken place in India, except for one study in Papua New Guinea (Willmer and Stone, 1989). With the increasing cultivation of cardamom in Mexico and parts of Central America, it is important to study the pollination ecology of cardamom in its introduced range, since effective pollination is required for viable fruit production in cardamom. The intention of this study was therefore to study the pollination ecology of cardamom in its introduced range in Mexico and to address 1) who are the pollinators of cardamom? 2) are the most frequent visitors also the most effective pollinators? 3) do nectar robbing stingless bee species also pollinate cardamom? and finally 4) if there is any temporal variation in the pollinators of cardamom?

We assessed the pollinator community of cardamom in a coffee plantation in southern Mexico by identifying pollinators and quantifying their visitation rates and pollen deposition effectiveness and their contribution to plant fitness. We expected that despite robbing the nectar from cardamom flowers, short-tongued stingless bee species would be as effective as the legitimate, long-tongued pollinators visiting cardamom plants due to their high visitation frequency and pollen collecting behavior.

5.3 Methods

5.3.1 Study System

Cardamom is a perennial plant that grows up to three meters tall with the flowers located on the panicle, a low-lying branch on the base of the plant. These panicles can house up to 45 flowers which can all flower at different times, with peak flowering taking place between May-September. Each flower only opens for a 24-hour period. Fruits usually take 40-45 days to mature. In its native range, cardamom is cultivated under the canopy of shade trees that provide mulch and organic matter and nesting sites for arboreal nesting pollinator species. It is usually cultivated as a mixed crop, often intercropped with coffee plants.

We conducted this study in Finca Irlanda (15.17358, −92.33827), located in Chiapas in the Socunusco region of southern Mexcio. Finca Irlanda is a 300-ha farm, a coffee plantation where coffee is grown under the canopy of diverse shade trees, and it includes several forest fragments. Cardamom occurs both within the farm, intercropped with coffee and in the forest fragments. Finca Irlanda first started growing cardamom in the forest fragments and then expanded cultivation within the coffee plantation. Therefore, cardamom that is growing in the forest fragments is now no longer managed.

5.3.2 Field data collection

We chose a total of 35 plants with flowers ranging from 5-37 flowers and observed them for a period of 10 minutes in June-July in 2017, in June 2019, and in May 2022. As cardamom cultivation is expanding every year in Finca Irlanda, plants were added each year to ensure representation of new sites in the observations. Although, each plant was not observed every year, we had a total of 900 minutes of plant observations pooled across the three years. Each time before the observations took place, we recorded the number of flowers that were easily visible after standing one meter away from the plant. During the 10 minute observation period, we recorded each species of visitor and counted the number of visits by each species to the observed flowers. We also recorded the behavior of the visitor on the flower into three categories – whether the visit was to collect nectar legitimately, to collect pollen or it was a robbing visit. Thus, we were able to identify visitors that were legitimate visitors and those that were nectar robbers. All flower visitors were identified to species whenever possible.

To measure single-visit pollen deposition, we bagged flowers in the bud stage one day prior to opening. Once the flowers opened, we removed the bags and observed the flower until the first visitor arrived and made contact with the reproductive organs. We recorded the visit as either a visit for nectar or for pollen collection. The visiting species was then recorded, and the flower was immediately collected and placed into 70% alcohol solution and brought back to the lab for further analysis. A total of 97 stigma samples were collected in June of 2019. In May of 2022, we followed the same protocol for single-visits, then rebagged the flowers after the single visit and recorded fruit set in June 2022 on 82 flowers.

We did not manipulate the experimental protocol to ensure equal representation of taxa for either pollen deposition or fruit set following single visits. We could not control which visitors visited flowers and therefore sampling of all taxa with equal frequency was not possible.

5.3.3 Lab data collection

In the lab, we transferred the collected stigmas in NaOH solution and softened them for 4 to 6 hours. After softening, stigmas were dipped in water and dried and subsequently mounted on a microscopic slide with decolorized aniline blue dye (Kearns and Inoye 1993). We squashed the stigmas with a coverslip and took pictures of the cup of the stigma using the Nikon Eclipse E800 UV microscope. From these images, was counted the total number of conspecific pollen grains in the stigma cup using the program "Dot Dot Goose".

Pollinator effectiveness

We recorded the total number of conspecific pollen grains after scoring single-visit stigmas for each species and averaged them to calculate the mean pollen deposition for each species for which we collected single visit data on. We used two measures of pollinator effectiveness –single visit pollen deposition and contribution to fruit set after a single visit. We calculated mean visitation rates of each visitor by dividing the number of visits (which involved contact with the reproductive organs only, i.e., nectar and pollen visits) by the total number of flowers observed, first at the plant level and then averaged across plants, pooled across the three years.

To calculate pollinator effectiveness for each of the species from which we recorded single-visit effectiveness (i.e., single-visit pollen deposition), we multiplied mean pollen deposition for each species by the mean visitation rate of that species. We then divided it by 10 minutes to get the measurement of pollinator effectiveness per unit time for each species. We also calculated pollinator effectiveness by multiplying mean visitation rates of each species by the single-visit fruit set for those species.

5.3.4 Data Analysis

To test whether there was any difference in visitation rates between the three years, we used a linear regression model (function "lm" from the package nlme) and conducted post hoc pairwise comparisons using the "emmeans" package. We modelled single visit pollen deposition by using GLMMs with visitor identity as the fixed effect, plant and site as random effects with a Poisson distribution. We also added an observation level random effect to account for overdispersion. We then made pairwise comparisons between all the species using the "emmeans" package. To test if there were differences in the fruit set among the five pollinators, we used a linear regression model (function "lm" from the package nlme) and conducted post hoc pairwise comparisons using the "emmeans" package.

5.4 Results

Pollinator assemblages and visitation rate

We observed a total of 23 species visiting cardamom, out of which 17 were bee species (Table 5.1). Out of the 23 species, the nine most abundant species (the most number of visits across all plants) were *Trigona fuliventris, Scaptotrigona mexicana, Plebia emerina, Eulaema spp.* (counted together), *Melissodes black, Augochlora spp., Tetragonisca angustula, Ceratina spp. and Euglossa I* (counted together). The native stingless bee species, *Trigona fuliventris* (42.9%) and *Scaptotrigona mexicana* (21.25%), made up more than 50% of those visits (Fig 5.2). But after including only those visits that made contact with the reproductive organs of the flowers, the 5 most abundant species were the nectar robbers, *Trigona fuliventris, Scaptotrigona mexicana*, and the legitimate visitors - *Melissodes black* and the orchid bees (*Euglossa* spp. and *Eulaema* spp.). These bees made up more than 80% of the legitimate visits (Fig 5.3) and therefore we have only included them in our analysis. We considered *Euglossa* spp. and *Eulaema* spp. as a group rather than individual species, largely because they would function in similar ways as pollinators due to the similarities in their foraging patterns and natural history, and to increase the sample size of other measures of pollinator effectiveness (single- visit pollen deposition and fruit/seed set).

There were some fluctuations in the mean visitation rates from one year to the next for all of the five most abundant species (Fig 5.4) but this temporal variation was only significant for the genus *Eulaema* ($F_{2,24}$: 4.732, p-value: 0.01852) and not for *T. fuliventris* ($F_{2,80}$=0.9699, p-value=0.3835), *S. mexicana* ($F_{2,58}$: 1.12, p-value: 0.3332), *Melissodes black* ($F_{2,14}$=0.2591, p-value=0.7754) or the genus *Euglossa* ($F_{2,16}$: 0.2056, p-value: 0.8163).

Pollinator effectiveness – pollen deposition and fruit

The number of pollen grains deposited by the most abundant species (except *Melissodes black*) and the resulting pollen deposition effectiveness is reported in Table 5.2. The group of species from the genus *Euglossa* deposited the maximum mean pollen load on cardamom stigmas. However, we found no significant differences between *Euglossa* and the other three most abundant species ($\chi2$ =5.25, d.f. = 3, p =0.15; Fig 5.5). *Melissodes black* did not visit virgin flowers in 2019 so we do not have pollen deposition data for it.

The most effective pollinator species was the stingless bee species *Scaptotrigona mexicana* followed by the orchid bee group from the genus *Eulaema*. Early fruit set after single visits was the highest in the *Euglossa* group of species ($F_{4,77}$= 3.072, p-value= 0.02, Table 5.2), but this was significantly more only than *T. fuliventris* and *Melissodes black*. The most effective pollinator in terms of its contribution to the female reproductive success of cardamom (mean visitation rate x fruitset) was also the stingless bee species *Scaptotrigona mexicana* followed by the orchid bee group from the genus *Eulaema* (Table 5.2).

5.5 Discussion

Our aim in this study was to investigate the pollination ecology of *Eletteria cardamomum* in its introduced range in Mexico. To the best of our knowledge, ours is the first study to examine the relationships between cardamom and its novel pollinators after its introduction in Mexico and other parts of Central America. Our results indicate that cardamom is visited by a wide variety of pollinators and is likely an important food resource for them since cardamom flowers during the portion of the rainy season when other flowering resources are low.

The most frequent visits to cardamom were made largely by bee species, though not all of them were legitimate visits. We considered visits to be legitimate when the visitors made contact with the reproductive parts of the flowers (anther and stigma). Surprisingly, we found that the native stingless bee species, *Scaptotrigona mexicana* and *Trigona fulviventris*, the most frequent visitors to cardamom (Figs 5.2, 5.3), despite robbing the nectar from the flowers, also collected pollen, and thus, had higher visitation rates than the legitimate pollinators.

Both *Scaptotrigona mexicana* and *Trigona fulviventris* have short tongues and have been previously described as nectar robbers of flowers with long corolla tubes (Fitch and Vandermeer, 2020). However, *T. fulviventris* is considered to be the primary robber since it is the one bee species in this group that is able make a slit at the base of the corolla tube using its mandibles; bee species other than *T. fuliventris* that rob nectar from flowers tend to only do secondary robbing (pers. observation and Fitch and Vandermeer, 2020). Though we found instances when both these bee species took nectar “legitimately”, these visits were much lower than when they made visits for pollen collection, thus the mechanism of pollination in cardamom by these two bee species is largely from pollen collection. Both were equally effective at single visit pollen deposition as the legitimate pollinators from the two orchid bee groups, *Euglossa spp.* and *Eualema spp.* Indeed, we found that *S. mexicana* was the most effective pollinator given its high visitation rates and effectiveness via both measures of single visit pollen deposition and single visit fruit set (Table 5.2). We used two different measures of pollinator effectiveness in this study. Pollen deposition is often used as a proxy for fruit set in most studies, but pollen deposition does not take into account the quality of pollen that is deposited. While it is a good first step in understanding the effectiveness of the pollinator. It is important to complement results from pollen deposition effectiveness with single-visit fruit set effectiveness to get a full-rounded understanding of the bees’ contribution to the pollination of cardamom.

Bees from the genus *Eulaema* were closely behind, though *Eulaema spp.* were the only bees that showed among-year variation with significant drop in their visitation rates after the first year of observations (Fig 5.3A). Thus, while they were effective at pollinating cardamom our data suggests that they are less reliable than the stingless bee species. Bees in the genus *Euglossa* had the highest mean pollen loads after single visits and set significantly more number of fruits than *T. fulviventris* and *Melissodes black,* but visited cardamom flowers at a lower rate, making up only 4.5% of total legitimate visits (Table 2). When only pollination effectiveness after single visit pollen deposition was considered, *T. fulviventris* was more effective than *Euglossa* spp. Its contribution to fruit set after a single visit was zero, questioning the quality of the pollen deposited by *T. fulviventris.* Though the behavior of both stingless bee species is similar in terms of frequency of visits and interactions with the flowers, *S. mexicana* spent more time in the flowers per visit than *T. fuliventris* (pers. observation and video data) which likely ensured reliable single-visit pollen deposition (9.9 pollen grains more per visit) and therefore higher pollination effectiveness. We found that the other legitimate pollinator with high visitation rates, *Melissodes black*, was also unsuccessful in setting fruit after single visits. This is likely because similar to some of the bee species in the *Euglossa spp.* group (particularly *E. cingulata* and *E. meriana)*, *Melissodes black* would sometimes approach the corolla tube from the side of the labellum and not make contact with the reproductive organs. Taken together, our results indicate that although cardamom is not morphologically adapted to pollination by short-tongued bees, *Scaptotrigona mexicana* is nonetheless the most effective pollinator of cardamom.

Stingless bee species have been found to be effective pollinators of cardamom in its native range as well. *Trigona iridipennis*, a native stingless bee species in India, was the most effective pollinator of cardamom compared with the native honeybee species *Apis cerana* (Kuriakose et al., 2009; Sinu and Shivanna, 2007). Like *Scaptotrigona mexicana*, *Trigona iridipennis* also has a short tongue and largely forages for pollen on cardamom flowers, although we did not encounter any mention of *T. iridipennis* robbing nectar from the flowers. In India, along with *T. iridipennis*, two native honeybee species, *Apis cerana* and *Apis dorsata* are effective pollinators of cardamom and are managed heavily for this purpose. Finca Irlanda has steadily increased the number of Africanized honeybee apiaries in recent years, but surprisingly there were practically no visits from honeybees to cardamom. We recorded only one visit in 2022 in all three years. While honeybees are the dominant visitors to coffee plants in Finca Irlanda (Chapter 3, Vaidya, Dominguez Martinez and Vandemeer, unpublished), they avoided foraging on cardamom flowers, choosing instead to forage on herbaceous flowering plants growing in the vicinity (pers. Observation).

Our study highlights the importance of pollination studies that examine the activity of visitors that on the face of it appear to only "rob" plants but may turn out to be the most effective pollinators (Navarro 2000). This is especially significant for plants that have been introduced outside their native regions and have no co-evolved pollinators in their new environments. Additionally, it will be important to continue these observations on cardamom in its introduced range to examine if flowers of intermediate corolla tube lengths that utilize both the legitimate pollinators and the stingless bee species will be favored. Future work should measure the lengths of corolla tubes over a period of 2-3 years at the minimum and continue to monitor the visitors of cardamom and determine whether *S. mexicana* continues to be its most frequent visitor. If visitation to cardamom is still dynamic, that is, it changes from year to year because of its recent introduction in Mexico (or on the farm where this study took place), then it might be too early to determine whether this change in the pollinator community of cardamom eventually leads to changes in the floral traits either via phenotypic plasticity or local adaptations to optimize traits for the most effective pollinators, as has been found in other systems (Mackin et al., 2021; Medel et al., 2018). There is also some ambiguity about floral trait evolvabilities, with morphological fit traits having the slowest rate of evolvability than mating-system shifts and floral display size (Opedal, 2019). Still, it is not uncommon and can happen rapidly. For example, substantial evolutionary change was found in the corolla length of *Ipomopsis aggregate* over only five generations of hummingbird mediated selection (Campbell et al., 2018), as well as in *Digitalis purpurea*, where after recent transcontinental range expansion and faced with a novel pollinator community, this specied evolved longer corolla tubes than in its native region, to match the long tongues of its most effective pollinator (Mackin et al., 2021). This evidence points to the possibility of corolla tube evolvability even in *E. cardamomum*, if *S. mexicana* remains the most consistent and effective pollinator of cardamom.

5.6 Acknowledgments

We are grateful to Peter Walters and Finca Irlanda for allowing us to conduct research on the farm. Funding was provided by the Rackham Graduate School and the Department of Ecology and Evolutionary Biology, University of Michigan.

5.7 References

Aigner PA. Optimality modeling and fitness trade-offs: When should plants become pollinator specialists? Oikos 2001. https://doi.org/10.1034/j.1600-0706.2001.950121.x.

Bernardello G, Anderson GJ, Stuessy TF, Crawford DJ. A survey of floral traits, breeding systems, floral visitors, and pollination systems of the angiosperms of the Juan Fernández islands (Chile). Bot Rev 2001. https://doi.org/10.1007/BF02858097.

Burkle LA, Irwin RE, Newman DA. Predicting the effects of nectar robbing on plant reproduction: Implications of pollen limitation and plant mating system. Am J Bot 2007. https://doi.org/10.3732/ajb.94.12.1935.

Fitch G, Vandermeer JH. Light availability influences the intensity of nectar robbery and its effects on reproduction in a tropical shrub via multiple pathways. Am J Bot 2020. https://doi.org/10.1002/ajb2.1559.

González-Varo JP, Vilà M. Spillover of managed honeybees from mass-flowering crops into natural habitats. Biol Conserv 2017;212:376–82. https://doi.org/10.1016/j.biocon.2017.06.018.

Goulson D. Effects of Introduced Bees on Native Ecosystems. Annu Rev Ecol Evol Syst 2003. https://doi.org/10.1146/annurev.ecolsys.34.011802.132355.

Grass I, Berens DG, Peter F, Farwig N. Additive effects of exotic plant abundance and land-use intensity on plant-pollinator interactions. Oecologia 2013. https://doi.org/10.1007/s00442-013-2688-6.

Kuriakose G, Sinu PA, Shivanna KR. Domestication of cardamom (Elettaria cardamomum) in Western Ghats, India: Divergence in productive traits and a shift in major pollinators. Ann Bot 2009;103:727–33. https://doi.org/10.1093/aob/mcn262.

Mackin CR, Peña JF, Blanco MA, Balfour NJ, Castellanos MC. Rapid evolution of a floral trait following acquisition of novel pollinators. J Ecol 2021. https://doi.org/10.1111/1365-2745.13636.

Maloof JE, Inouye DW. Are nectar robbers cheaters or mutualists? Ecology 2000. https://doi.org/10.1890/0012-9658(2000)081[2651:ANRCOM]2.0.CO;2.

Medel R, González-Browne C, Salazar D, Ferrer P, Ehrenfeld M. The most effective pollinator principle applies to new invasive pollinators. Biol Lett 2018;14. https://doi.org/10.1098/rsbl.2018.0132.

Nair KPP. The Agronomy and Economy of Cardamom (Elettaria cardamomum M.): The "Queen of Spices." Adv Agron 2006. https://doi.org/10.1016/S0065-2113(06)91004-9.

Navarro L. Pollination ecology of Anthyllis vulneraria subsp. Vulgaris (Fabaceae): Nectar robbers as pollinators. Am J Bot 2000;87:980–5. https://doi.org/10.2307/2656997.

Opedal ØH. The evolvability of animal-pollinated flowers: towards predicting adaptation to novel pollinator communities. New Phytol 2019. https://doi.org/10.1111/nph.15403.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. Global pollinator declines: Trends, impacts and drivers. Trends Ecol Evol 2010;25:345–53. https://doi.org/10.1016/j.tree.2010.01.007.

Sinu PA, Shivanna KR. Pollination ecology of cardamom (Elettaria cardamomum) in the Western Ghats, India. J Trop Ecol 2007;23:493–6. https://doi.org/10.1017/S0266467407004191.

Stebbins GL. Adaptive Radiation of Reproductive Characteristics in Angiosperms, I: Pollination Mechanisms. Annu Rev Ecol Syst 1970. https://doi.org/10.1146/annurev.es.01.110170.001515.

Waser NM, Chittka L, Price M V., Williams NM, Ollerton J. Generalization in pollination systems, and why it matters. Ecology 1996. https://doi.org/10.2307/2265575.

Willmer PG, Stone GN. Incidence of entomophilous pollination of lowland coffee (Coffea canephora); the role of leaf cutter bees in Papua New Guinea. Entomol Exp Appl 1989;50:113–24. *https://doi.org/10.1111/j.1570-7458.1989.tb02380.x*.

Tables and Figures

Table 5.1. Taxa observed visiting *E. cardamomum* in Finca Irlanda, Chiapas, Mexico and their behavior in the flowers.

Order	Family	Species	Visitation
Hymenoptera	Apidae	*Apis mellifera, scutellata*	Legitimate
		Ceratina sp.	Robber
		Melipona beechii	Legitimate
		Melissodes black	Legitimate
		Euglossa viridissima	Legitimate
		Euglossa sp.	Legitimate
		Eulaema cingulata	Legitimate
		Eulaema meriana	Legitimate
		Eulaema polychroma	Legitimate
		Plebia emerina	Robber
		Scaptotrigona mexicana	Legitimate and Robber
		Tetragonisca angustula	Legitimate and Robber
		Trigona fulviventris	Legitimate and Robber
		Trigona nigerrima	Legitimate and Robber
	Formicidae	*Unidentified ant*	Robber
	Halictidae	*Augochlora sp.*	Legitimate and Robber
		Lasioglossum sp.	Legitimate and Robber
		Agapostemon sp.	Legitimate and Robber
	Unknown	*Unidentified wasp*	Legitimate

Lepidoptera	Unknown	*Butterfly*	Legitimate
	Unknown	*Moth*	Legitimate
Diptera	Unknown	*Syrphid*	Legitimate
Dermaptera	Unknown	*Earwig*	Robber
Apodiformes	Trochilidae	*Amazilia cyanura*	Legitimate

Table 5.2. Visitation rates (mean ± std. dev), pollen deposition per single visit (mean ± std. dev) and pollination effectiveness of each of the five most abundant species visiting E. cardamomum. Dashes in columns indicate lack of stigmas for pollen counting. Numbers in parenthesis show the number of stigmas analyzed per species for single visit pollen deposition and number of flowers followed to fruit formation for single visit fruit set.

Species	**Visitation rate visits per flower**	**Pollen deposition pollen load per single visit**	**Single visit effectiveness per unit time visitation rate x mean pollen load**	**Fruit set**	**Single visit effectiveness towards fruit set visitation rate x fruit set**
S. mexicana	0.497 ± 0.46	48.2 ± 34.02 (39)	2.397	0.29 (35)	0.142
T. fuliventris	0.411 ± 0.48	39.3 ± 31.16 (34)	1.614	0 (22)	0
Euglossa spp.	0.229 ± 0.16	55.9 ± 24.07 (8)	1.278	0.43 (7)	0.098
Eulema spp.	0.412 ± 0.39	46 + 31.93 (7)	1.896	0.33 (6)	0.137
Melissodes black	0.416 ± 0.36	-	-	0 (8)	0

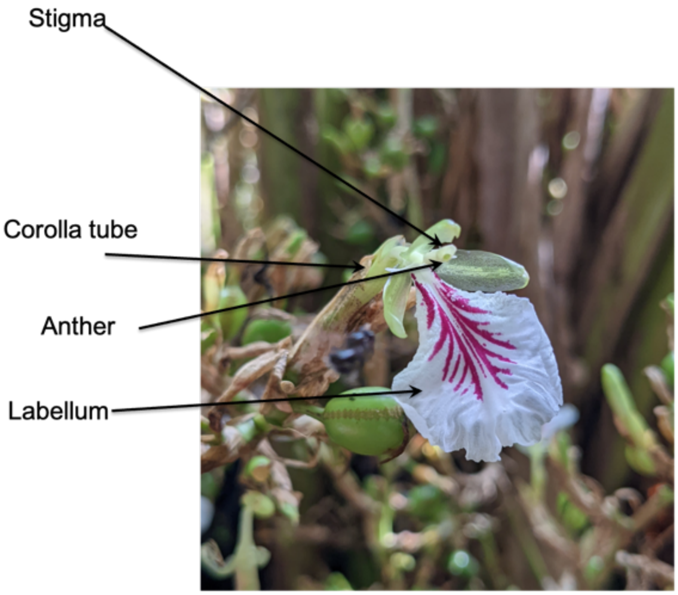

Figure 5.1. Floral morphology of *Eletteria cardamomum.*

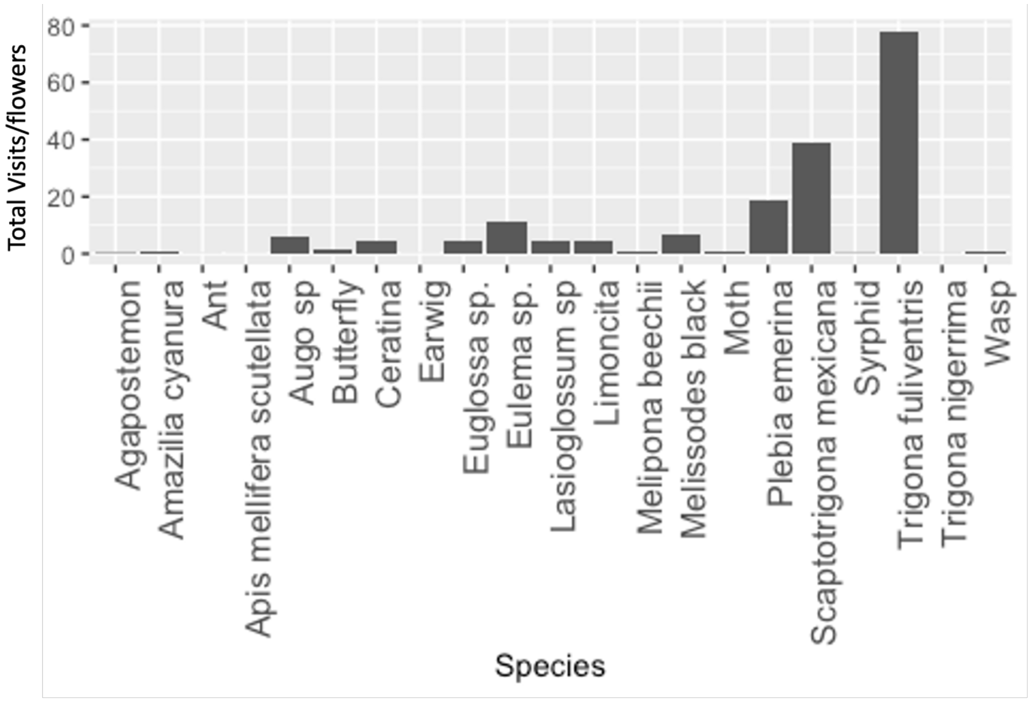

Figure 5.2. Visitors of cardamom and their corresponding visits pooled across plants and 3 years. Nectar robbing stingless bee species *Trigona fuliventris* and *Scaptotrigona Mexicana* were the most frequent visitors of cardamom.

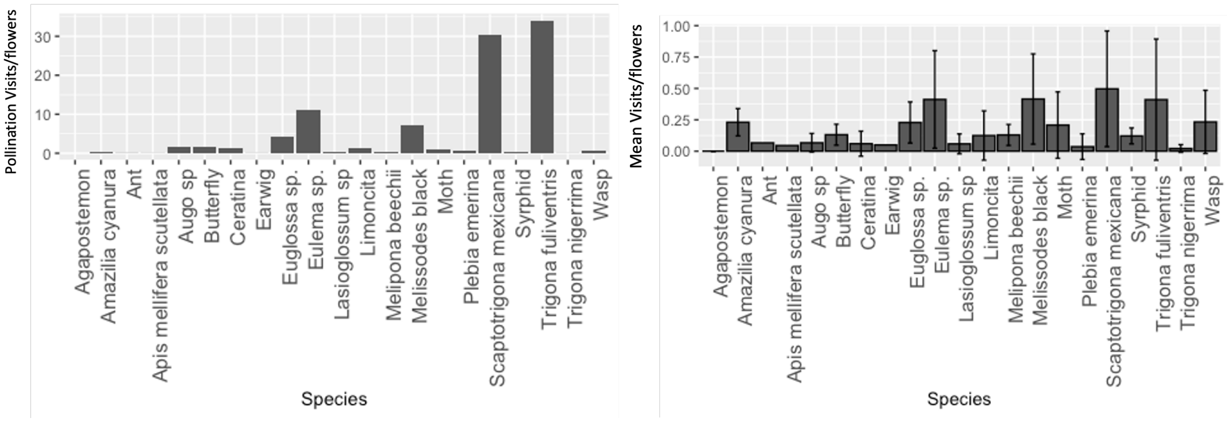

Figure 5.3. A) Visitors of cardamom and their corresponding total legitimate visits (pollination visits) pooled across plants and 3 years. Stingless bee species *Trigona fuliventris* and *Scaptotrigona mexicana* were still the most frequent visitors of cardamom, with a large proportion of those visits consisting of pollen collection visits. B) Mean visitation rates (average visits/flower) of all the visitors of cardamom.

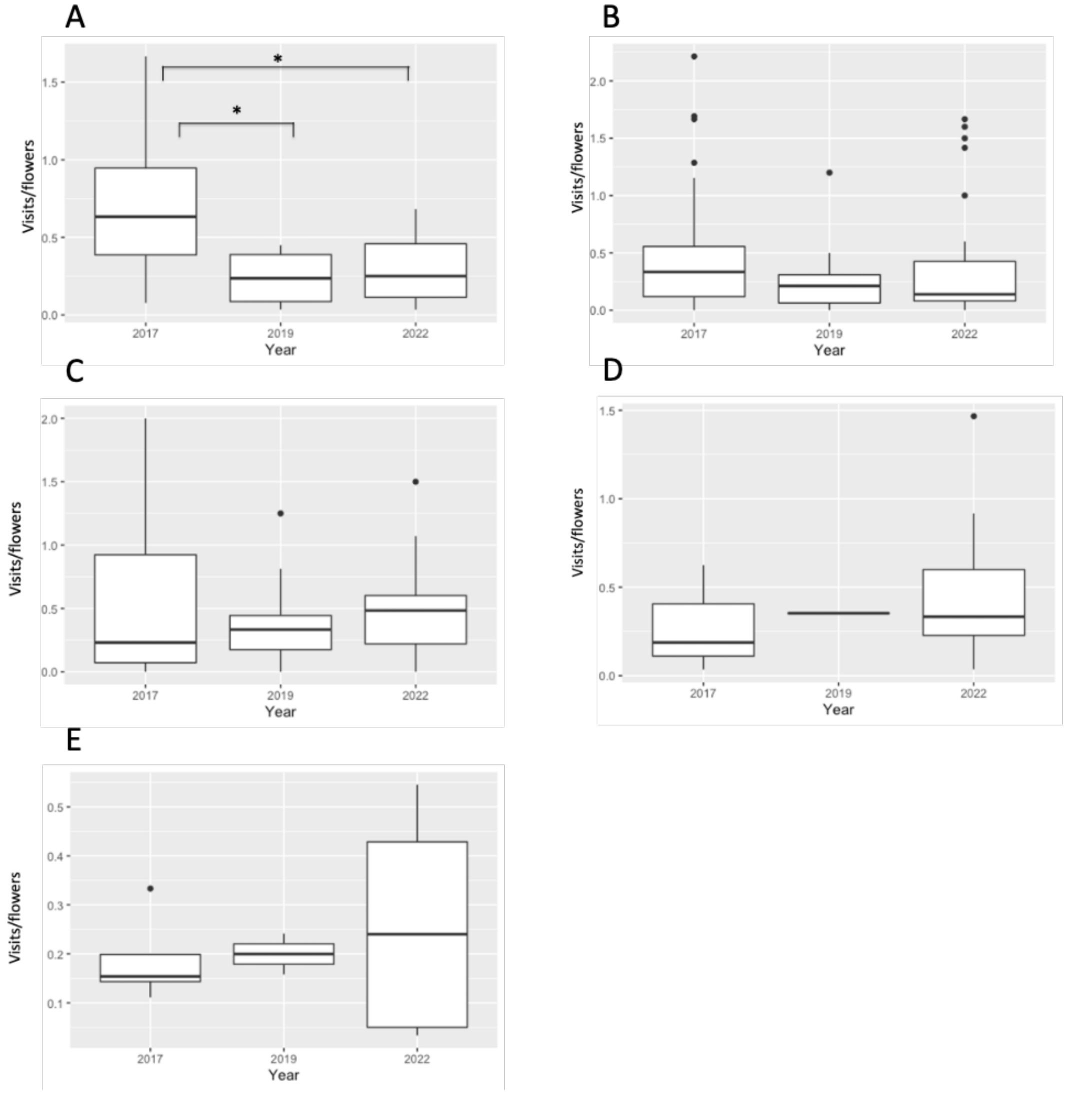

Figure 5.4. Temporal variation in visitation rates of the five most abundant species between the three sampled years. A) *Eulaema.* B) *Trigona fuliventris* C) *Scaptotrigona mexicana* D) *Melissodes black* and E) *Euglossa.* Only the *Eulaema* group showed a decrease in visitation rates from the year 2017 to later years.

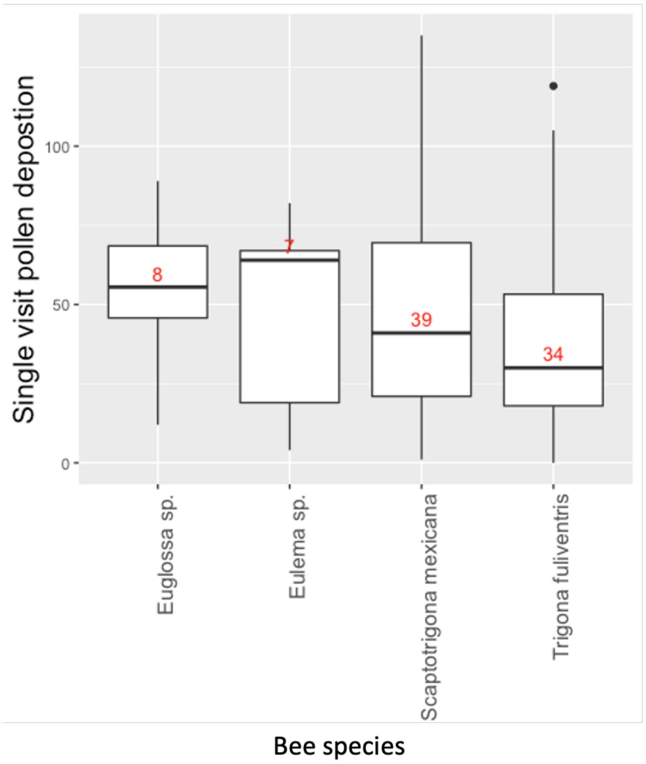

Figure 5.5. Pollen loads deposited by four out of the five most abundant visitors of E. cardamomum. Numbers in red denote sample size for each species.

Chapter 6 Roads Pose a Significant Barrier to Pollinator Movement, Mediated by Road Size, Traffic, and Pollinator Identity[5].

6.1 Abstract

1. Road development is a ubiquitous and rapidly expanding driver of environmental stress, yet we know surprisingly little about how roads impact the movement of insect pollinators, and consequent pollination.

2. We investigated the influence of roads on pollinator movement and pollination by examining patterns of pigment transfer between focal plants of two species, *Coreopsis verticillata* and *Monarda fistulosa*. We tested whether roads reduced pigment transfer, and if this effect was mediated by road characteristics, roadside habitat, or pollinator assemblage.

3. We found that plants across a road from a pigment-added plant received significantly less pigment than plants alongside the road. This effect was mediated by pollinator size, with smaller pollinators being more impacted.

4. Road width was the best predictor of pigment transfer, with a smaller effect of traffic volume; we saw no pigment transfer across large roads. Roadside habitat had little influence on pigment transfer.

---

[5] This paper was published as: Fitch, G*., & Vaidya, C*. (2021). Roads pose a significant barrier to bee movement, mediated by road size, traffic and bee identity. *Journal of Applied Ecology*, *58*(6), 1177-1186.
* *denotes co-first authorship*

Synthesis and applications. Roads pose substantial, species-specific barriers to pollinator movement and pollination, with potentially serious implications for the plants that depend on pollination by small-bodied insects. The effects of roads can be mitigated by constricting road width and installing habitat corridors across roadways or pollinator-attracting plantings in road medians.

6.2 Introduction

Road development is a ubiquitous driver of environmental stress. Currently, the road network extends to about 32 million km across the globe (IRF, 2017), and is projected to increase by 25 million km globally by 2050 (Dulac, 2013). Roads can have profound impacts on populations and ecological communities. While the bulk of studies measuring the effect of roads have focused on vertebrate animals (Bennett, 2017), there is increasing evidence that roads also impact invertebrates – and particularly insects – both via mortality from vehicle collisions (Baxter-Gilbert et al., 2015) and by altering movement patterns (Andersson et al., 2017; Bhattacharya et al., 2003). Yet the small number of studies evaluating the degree to which roads represent barriers to insect movement are largely anecdotal or poorly replicated (Bhattacharya et al., 2003; Franzén et al., 2009; Remon et al., 2018; Zurbuchen et al., 2010), save for a small number of studies on butterflies (Munguira & Thomas, 1992; Ries & Debinski, 2001). We lack a rigorous quantification of the extent to which roads limit insect movement.

Further complicating our understanding of the effects of roads on insects, in landscapes otherwise dominated by agriculture, roadsides are important habitat for insects (Gardiner et al., 2018; Phillips et al., 2020), particularly when they are managed to resemble semi-natural meadow or prairie habitat, and especially for pollinators (Hopwood, 2008; Phillips et al., 2019;

Ries et al., 2001). Thus, fully understanding the effects of roadways requires disentangling the effects of the roads themselves from management of road verges. Moreover, all roads are not created equal. The degree to which a road represents a barrier to the movement of insect pollinators likely depends on the characteristics of the road – particularly its width and the speed and volume of the traffic traveling along it. Yet despite widespread recognition that roads pose a barrier to insect pollinator movement, little research has been done to determine how particular road attributes mediate this effect.

Understanding the effects of roads on pollinating insects is particularly crucial. Since insect pollination is essential to reproduction in many plant species, road impacts on pollinators are likely to have cascading effects on pollination and plant populations. Indeed, studies have documented reduced pollination (Cunningham, 2000; Dargas et al., 2016; Geerts & Pauw, 2011) and seed set (Cunningham, 2000) in both insect- and bird-pollinated plants growing alongside roads (but see (Grobler & Campbell, 2020)). As barriers to pollinator – and therefore pollen – movement, roads may also genetically isolate plant populations (Nobarinezhad et al., 2019). While urbanization - which includes but is not limited to increasing road density - has been shown to result in high rates of local extinction and increased selfing (Cheptou & Avendaño V, 2006; Dornier & Cheptou, 2012), the role of roads *per se* in driving pollen limitation and population fragmentation in plants has been scarcely evaluated.

Here, we begin to fill these research gaps by reporting the results from an investigation into how roads of varying characteristics affect pollen movement in two plant species. We examined patterns of transfer of fluorescent pigment (an analogue for pollen) between focal plants of two native, insect-pollinated species [*Monarda fistulosa* (wild bergamot; Lamiaceae; hereafter “monarda”) and *Coreopsis verticillata* ‘Zagreb’ (threadleaf coreopsis; Asteraceae;

hereafter “coreopsis”)] at 47 road- or pathside sites in and around Ann Arbor, Michigan, USA. Using these experimental plants, we asked 1) if patterns of pigment transfer differ depending on whether a plant is located across the road versus along the road from a pigment-added plant, and 2) if this effect is mediated by pollinator assemblage. Additionally, we asked 3) which road characteristics (lane number, traffic volume, traffic speed, roadside habitat) determine the degree to which roads serve as barriers to pollinator movement. Finally, we sought to determine 4) whether proximity to a road or path influenced the magnitude of pigment transfer, relative to pigment transfer through contiguous habitat not adjacent to a road.

Consistent with the idea that roads serve as barriers to pollinator movement, we hypothesized that plants across a road from the pigment-added plant would receive less pigment than plants alongside the road. We expected the magnitude of the barrier posed by roads to differ based on pollinator body size, which in bees is strongly correlated with flight ability (Greenleaf et al., 2007). Since we expected the pollinator assemblage of coreopsis to be comprised of smaller-bodied insects than that of monarda, we hypothesized that coreopsis would see greater declines in pollen transfer. We further hypothesized that road width, traffic volume and speed would mediate the degree to which roads impede pollinator movement. Finally, we hypothesized that roadsides would support fewer pollinators than contiguous habitats away from a roadway, and those pollinators present would experience greater disruption to foraging, so pigment transfer along roadways would be reduced relative to contiguous sites.

6.3 Methods

6.3.1 Study system

This study was conducted in and around Ann Arbor, Michigan, USA, a small city (population 130,000), from July 6 to August 10 2020. We used two species of flowering plants native to the region: *Monarda fistulosa* and *Coreopsis verticillata* 'Zagreb'. Differences in flower morphology between the species suggest that they are visited by distinct assemblages of pollinators (see insets in Figures 6.1 and 6.2). We purchased plants from Bluestone Perennials, Inc. (Madison, Ohio, USA) and maintained them individually in 1.5-gallon plastic pots until flowering began.

6.3.2 Data collection

We selected 47 sites adjacent to a road or path (hereafter road/path site). We selected road sites to represent the widest possible spectrum of road sizes and traffic volumes and speeds, with additional sites adjacent to paved cycling paths, paved sidewalks, and unpaved walking paths to evaluate the effect of car-free transportation. Sites were separated from one another by at least 500m, with the exception of paved sidewalk sites, which were located within 50m of a road site (but set out on a different day).

A set of three conspecific plants (either alone or together with a set of plants from the other species) were deployed at each road/path site. We collected data from coreopsis at a total of 41 sites, and from monarda at 25 sites. On one plant of the three, we applied luminous pigment (BioQuip, Rancho Dominguez, California, USA) to all floral units (red for coreopsis, blue for monarda). This plant was placed alongside the road/path. We placed a second plant (the 'across' plant) across the road/path from the pigment-added plant, and measured the distance between these two plants. The third plant (the 'along' plant) was placed alongside the road/path on the same side as the pigment-added plant, and at the same distance from the pigment-added plant as the ‘across’ plant (see Supplementary Figure 1 for a schematic diagram illustrating the experimental setup). The distance between experimental plants and the road or path edge varied somewhat depending on site conditions, but was always <3.5m. Plant set-up occurred between 0800-1030h on warm, sunny days during the work week (Monday-Friday) and collection between 1630-1900h on the same day.

Upon collection, we took the ‘across’ and ‘along’ plants from each site into a dark location and used a UV flashlight to detect pigment deposited on flowers. On each plant, we counted the total number of inflorescences (and, for monarda, individual flowers) that held pigment on reproductive structures, as well as the total number of open inflorescences (and flowers for monarda). Because we observed pigment being moved by both wind and non-pollinating insects, we only counted inflorescences where the pigment was placed in a location consistent with movement patterns of pollinators. After all flowers were tallied, we removed inflorescences (for coreopsis) or flowers (for monarda) that held pigment, so the plants could be used for data collection again on subsequent days. We also counted the number of open inflorescences and flowers with pigment on the pigment-added plants; these were not removed, though fresh pigment was added each sampling day. Thus pigment-added plants were always pigment-added, while across and along plants were randomly assigned to location from one day to the next.

At each road/path site, we recorded 1) GPS coordinates for the center of the arranged plants, 2) the distance between plants, 3) the number of striped lanes for vehicular traffic, 4) the posted speed limit (measure of traffic speed), 5) traffic volume and 6) three binary attributes of roadside vegetation that we hypothesized might influence pigment transfer (presence of significant floral resources, presence of semi-natural meadow or prairie habitat, and maintenance via regular mowing). For 'along' plants at road sites, we also recorded whether their orientation relative to the pigment-added plant was with or against the flow of traffic in the adjacent lane. Traffic volume was determined by recording the number of vehicles passing by on the road for 5 minutes, either at the time of set-up or collection which corresponded to high-traffic commuting hours. For car-free roads, traffic surveys separately tallied the number of bicycles and pedestrians passing the plants.

To see whether proximity to a road or path influenced pigment transfer, we selected 6 sites ≥100m from a road with contiguous, unbroken habitat (either mowed lawn [3 sites] or semi-natural meadow [3 sites]). At each site, we set up an identical array of 5 plants, with a pigment-added plant at the center, and 4 plants situated perpendicular to one another at distances of 5m, 15m, 25m and 35m from the pigment-added plant in each cardinal direction (Supplementary Figure 1). This range of distances nearly spans the range of distances at which our road/pathside plants were placed (1.7-41.1m). Due to its shorter flowering period, monarda was included at only 3 of the 6 sites (2 lawns and 1 meadow). Protocols for quantifying pigment transfer were identical to those used at road/path sites.

We also conducted pollinator observations at 46 sites. For pollinator observations, we observed one plant for five minutes, and recorded all floral visitors. We identified visitors to morphospecies on the wing, and recorded the number of visits by each morphospecies. Any contact with the reproductive parts of the floral unit constituted a visit. Visits by the same individual to multiple floral units were recorded separately. For coreopsis, we recorded visits to inflorescences, while for monarda we recorded visits to flowers, including multiple flowers within the same inflorescence. We also recorded the number of open floral units at the time of the observation. Pollinator observations were used to determine the composition of the pollinator assemblage for each species.

6.3.3 Data Analysis

We performed all analyses using R v.4.0.2 (R Core Team, 2020). All analyses were conducted separately for the two plant species.

Do pollinator assemblages between the two plant species differ by body size?

To test whether the pollinator assemblages of the two plant species differed by body size, we assigned each pollinator species that visited the two plant species during the observation period to a size class. We used intertegular distance (ITD), a close correlate of body size and flight ability (Greenleaf et al., 2007; Ricketts et al., 2008), as our measure of body size. Observed pollinator species were assigned to two size classes - “small” (ITD < 2mm) and “large” (ITD > 3mm), based on body size measurements given in (Benjamin et al., 2014; Fitch, Glaum, et al., 2019). Most visits to both species were made by bees (97%), with additional visits from Coleoptera, small Diptera, a wasp species and a large butterfly species. Unlike the observed bee species, we did not have measurements for ITD for these pollinators (nor is this measure necessarily a good proxy for body size in these taxa); based on size comparisons with bees with known ITD, we assigned them to either small or large size classes. In our data set, all bees had ITD either less than 2mm or more than 3mm (Supplementary Table 1). We calculated the proportion of visits to each plant species by the pollinators. For each observation at which a visit occurred we calculated the proportion of visits by small pollinators. We then performed a

Wilcoxon rank sum test to test whether the distribution of proportion of visits differed between the two plant species.

Do roads and paths influence pigment transfer?

We calculated the proportion of inflorescences with pigment by dividing the number of inflorescences with pigment by the number of inflorescences with open flowers. For monarda, we additionally calculate the proportion of flowers that received pigment. Results were qualitatively similar for flowers and inflorescences. Since inflorescence data are more easily compared with data from coreopsis, we only report inflorescence-level data here (see Supplementary Table 2 for flower-level results) To determine whether the proportion of inflorescences with pigment differed between 'across' and 'along' plants, we used a paired t-test, both with data from all sites combined and separately considering road, bike path, and pedestrian path sites. We compared overall levels of pigment transfer between the two species using a t-test.

Which road characteristics influence pigment transfer?

We evaluated the effect of four road characteristics (interplant distance, number of lanes of vehicular traffic, speed limit, and traffic volume) on the proportion of flowers with pigment using binomial GLMs. We counted bike paths as 0.5 lanes, and pedestrian paths as 0 lanes. Because of the high degree of collinearity among the four road characteristics (Supplementary Table 3), including more than one variable in a model resulted in variance inflation factors >4. Therefore, we constructed four separate models, one for each road characteristic. All models had the proportion of flowers with pigment as the dependent variable, and plant position ('along' or 'across') and the relevant road variable as predictors. Analysis of model residuals indicated significant heteroscedasticity in all models for coreopsis. Thus, we ran updated models with observations weighted by the reciprocal of the absolute value of the residuals from the unweighted model. After weighting, all models conformed to assumptions of normality and heteroscedasticity. To evaluate the relative importance of the four road characteristics in determining patterns of pigment transfer, we compared pseudo-R2 and AICC values for the four models. To determine whether the effect of the road characteristics depended on plant position, we ran another set of models that included a plant position × road characteristic interaction term and checked for significance of the interaction term and overall model fit.

Because the distance between plants, while related to road width, was experimentally imposed, we examined the effect of the other three road variables on pigment deposition while controlling for the effect of interplant distance. To do so, we ran linear models with the residuals from the binomial GLM for interplant distance as the response variable, and one of the remaining three road variables (number of lanes, traffic volume, and speed limit) as the predictor. Because lane number and traffic volume had similar relationships with pigment deposition (see Results), we did the same procedure with these two variables (i.e., test the effect of one using the residuals from a model of the other) to identify which was a more important determinant of pigment movement patterns.

To test for the effect of road/path verge vegetation attributes, we updated the best model (as selected by the procedure outlined above) to include the three habitat attributes. We compared this model to the model without habitat attributes using AICc, and conducted stepwise model simplification, removing the habitat attribute with the smallest effect on pigment transfer, until the best model was found.

To test whether traffic-induced airflow impacted pigment transfer, we used only data from 'along' plants at road sites. Using the best model for pigment transfer on this subsetted dataset, we added a binary variable indicating orientation to the pigment-added plant relative to flow of traffic ('with' or 'against' traffic),and checked for significance.

Does pigment transfer differ between road/path sites and contiguous sites?

To test whether being adjacent to a road affected pigment transfer, we used the data from contiguous sites to regress the proportion of flowers with pigment against distance from the pigment-added plant, again using weighted binomial GLMs. We then compared the predicted relationship to that predicted using data drawn from the 'along' plants.

6.4 Results

Do pollinator assemblages between the two plant species differ by body size?

Pollinator visits to both plant species were largely by bees (97% of the visits), with additional visits from small flies, butterflies, beetles and wasps. The ITD of observed bees ranged from 1.2 to 3.3mm (Supplementary Table 1); this represents nearly the full range of body sizes for locally-occurring bees (Fitch, Glaum, et al., 2019).

Small-bodied pollinators tended to visit coreopsis more (82% of total visits) and large-bodied pollinators visited monarda more (93% of the total visits; W=96; p=0.002). Coreopsis had the greatest number of visits from sweat bees (*Augochlorella* spp. followed by *Lasioglossum* spp.) and eusocial bees dominated visitation to monarda (*Bombus* spp. [primarily *B. impatiens*] followed by *Apis mellifera*).

Do roads and paths influence pigment transfer?

Plants located across a road from a pigment-added plant received less pigment than plants located alongside the road (Figure 6.1a-b). The magnitude of this difference was greater for coreopsis than for monarda, with a 50% reduction in the number of inflorescences receiving pigment for coreopsis (paired t-test: $t = -3.89$, d.f. = 26, $p < 0.001$), compared to a 34% reduction for monarda (paired t-test: $t = -2.22$, d.f. = 21, $p = 0.04$). This trend was consistent for nearly all roads, and was not driven by roads with particular characteristics (Figure 6.2). For coreopsis, the effect of bike paths on pigment transfer was similar to the effect of roads, while for monarda plant location relative to the bike path had no effect on pigment transfer (Figure 6.1c-d). Position relative to pedestrian paths, whether unpaved or paved, had no effect on pigment transfer in coreopsis (Figure 6.1e; we did not deploy monarda at any pedestrian path sites). Across all sites, the proportion of inflorescences receiving pigment was significantly lower for monarda (0.25 ± 0.04) than for coreopsis (0.46 ± 0.04; t-test: $t = 3.59$, d.f. = 126.5, $p < 0.001$).

Which road characteristics influence pigment transfer?

For coreopsis, all four measured road characteristics (interplant distance, number of lanes, traffic volume, traffic speed) had a significant negative relationship with the proportion of inflorescences receiving pigment (Figure 6.2a-d; Figure 6.3). However, once the effect of interplant distance was controlled for, traffic speed no longer had a significant effect on pigment transfer (Figure 6.3). Similarly, when the effect of lane number was controlled for, traffic volume no longer affected pigment transfer, while lane number still had a significant negative effect on pigment transfer even after controlling for traffic volume (Figure 6.3). For all models, while plant position had a significant effect, there was no effect of a plant position × road characteristic interaction (Supplementary Table 3). For monarda, only interplant distance had a significant effect on the proportion of inflorescences receiving pigment (Figure 6.2e-h; Figure 6.3) Neither plant position nor the plant position × road characteristic interaction term significantly influenced pigment transfer in any model. Supplementary Table 3). This was true when all sites were considered together and when road sites were considered separately from path sites (Supplementary Table 3).

While the magnitude of the difference in levels of pigment transfer between ‘across’ and ‘along’ plants was greatest for smaller roadways, ‘across’ plants on smaller roads nevertheless received substantial pigment in both species. On larger roads, by contrast, few ‘across’ plants received any pigment: for roads with 3 or more lanes, 71% of ‘across’ coreopsis plants received no pigment, compared to 19% of plants on roads/paths with 2 or fewer lanes; for monarda, the numbers were 75% and 1%, respectively.

None of the vegetation attributes of road/path verges (regular mowing, presence of semi-natural habitat, presence of significant floral resources) affected pigment transfer in coreopsis ($p > 0.2$ in all cases). For monarda, regular mowing had a marginally significant positive effect on pigment transfer ($ß = 3.7\pm2.0$, $z = 1.81$, $p = 0.07$); including this habitat attribute as a predictor of pigment transfer improved model fit over the model including only interplant distance ($\Delta AICc = 5.3$).

For coreopsis, the orientation of the ‘along’ plant relative to the flow of traffic had a significant effect on pigment transfer, with plants oriented with the flow of traffic relative to the pigment-added plant having a higher proportion of inflorescences with pigment (mean±s.e. = 0.52 ± 0.13, $N = 9$) than those oriented against traffic (0.40 ± 0.08, $N = 18$; $z = 2.50$, $p = 0.01$). There was no equivalent effect on pigment transfer for monarda (with traffic: 0.17 ± 0.14, $N = 6$; against traffic: 0.31 ± 0.06, $N = 15$; $z = 0.21$, $p = 0.8$).

Does pigment transfer differ between road/path sites and contiguous sites?

For coreopsis, the proportion of inflorescences with pigment was, unexpectedly, lower on average in contiguous sites than in road or path sites (mean±s.e., contiguous sites: 0.13±0.05; road/path sites: 0.46±0.04; Mann-Whitney U-test: W = 593.5, $p < 0.001$). This was driven primarily by low levels of pigment transfer to nearby plants (5m from pigment-added plant; Supplementary Figure 2). Plants in lawn contiguous sites received substantially less pigment than those in meadow contiguous sites (lawn: 0.04±0.02; meadow: 0.26±0.11), though due to the small sample size this difference was not significant (Mann-Whitney U-test: W = 70, p = 0.2). When the two contiguous site habitats were considered separately, the estimated relationship between distance and pigment transfer for meadow sites was indistinguishable from that for road/path sites, while that for lawn sites had a significantly reduced intercept (Supplementary Figure 2).

For monarda, there was only a slight, non-significant difference in the proportion of inflorescences with pigment between contiguous sites and road sites (contiguous sites: 0.17±0.03; road/path sites: 0.25±0.04; Mann-Whitney U-test: W = 297, p = 0.9; Supplementary Figure 2).

6.5 Discussion

Our study shows that roads and paths pose a significant barrier to pollinator movement, and therefore substantially reduce pollen transfer, but that the magnitude of this effect is species-dependent. Separation by a road reduced pigment transfer (a proxy for pollen transfer) between coreopsis individuals by half and between monarda individuals by one third. Surprisingly, the negative effect on pigment transfer persisted even on narrow and quiet roads and paths – though, again, the effect differed between plant species. For monarda, we detected an effect of roads but not of dedicated cycling and walking paths, whereas for coreopsis, even narrow cycling paths impeded pigment transfer, though pedestrian-only paths and sidewalks did not (Fig 6.3).

Differences between coreopsis and monarda in the effect of roads and paths on pigment transfer were due to differences in pollinator assemblages. Coreopsis was mostly visited by small-bodied pollinators (primarily sweat bees), while monarda was mostly visited by large-bodied pollinators (primarily eusocial bees). In bees at least, foraging distance is correlated with body size (Greenleaf et al., 2007), and small bees are also likely to be less efficient and strong fliers than large bees. Thus, a road that is devoid of floral resources is likely to pose more of a barrier to smaller bees. It may also be that smaller pollinators are more vulnerable to vehicle collisions, since evidence suggests that they cross roads at lower heights (Munguira & Thomas, 1992; Ries et al., 2001; Saarinen et al., 2005). Our results are consistent with at least one study that found that only 10% of marked individuals of a solitary bee, *Andrena hattorfiona*, crossed a road <10m wide, even though it was unpaved (Franzén et al., 2009). Additionally, smaller pollinators may also be more vulnerable to traffic-induced air turbulence. The fact that 'along' coreopsis plants oriented downstream from pigment-added plants (i.e. in the direction of vehicle travel) received more pigment than plants located upstream, suggests that traffic-induced airflow influenced small pollinator movement alongside roads. This suggests that plants primarily pollinated by small insects are more likely to face more genetic isolation due to habitat fragmentation.

We hypothesized that traffic characteristics (traffic volume and, to a lesser extent, speed) would be the road characteristic that best explained pigment transfer patterns – that is, that roads and paths impede pollinator movement primarily by acting as conduits for fast-moving vehicles.

Instead, we found that road width, not traffic volume or speed, was the best predictor of pigment transfer in coreopsis, and that in monarda only interplant distance was a significant predictor. This suggests that the physical presence of the road, rather than the vehicles that travel along it, is the primary impediment to pollinator movement. However, this result should be interpreted with caution. Traffic volume and road width were highly correlated ($R^2 = 0.56$). Moreover, we measured traffic volume over only a 5-minute interval, during peak traffic, which may not accurately capture traffic volume over the course of the day at all sites (e.g., our traffic survey of a site close to a hospital, with a shift schedule that differs from the typical 0900-1700, may have underestimated total traffic volume). Similarly, our measure of traffic speed was the posted legal speed limit; the degree to which traffic obeyed these limits, and thus the accuracy of this measure, varied across sites (authors' personal observation). Thus, while we cannot entirely disentangle the effects of the physical presence of roads from the effects of traffic, our results suggest that both play a role. Further work (e.g. comparing pollinator or pigment movement between high- and low- traffic days) is needed to disentangle the relative importance of physical infrastructure and traffic.

We had expected that higher quality habitats, characterized by the presence of substantial floral resources and areas of semi-natural habitat, would support higher pollinator activity and thus promote pigment transfer. Instead, we found no effect of vegetation attributes on pigment transfer in coreopsis, and a weak but counterintuitively positive effect of mowing on pigment transfer in monarda. It may be that the conflicting effects of high- and low-quality habitats on flower visitation (high floral resource habitats support larger pollinator populations, but may dilute visitation; low floral resource habitats support fewer pollinators, but concentrate visitation to the small number of available flowers) may have effectively canceled one another, leading to no overall effect of habitat on pigment transfer. This study occurred in an urban area, while most other studies of the effects of roadside vegetation on pollinator communities have been conducted in rural landscapes (but see (Baldock et al., 2019)). Perhaps aspects of the urban environment (e.g. ecological filtering (Fitch, Glaum, et al., 2019; Fitch, Wilson, et al., 2019); impediments to dispersal (Jha & Kremen, 2013)) change the relationship between road/path verge management practices and pollinator communities in a way that reduces the effect of local vegetation characteristics.

Finally, we hypothesized that plants alongside a road would experience reduced pigment transfer in comparison to plants in contiguous habitat away from a road. We did not find that to be the case for either species (Supplementary Figure 2). For monarda, there was no difference in pigment transfer between contiguous and road/path sites. Even more surprising, for coreopsis pigment transfer was significantly lower at contiguous sites than at road/path sites. Our contiguous sites comprised two habitats, turfgrass lawn and semi-natural meadow. Our finding of low pigment transfer at contiguous sites was largely driven by lawn sites; levels of pigment transfer at meadow sites were indistinguishable from road/path sites. It is not surprising that isolated plants within lawns – a habitat that typically has few flowers and thus attracts and supports few pollinators – would be visited less than plants in higher-quality meadow habitats. Lawns are also heavily disturbed areas due to the high frequency of mowing as well as the use of chemicals such as herbicides and pesticides. But it is puzzling that road/path sites, many of which also included lawns, did not show parallel habitat-based differences in pigment transfer or overall lower levels of pigment transfer. It may be that road/path verges, even those maintained as lawns, are less intensively managed than equivalent lawns in city parks, and therefore provide better habitat for pollinators than the contiguous site lawns. Indeed, studies have found that frequent mowing reduces abundances of both flowers and pollinators (Phillips et al., 2019, 2020). The idea that road verges provide good habitat for pollinators is supported by several studies showing that the density and species richness of both flowers and pollinators are generally higher in road verges as compared to agricultural (Hanley & Wilkins, 2015; Osgathorpe et al., 2012; Phillips et al., 2019) and forest and woodland habitats (Riva et al., 2018). Moreover, Baldock and colleagues found that the density and species richness of flowers and pollinators in road verges in 3 cities in the UK were similar to several other urban habitats (Baldock et al., 2019). In addition, bees may use road and path edges as navigational aids (Menzel et al., 2019), and, as our research suggests, traffic-induced patterns of airflow may channel pollinator flight along roadsides. Such channeling would help explain increased pigment transfer to 'along' plants over plants in contiguous sites where pollinator flight direction is not constrained. This latter explanation is supported by our finding that pigment transfer to coreopsis in contiguous sites was much lower than expected for plants close to the pigment-added plant, but was equivalent to levels seen at road/path sites at greater distances from the pigment-added plant.

Taken together, the results of this study indicate that although roadsides may attract and support sizable pollinator populations, roads pose substantial barriers to pollinator movement, and consequently pollen vectoring. While our findings suggest that even small roads and bicycle paths are barriers to pollinator movement, we nevertheless observed substantial pigment transfer across these roads, suggesting that this barrier does not preclude dispersal of insect pollinators and insect-vectored pollen. However, pigment transfer across roads was rare in either species for roads with 3 or more lanes of traffic, and for 5-lane roads was observed only once in coreopsis and never in monarda. While we surveyed roads only up to 5 lanes wide, it should be noted that many roads, particularly highways, often exceed this width. Moreover, our estimate of the effect of roads is likely conservative, since the study took place during the COVID-19 pandemic, when traffic was substantially reduced from pre-pandemic levels. Therefore, we suggest that medium-sized and large roads may impede the movement of insect pollinators sufficiently to impact dispersal and population connectivity. We predict that this is particularly likely in plants that rely on insect-vectored cross-pollination for successful reproduction, and in urban areas where habitat fragments are commonly surrounded on all sides by busy roadways. Further research on the genetic structure of populations of pollinating insects (particularly smaller species) and, insect-pollinated plants, with explicit reference to roads as potential barriers, is needed to evaluate this claim.

In light of our findings, we recommend the evaluation and implementation of strategies to make roads less of a barrier to pollinators. Habitat corridors have been effective in reducing the impact of roads for many vertebrate taxa (Gilbert-Norton et al., 2010), and this concept can be adapted for pollinators. For example, including pollinator-attracting flowers in medians and pedestrian crossings whenever possible, but especially on large roads, may aid pollinator movement and reduce the likelihood of population fragmentation. With any such measure that encourages pollinators to cross the road, it will be important to evaluate whether it results in elevated mortality due to increased vehicle collisions, and if so whether this outweighs the benefits of increased population connectivity. These interventions have the potential to dovetail with efforts to promote alternative modes of transportation and reduce traffic accidents via so-called “road diets”, which reduce the area of a roadway dedicated to vehicular traffic (Ewing, 2008; Huang et al., 2002) and may open space for pollinator-attracting plantings. These and related efforts have the potential to reduce the environmental stress roads exert on all of us, human and non-human alike.

6.6 Acknowledgements

We thank the homeowners who allowed us to use their sidewalks for this study. Thanks to M.D. Palmer for assistance with selecting and procuring plants, and to J.H. Vandermeer, K. Sanchez, Z. Hajian-Forooshani, I. Saraeny Rivera Salinas, J.R. Morris and N. Medina for helpful comments on earlier versions of the manuscript.

6.7 References

Andersson, P., Koffman, A., Sjödin, N. E., & Johansson, V. (2017). Roads may act as barriers to flying insects: Species composition of bees and wasps differs on two sides of a large highway. Nature Conservation, 18, 47–59. https://doi.org/10.3897/natureconservation.18.12314

Baldock, K. C. R., Goddard, M. A., Hicks, D. M., Kunin, W. E., Mitschunas, N., Morse, H., Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V., Staniczenko, P. P. A., Stone, G. N., Vaughan, I. P., & Memmott, J. (2019). A systems approach reveals urban pollinator hotspots and conservation opportunities. Nature Ecology & Evolution, 3(3), 363–373. https://doi.org/10.1038/s41559-018-0769-y

Baxter-Gilbert, J. H., Riley, J. L., Neufeld, C. J. H., Litzgus, J. D., & Lesbarrères, D. (2015). Road mortality potentially responsible for billions of pollinating insect deaths annually. Journal of Insect Conservation, 19(5), 1029–1035. https://doi.org/10.1007/s10841-015-9808-z

Benjamin, F. E., Reilly, J. R., & Winfree, R. (2014). Pollinator body size mediates the scale at which land use drives crop pollination services. Journal of Applied Ecology, 51(2), 440–449. https://doi.org/10.1111/1365-2664.12198

Bennett, V. J. (2017). Effects of Road Density and Pattern on the Conservation of Species and Biodiversity. Current Landscape Ecology Reports, 2(1), 1–11. https://doi.org/10.1007/s40823-017-0020-6

Bhattacharya, M., Primack, R. B., & Gerwein, J. (2003). Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? Biological Conservation, 109(1), 37–45. https://doi.org/10.1016/S0006-3207(02)00130-1

Cheptou, P. O., & Avendaño V, L. G. (2006). Pollination processes and the Allee effect in highly fragmented populations: Consequences for the mating system in urban environments. New Phytologist, 172(4), 774–783. https://doi.org/10.1111/j.1469-8137.2006.01880.x

Cunningham, S. A. (2000). Depressed pollination in habitat fragments causes low fruit set. Proceedings of the Royal Society of London. Series B: Biological Sciences, 267(1448), 1149–1152. https://doi.org/10.1098/rspb.2000.1121

Dargas, J. H. F., Chaves, S. R., & Fischer, E. (2016). Pollination of lark daisy on roadsides declines as traffic speed increases along an Amazonian highway. Plant Biology, 18(3), 542–544. https://doi.org/10.1111/plb.12437

Dornier, A., & Cheptou, P.-O. (2012). Determinants of extinction in fragmented plant populations: Crepis sancta (asteraceae) in urban environments. Oecologia, 169(3), 703–712. https://doi.org/10.1007/s00442-011-2229-0

Dulac, J. (2013). Global Land Transport Infrastructure Requirements: Estimating Road and Railway Infrastructure Capacity and Costs to 2050: Information Paper. International Energy Agency;

Ewing, R. (2008). Traffic calming in the United States: Are we following Europe's lead? URBAN DESIGN International, 13(2), 90–104. https://doi.org/10.1057/udi.2008.20

Fitch, G., Glaum, P., Simao, M.-C., Vaidya, C., Matthijs, J., Iuliano, B., & Perfecto, I. (2019). Changes in adult sex ratio in wild bee communities are linked to urbanization. Scientific Reports, 9(1), 1–10. https://doi.org/10.1038/s41598-019-39601-8

Fitch, G., Wilson, C. J., Glaum, P., Vaidya, C., Simao, M.-C., & Jamieson, M. A. (2019). Does urbanization favour exotic bee species? Implications for the conservation of native bees in cities. Biology Letters, 15(12), 20190574. https://doi.org/10.1098/rsbl.2019.0574

Franzén, M., Larsson, M., & Nilsson, S. G. (2009). Small local population sizes and high habitat patch fidelity in a specialised solitary bee. Journal of Insect Conservation, 13(1), 89–95. https://doi.org/10.1007/s10841-007-9123-4

Gardiner, M. M., Riley, C. B., Bommarco, R., & Öckinger, E. (2018). Rights-of-way: A potential conservation resource. Frontiers in Ecology and the Environment, 16(3), 149–158. https://doi.org/10.1002/fee.1778

Geerts, S., & Pauw, A. (2011). Easy technique for assessing pollination rates in the genus Erica reveals road impact on bird pollination in the Cape fynbos, South Africa. Austral Ecology, 36(6), 656–662. https://doi.org/10.1111/j.1442-9993.2010.02201.x

Gilbert-Norton, L., Wilson, R., Stevens, J. R., & Beard, K. H. (2010). A Meta-Analytic Review of Corridor Effectiveness. Conservation Biology, 24(3), 660–668. https://doi.org/10.1111/j.1523-1739.2010.01450.x

Greenleaf, S. S., Williams, N. M., Winfree, R., Kremen, C., Greenleaf, S. S., & Williams, N. M. (2007). Bee foraging ranges and their relationship to body size. Oecologia, 153(3), 589–596. https://doi.org/10.1007/s00442-007

Grobler, B. A., & Campbell, E. E. (2020). Pollinator Activity and the Fecundity of a Rare and Highly Threatened Honeybush Species along a Highway in the Cape Floristic Region. International Journal of Plant Sciences, 181(6), 581–593. https://doi.org/10.1086/708385

Hanley, M. E., & Wilkins, J. P. (2015). On the verge? Preferential use of road-facing hedgerow margins by bumblebees in agro-ecosystems. Journal of Insect Conservation, 19(1), 67–74. https://doi.org/10.1007/s10841-014-9744-3

Hopwood, J. L. (2008). The contribution of roadside grassland restorations to native bee conservation. Biological Conservation, 141(10), 2632–2640. https://doi.org/10.1016/j.biocon.2008.07.026

Huang, H. F., Stewart, J. R., & Zegeer, C. V. (2002). Evaluation of Lane Reduction "Road Diet" Measures on Crashes and Injuries. Transportation Research Record, 1784(1), 80–90. https://doi.org/10.3141/1784-11

IRF. (2017). World Road Statistics 2016. International Road Federation.

Jha, S., & Kremen, C. (2013). Urban land use limits regional bumble bee gene flow. Molecular Ecology, 22(9), 2483–2495. https://doi.org/10.1111/mec.12275

Menzel, R., Tison, L., Fischer-Nakai, J., Cheeseman, J., Balbuena, M. S., Chen, X., Landgraf, T., Petrasch, J., Polster, J., & Greggers, U. (2019). Guidance of Navigating Honeybees by Learned Elongated Ground Structures. Frontiers in Behavioral Neuroscience, 12. https://doi.org/10.3389/fnbeh.2018.00322

Munguira, M. L., & Thomas, J. A. (1992). Use of Road Verges by Butterfly and Burnet Populations, and the Effect of Roads on Adult Dispersal and Mortality. Journal of Applied Ecology, 29(2), 316–329. https://doi.org/10.2307/2404501

Nobarinezhad, M. H., Challagundla, L., & Wallace, L. E. (2019). Small-Scale Population Connectivity and Genetic Structure in Canada Thistle (Cirsium arvense). International Journal of Plant Sciences, 181(4), 473–484. https://doi.org/10.1086/706882

Osgathorpe, L. M., Park, K., & Goulson, D. (2012). The use of off-farm habitats by foraging bumblebees in agricultural landscapes: Implications for conservation management. Apidologie, 43(2), 113–127. https://doi.org/10.1007/s13592-011-0083-z

Phillips, B. B., Gaston, K. J., Bullock, J. M., & Osborne, J. L. (2019). Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting. Journal of Applied Ecology, 56(10), 2316–2327. https://doi.org/10.1111/1365-2664.13470

Phillips, B. B., Wallace, C., Roberts, B. R., Whitehouse, A. T., Gaston, K. J., Bullock, J. M., Dicks, L. V., & Osborne, J. L. (2020). Enhancing road verges to aid pollinator conservation: A review. Biological Conservation, 108687. https://doi.org/10.1016/j.biocon.2020.108687

R Core Team. (2020). R: a language and environment for statistical computing (4.0.2) [Computer software].

Remon, J., Chevallier, E., Prunier, J. G., Baguette, M., & Moulherat, S. (2018). Estimating the permeability of linear infrastructures using recapture data. Landscape Ecology, 33(10), 1697–1710. https://doi.org/10.1007/s10980-018-0694-0

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng', A., & Viana, B. F. (2008). Landscape effects on crop pollination services: Are there general patterns? Ecology Letters, 11(5), 499–515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Ries, L., & Debinski, D. M. (2001). Butterfly responses to habitat edges in the highly fragmented prairies of Central Iowa. Journal of Animal Ecology, 70(5), 840–852. https://doi.org/10.1046/j.0021-8790.2001.00546.x

Ries, L., Debinski, D. M., & Wieland, M. L. (2001). Conservation Value of Roadside Prairie Restoration to Butterfly Communities. Conservation Biology, 15(2), 401–411. https://doi.org/10.1046/j.1523-1739.2001.015002401.x

Riva, F., Acorn, J. H., & Nielsen, S. E. (2018). Localized disturbances from oil sands developments increase butterfly diversity and abundance in Alberta's boreal forests. Biological Conservation, 217, 173–180. https://doi.org/10.1016/j.biocon.2017.10.022

Saarinen, K., Valtonen, A., Jantunen, J., & Saarnio, S. (2005). Butterflies and diurnal moths along road verges: Does road type affect diversity and abundance? Biological Conservation, 123(3), 403–412. https://doi.org/10.1016/j.biocon.2004.12.012

Zurbuchen, A., Bachofen, C., Müller, A., Hein, S., & Dorn, S. (2010). Are landscape structures insurmountable barriers for foraging bees? A mark-recapture study with two solitary pollen specialist species. Apidologie, 41(4), 497–508. https://doi.org/10.1051/apido/2009084

6.8 Tables and Figures

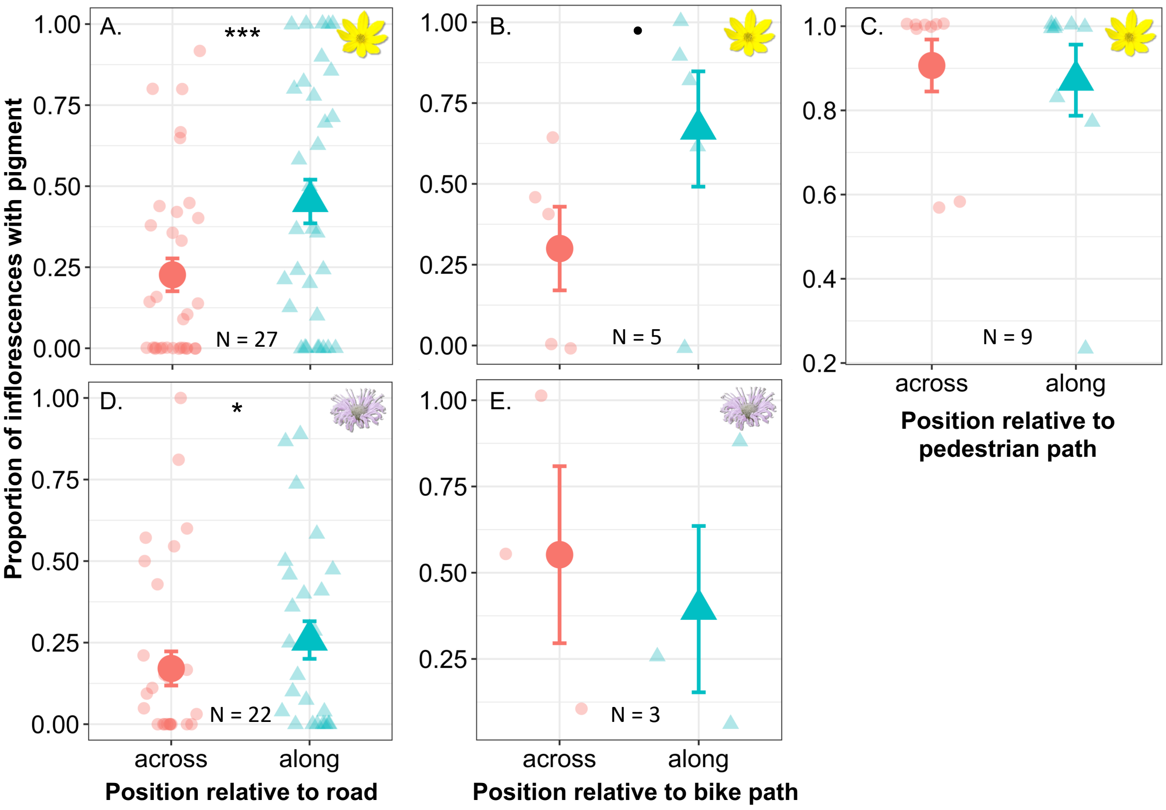

Figure 6.1. Effect of position relative to road or path on pigment deposition in coreopsis (A,C,E) and monarda (B,D). Large points represent means and error bars ± 1 SE; small points represent observations from individual sites, jittered to improve legibility. Significance codes, according to paired t-tests: ***p < 0.001; *p < 0.05; •p = 0.05.

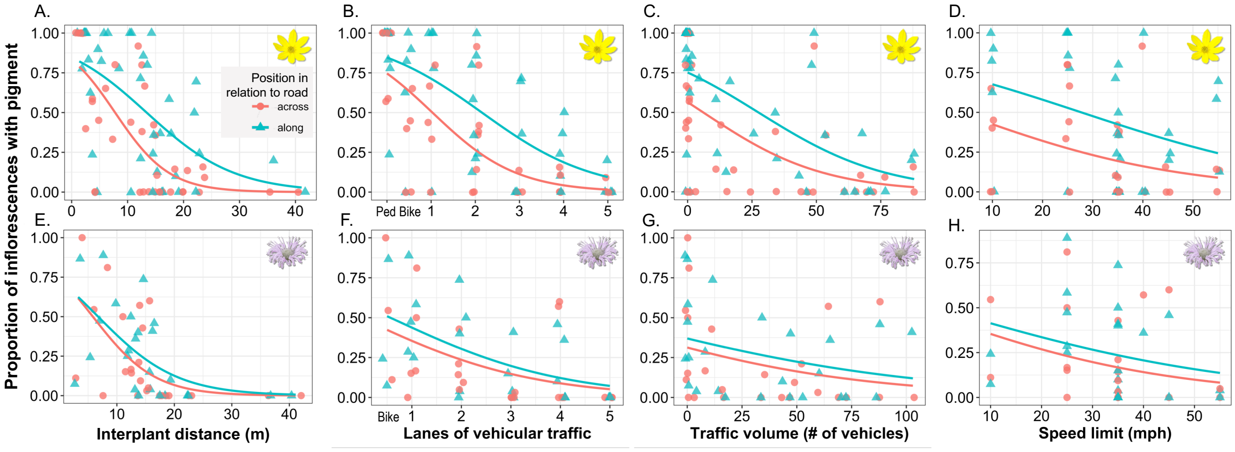

Figure 6.2. Relationship between road characteristics and pigment transfer in coreopsis (A-D) and monarda (E-H). Red circles represent data from plants located across the road/path from the pigment-laden plant, while green triangles represent plants alongside the road/path. Lines show best-fit regression according to binomial GLMs.

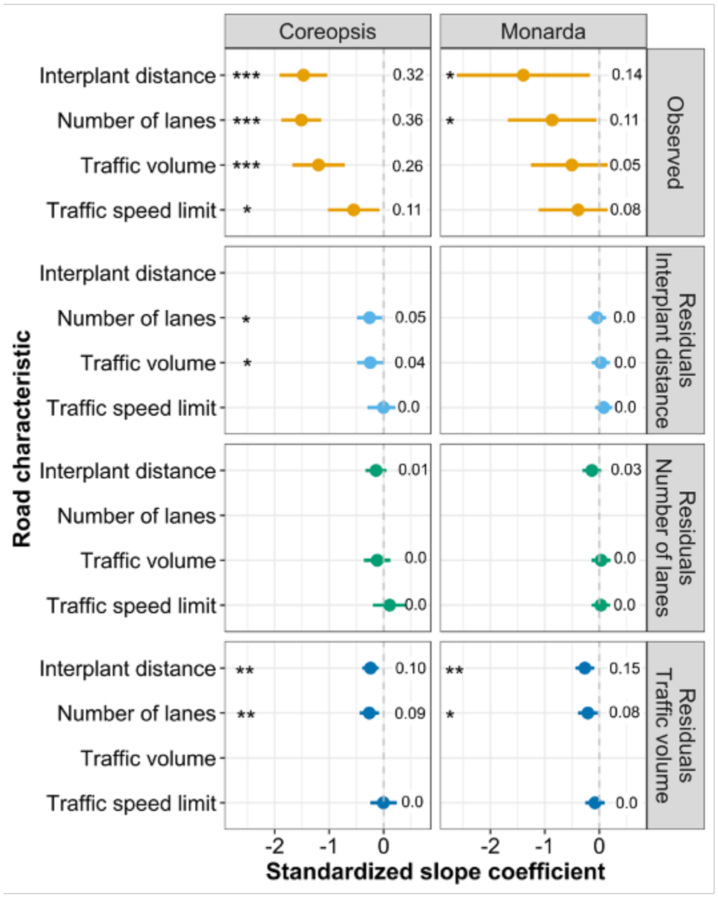

Figure 6.3. Effect of road characteristics on pigment transfer in coreopsis and monarda. Points represent ß estimates and bars represent ± standard error for each predictor, derived from binomial GLMs. Numbers to the right are R2 values for that model. In the top panel, ß and R2 values are for model including plant position; all other panels show values for model using residuals from models in top panel as the response variable. Significance codes: *p<0.05, **p<0.005, ***p<0.001.

Chapter 7 : Conclusion

Global change has had profound effects on the world's biodiversity and has generated novel communities (Hobbs et al., 2006; Ohlemüller et al., 2006; Williams and Jackson, 2007). However, research on the effects of global change on biotic interactions in these novel communities is still in its nascent stages. Biotic interactions are the foundation for the functioning of ecosystems and the provisioning of ecosystem services, and we need a better understanding of how global change may affect these interactions. This work examines the role of land-use change, focusing on agriculture and urbanization, on biotic interactions and the ecosystem services they provide.

In the first part of my dissertation, I focused on agriculture as a global change driver. Chapter 2 tested the effects of increasing agricultural intensification on the colony dynamics of a key pollinator species in the coffee growing region in Mexico. This region is marked by two distinct seasons and the wet season is a particularly stressful period for pollinators, with low flowering resources and a reduced foraging period. The joint influence of these two drivers – seasonality and habitat - revealed the importance of the quality of the habitat in supporting wild pollinators during stressful periods. Stingless bee colony performance (weight, foraging activity) did not differ between low-intensive organic shaded coffee plantation and forest, while colony performance was dramatically worse in the high-intensive, conventional sun coffee plantation. This is the first study to compare colony dynamics of a bee species, especially stingless bee species, between these habitats and to show that organic shaded coffee farms are equivalent to forest fragments in supporting stingless bee colonies, while more intensified coffee production strongly negatively influences colony performance and survival. This reality necessitates taking a landscape-scale approach to thinking about pro-pollinator management interventions (Kennedy et al., 2013), which involves collaboration and partnership among landowners and users with potentially conflicting aims and priorities. Such an approach is beneficial not just for bees and pollination, but also for other organisms and ecosystem services of conservation concern (Rusch et al., 2016; Tscharntke et al., 2021).

Moving from the scale of the landscape to a finer scale, I tested the effects of shade tree management on biotic interactions and the outcomes of these interactions on two ecosystem services – pest control and pollination in an organic shaded coffee plantation. Understanding how multiple ecosystem services interact, especially ecologically, is an understudied area of research, with most research focusing on one ecosystem service only. In chapter 4, I investigated the outcome of the interactions between a dominant aggressive ant species, *Azteca sericeasur,* and the pollinators of *Coffea arabica.* Even though *A. sericeasur* interfered with pollinator visits to coffee flowers, it still had a positive effect on both fruit set and fruit weight and controlled a major coffee pest. These interactions of A. *sericeasur* with pollinators and coffee pests are the result of a mutualism between *A. sericeasur* and a hemipteran scale insect, *Coccus viridis*. The mutualism of *A. sericeasur* and its hemipteran partners are in turn influenced by plant nutrients, with *A. sericeasur* choosing hempiterans foraging on higher quality (high-fertilized coffee plants over medium or low-fertilized plants) resources, which I showed in Chapter 3 through a lab experiment. In the field, the strength of the mutualism between *A. sericeasur* and its hemipteran partners is governed by the shade trees the ants reside in, whether the trees are nitrogen fixing trees or not. On nitrogen fixing shade trees, *A. sericeasur* chooses to partner with another scale insect species rather than *C. viridis*, changing its interaction strength with the coffee pests. I found strong signals of interannual variation in yield parameters and the activity of all the key organisms, stressing the importance of giving explicit consideration to temporal variability. Through this chapter, I showed that the provisioning of ecosystem services are a result of complex interspecific interactions, which are themselves influenced by management practices.

In chapter 5, I studied the pollination ecology of *E. cardamomum,* as a case study to investigate how plants interact with novel pollinator communities. Cardamom has been recently introduced in Mexico and there is no research on its pollination ecology it in Mexico. I found that there were several species visiting cardamom and cardamom is an important food source, especially since it flowers in the rainy season, a time of floral scarcity. Much as in other plants, I found that nectar robbing commonly took place in cardamom due to the tubular morphology of its flowers. While cardamom was visited by both legitimate visitors as well as nectar robbers, nectar robbers made far more visits to cardamom flowers in comparison. Additionally, nectar robbers were also found collecting pollen from the flowers, questioning whether they were really "robbers" of cardamom. The most effective pollinator of *E. cardamomum* in its introduced range was indeed a native stingless nectar robbing bee species, making the most frequent visits to the flowers and reliably depositing pollen grains on the stigma during pollen collection. These studies are important for plants that have been introduced outside their native regions and have no co-evolved relationships with pollinators in their new environments. It is only through studies that we can begin to understand how introduced plants can persist in the face of global environmental challenge.

Finally, chapter 6 revealed the impact of roads on pollinator movement and pollination of plants in Ann Arbor, Michigan. Separation by a road reduced transfer of pigment on plants across the road and the negative effect was species-dependent. Plants that were visited largely by smaller bees were more negatively impacted than those visited by larger bee species such that even narrow and quiet roads saw the same patterns of reduction in pigment transfer. Width of the road was also the best predictor of impediment to bee movement, though road characteristics (traffic volume, speed and width) were all correlated and difficult to distentangle the effect of each from the other. Still, bees dispersed across small roads and paths and carried pollen with them, but as the size of the road increased (beyond three lanes) there was practically no bee movement. These findings suggest that there is a real chance of isolating plant populations and reducing genetic diversity of insect-pollinated plants.

7.1 Implications

Through this work, I have tried to demonstrate the consequences of land-use change on biodiversity and species interactions. As is the case in ecology, generalizing patterns is often difficult due to context dependency. But if there is one pattern that can be generalized, it is that intensification of agriculture is a strong driver of biodiversity loss and that agroecological or agroforestry systems are equivalent to remnant forests in supporting biodiversity and therefore biotic interactions (Kremen and Miles, 2012; Perfecto and Vandermeer, 2010). What is also important to note here is that even though there might be a lot of organisms that exclusively require forested areas and cannot survive in "rustic" farms, these kinds of farms are essential habitats for organisms dispersing from one forest fragment to another (Perfecto et al., 2019).

Even though my research has focused on one global change factor, it is imperative to study the interactive effects of multiple drivers of biodiversity loss. The interaction of climate change with land-use change will lead to the creation of more novel communities through introductions and range shifts and the effects of these drivers acting in combination will be hard to predict. Thus, supporting small and medium scale agroecological farms as important components of climate change adaptation and mitigation is important. This narrative also extends towards managing urban areas to promote conservation of biodiversity in areas that are often overlooked such as vacant lands (Glaum et al., 2017), along rail lines, highway verges, and vegetated storm drain channels (Faeth et al., 2011), encouraging community gardening (Vaidya et al., 2018) and eliminating the use of chemicals. The need to transform the way we use our lands is urgent before we lose more biodiversity and even more species interactions as a result.

7.2 References

Faeth SH, Bang C, Saari S. Urban biodiversity: Patterns and mechanisms. Ann N Y Acad Sci 2011. https://doi.org/10.1111/j.1749-6632.2010.05925.x.

Glaum P, Simao MC, Vaidya C, Fitch G, Iulinao B. Big city Bombus: Using natural history and land-use history to find significant environmental drivers in bumble-bee declines in urban development. R Soc Open Sci 2017. https://doi.org/10.1098/rsos.170156.

Hobbs RJ, Arico S, Aronson J, Baron JS, Bridgewater P, Cramer VA, et al. Novel ecosystems: Theoretical and management aspects of the new ecological world order. Glob Ecol Biogeogr 2006. https://doi.org/10.1111/j.1466-822X.2006.00212.x.

Kennedy CM, Lonsdorf E, Neel MC, Williams NM, Ricketts TH, Winfree R, et al. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecol Lett 2013. https://doi.org/10.1111/ele.12082.

Kremen C, Miles A. Ecosystem Services in Biologically Diversified versus Conventional Farming Systems : Benefits , Externalities , and Trade-Offs 2012;17.

Ohlemüller R, Gritti ES, Sykes MT, Thomas CD. Towards European climate risk surfaces: The extent and distribution of analogous and non-analogous climates 1931-2100. Glob Ecol Biogeogr 2006. https://doi.org/10.1111/j.1466-822X.2006.00245.x.

Perfecto I, Vandermeer J. The agroecological matrix as alternative to the land-sparing/agriculture intensification model. Proc Natl Acad Sci U S A 2010. https://doi.org/10.1073/pnas.0905455107.

Perfecto I, Vandermeer J, Wright A. Nature’s Matrix: Linking Agriculture, Biodiversity

Conservation and Food Sovereignty. 2019. https://doi.org/10.4324/9780429028557.

Rusch A, Chaplin-Kramer R, Gardiner MM, Hawro V, Holland J, Landis D, et al. Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. Agric Ecosyst Environ 2016. https://doi.org/10.1016/j.agee.2016.01.039.

Tscharntke T, Grass I, Wanger TC, Westphal C, Batáry P. Beyond organic farming – harnessing biodiversity-friendly landscapes. Trends Ecol Evol 2021. https://doi.org/10.1016/j.tree.2021.06.010.

Vaidya C, Fisher K, Vandermeer J. Colony development and reproductive success of bumblebees in an urban gradient. Sustain 2018. https://doi.org/10.3390/su10061936.

Williams JW, Jackson ST. Novel climates, no-analog communities, and ecological surprises. Front Ecol Environ 2007. https://doi.org/10.1890/070037.

Appendices

Appendix A. Supplementary figures of Chapter 2.

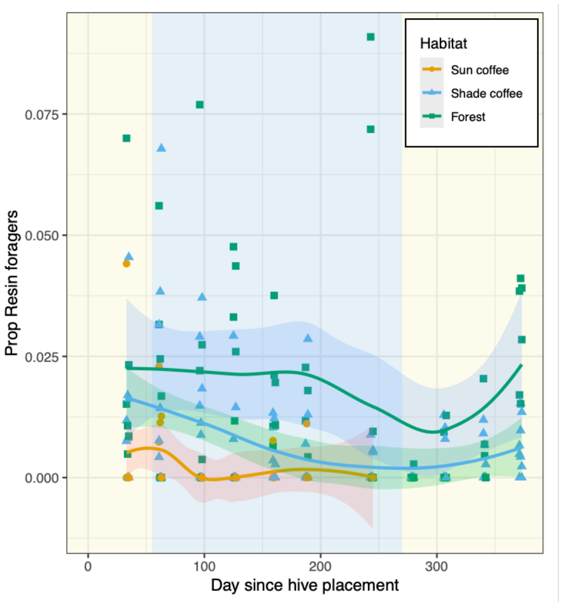

Figure A1. Proportion of total returning foragers carrying resin over the course of the study. Points represent proportion of foragers carrying resin recorded from each observation; solid line represents best-fit line, with shading indicating 95% CI, fit is with LOESS with span = 1;Yellow-shaded region indicates dry season; blue-shaded region indicated wet season.

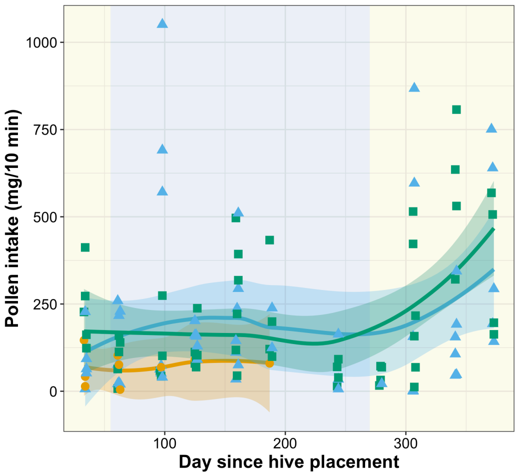

Figure A2. Colony-level pollen intake by habitat over the span of the study. Pollen intake was calculated as the mean per-forager corbicular pollen load multiplied by the number of pollen foragers observed during a single 10min observation. Points represent intake calculated for each observation; lines represent best-fit as calculated by LOESS with span = 1; shading about lines indicates 95% CI. Yellow-shaded region indicates dry season; blue-shaded region indicated wet season.

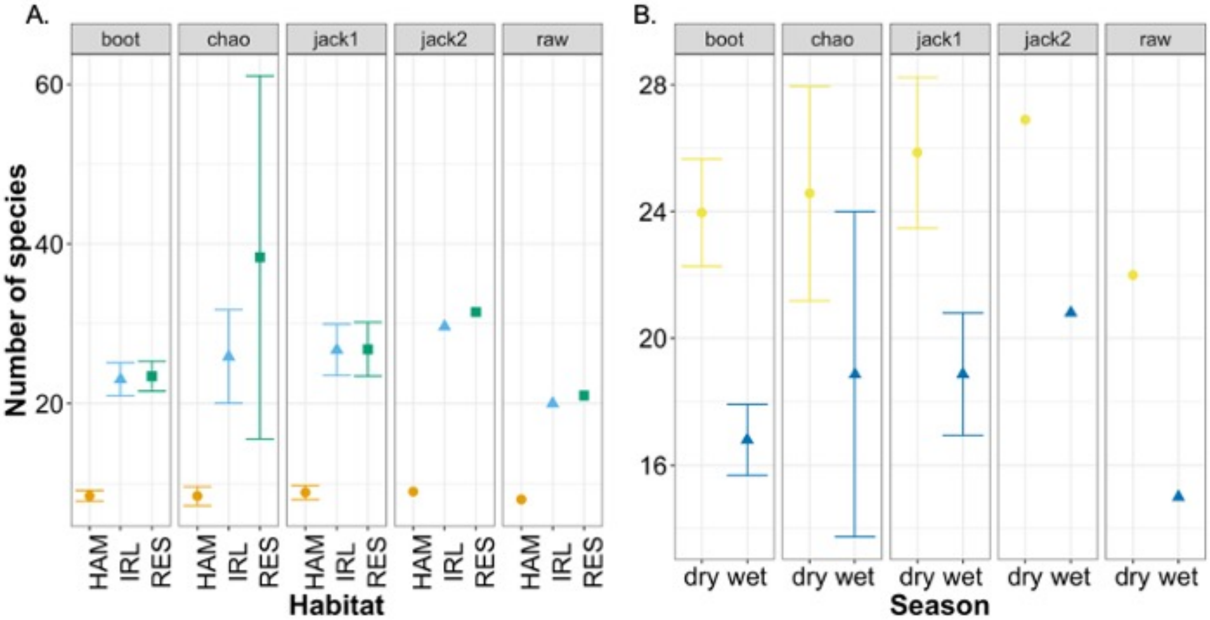

Figure A3. Comparison of pollen morphospecies pool size calculated with four estimators (bootstrap, chao, jackknife 1, jackknife 2, implemented with the ‘specpool()’ function in the `vegan` package) and raw richness for a) habitat and b) season. Note qualitative consistency of results across estimators. In a), “HAM” refers to conventional sun coffee farm, “IRL” refers to shaded organic coffee farm, and “RES” refers to forest fragment.

Appendix B. Supplementary tables and figures of Chapter 4

Table B1. Output of (generalized) mixed effects models with fruit weight as response variable and the number of beans and the presence/absence of CBB as explanatory variables. With each of bean, fruit weight increased by 0.57g and CBB presence decreased fruit weight by 0.11g.

	WEIGHT		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.62	0.54 – 0.69	**<0.001**
BEANS	0.57	0.55 – 0.60	**<0.001**
BROCA	-0.11	-0.15 – -0.07	**<0.001**
Random Effects			
σ^2	0.13		
τ_{00} Branch.id	0.03		
τ_{00} Tree.id	0.03		
τ_{00} Site	0.00		
ICC	0.34		
N Site	11		
N Tree.id	64		
N Branch.id	178		
Observations	8083		

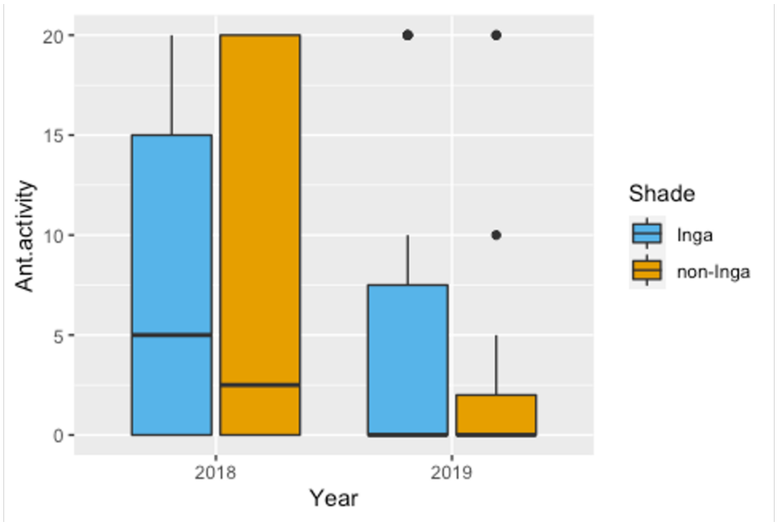

Figure B2. Change in *Azteca sericeasur* activity on shade trees from year 2018 to 2019.

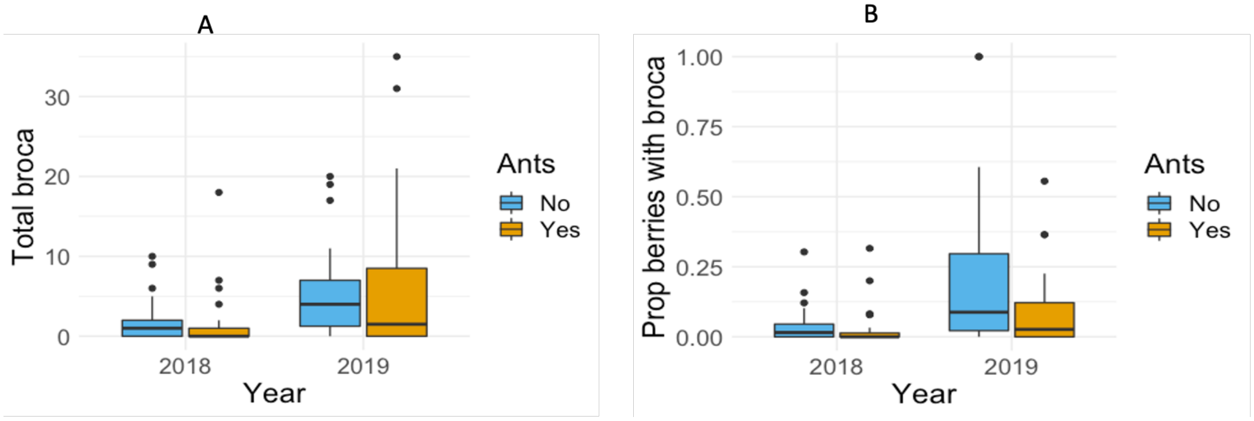

Figure B3. A) Number of berry borers with and without ants in years 2018 and 2019. B) Proportion of berries with berry borer with and without ants in 2018 and 2019.

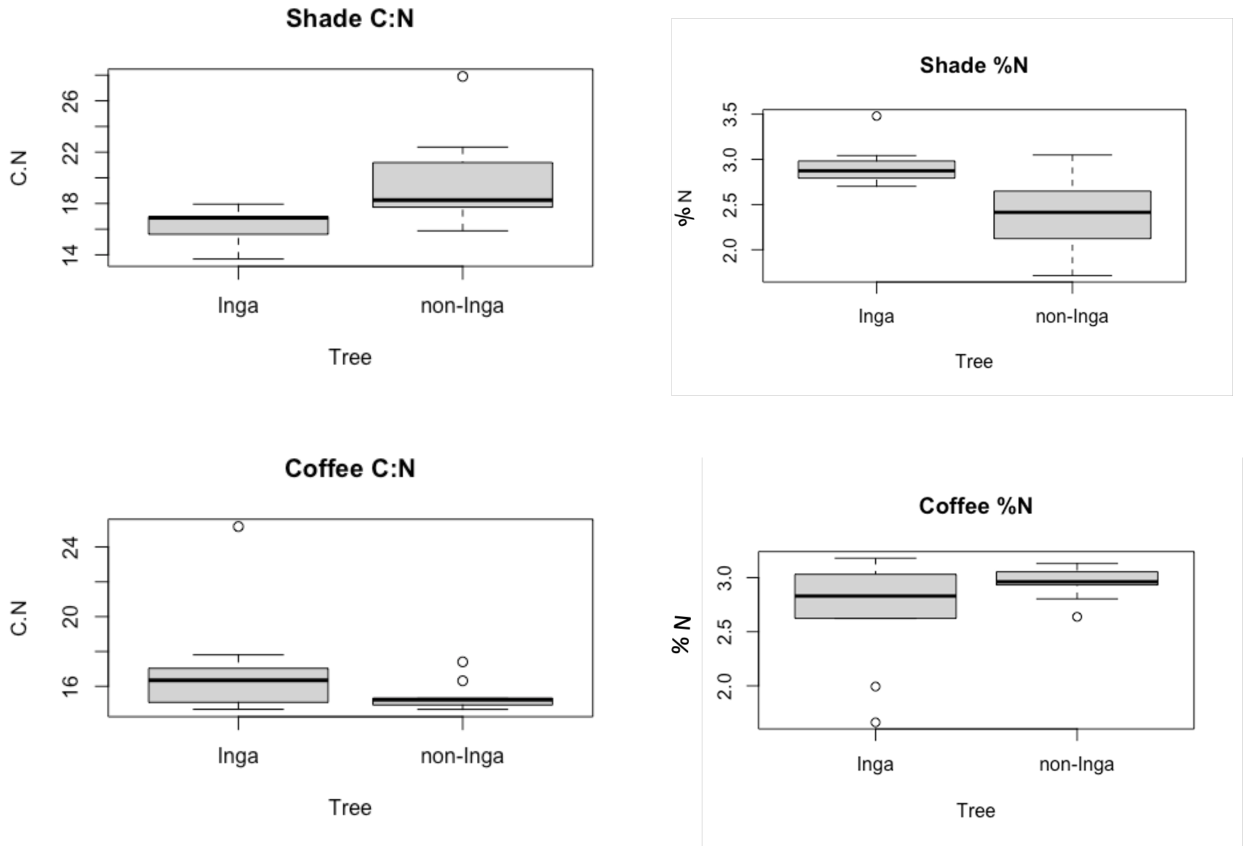

Figure B4. C:N ratios and %N in shade trees and coffee plants paired with *Inga* and non-*Inga* spp shade trees.

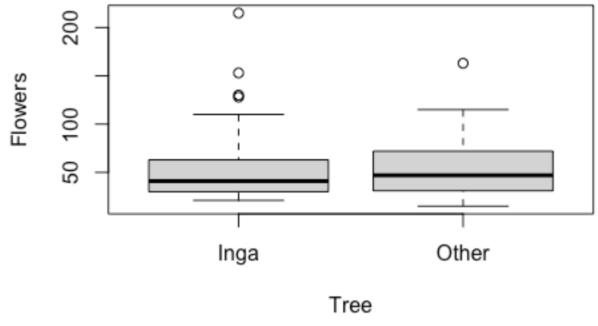

Figure B5. Number of flowers on coffee bushes close to *Inga* spp. and non- *Inga* spp. shade trees.