The Community Ecology and Management of Natural Pest Control: Investigating Interaction Complexity and Local Farm Perturbations in Coffee Agroecosystems

by

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Dedication

To my parents, for dragging me to the museum on Saturday morning.

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In the classic PBS series *Cosmos*, Carl Sagan astutely pointed out that, “Science is a collaborative enterprise, spanning the generations. When it permits us to see the far side of some new horizon, we remember those who prepared the way - seeing for them also.” This has undeniably been my experience as a graduate student and accordingly, there is a long list of people to whom I owe my gratitude for helping me through this collaborative journey.

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Table of Contents

List of Tables

List of Figures

Abstract

Agricultural intensification is the principal driver of many of our global environmental crises, including the alarming declines of terrestrial insect populations. To curb its negative impacts, it is imperative that we reduce pesticide use and shift agriculture to sustainable, agroecological pest control methods. Natural pest control (NPC) can reduce pest damage and make agriculture more biodiversity (and people) friendly by conserving natural enemy habitat in and around farms. However, a general approach to natural pest control has proved challenging to develop. More understanding is needed of the community ecology of these complex systems and of the impacts of farm management, both at the local and landscape scale. My dissertation explores key components of these knowledge gaps, investigating the role of interaction complexity, through non-consumptive effects, and the impact of local farm management perturbations on natural pest control communities. I use the coffee agroecosystem and ant-mediated natural pest control as a model system to explore these questions, prioritizing an experimental approach to promote mechanistic understanding.

In my first Chapter, I expand on the broader issues above and situate my work in the context of knowledge gaps in this field. In Chapter 2, I conduct an empirical experiment investigating the non-consumptive effect of an aggressive, coffee-foraging ant that removes pests from plants and throws them to the ground. I show how this effect mediates multi-predator interactions and spatial complementarity, and also enhances overall pest damage suppression. I also demonstrate how this interaction can be driven by variation in alternative resources for the

non-consumptive predator. In Chapter 3, I model the dynamics of this interaction more generally and demonstrate how non-consumptive effects can stabilize the complex dynamics of spatially coupled oscillators, mediate multi-predator interactions, and enhance pest regulation. In Chapter 4, I explore the broad impacts of disturbance through coffee cutting and renovation. This disturbance disrupted ground cover and coffee plant density, which reduced diversity and shifted the composition of the whole ant community. Arboreal ants were especially affected, with a significant reduction in species richness and abundance for several years, until an eventual recovery five years after disturbance. In Chapter 5, I show how contrasting farm management (high-input sun monoculture vs low-input shaded polyculture) impacts ant habitat through the epiphyte communities on coffee plants, where higher diversity and abundance of epiphytes in the less intensive farm may be driven by differences in the frequency of coffee plant pruning and distance from nearby forest patches. In Chapter 6, I demonstrate the impacts of experimental removal of coffee epiphytes on ant communities and pest control. I show that this intensive practice significantly reduces ant diversity, activity, and abundance on coffee, and ant-mediated pest damage reduction. I also show how this disturbance may interact with climate stress to exacerbate impacts on ants. In Chapter 7, I attempt to synthesize my results and place them in the context of our broader understanding. I explore how these findings illuminate the importance of interaction complexity (non-consumptive effects), which can result in non-intuitive impacts, compared to direct consumptive pest regulation. I also provide greater mechanistic understanding of the impacts of local farm intensification and perturbations, by experimentally testing several types of disturbance on ant natural pest control. I conclude with a discussion of the broader implications and suggestions for future research on these subjects.

Chapter 1 Introduction

Long before Charles Elton pointed out that the management of agricultural systems could benefit from a more holistic, ecological approach (Elton 1958), indigenous peoples worldwide were already practicing it (Carlisle 2022). The Mexica of central modern-day Mexico built one of the most populous societies of the new world on the foundations of whole-system agroecological crop management – the famed *chinampas* (Carlisle 2022). These systems employed “closed loop” and “integrative” practices (Altieri & Nicholls 2017) that are the subject of much of the current academic literature on sustainable farming systems (Kremen, Iles, and Bacon 2012). Yet, while these practices were developed many centuries ago, long before the arrival of European colonizers, today, the reductive approach of modern industrial agriculture is fueling many of the environmental crises which threaten the fabric of society (Foley et al. 2011; Kremen and Merenlender 2018; Vandermeer et al. 2018; Tscharntke et al. 2005).

Agricultural intensification, through increased mechanization and use of chemical inputs, reduces natural habitat and drives the loss of biodiversity (Tscharntke *et al.* 2005). Globally, industrial agriculture is considered one of the leading causes of the precipitous decline of terrestrial insects that only recently caught the attention of society, the so-called “insect apocalypse” (Raven & Wagner 2021; Wagner 2020; Wagner *et al.* 2021). The heavy use of chemical pesticides in these systems causes environmental pollution which increases birth defects in agricultural workers and other adverse health conditions (Larsen *et al.* 2017). Agricultural intensification is also a leading source of greenhouse-gas emissions (Vandermeer *et al.* 2018), through drivers such as deforestation, the by-products of chemical fertilizer inputs, and soil degradation, among others. Collectively, these trends threaten the many ecological processes and services upon which human society depends (Cardinale *et al.* 2012).

As we face the sixth mass extinction of biodiversity, some conservation ideas have gained traction, such as the call to preserve 30% of Earth's surface by 2030 (the 30-by-30 objective), and E. O. Wilson's "Half-Earth." While these proposals may be well-intentioned, they often miss a central part of the problem – much of the space between those areas: the agricultural matrix. Indeed, one of the seminal studies on insect declines to capture the world's imagination found a perplexing trend (Hallmann *et al.* 2017). As was reported by Hallmann et al. in 2017, in conservation areas in Europe, terrestrial volant (flying) insect populations have declined by more than 75% over roughly the last 30 years. The authors analyzed detailed historical data on landscape composition and climate change, but did not find that either of these significantly explained the drop-off. They concluded their study by suggesting that it has not been the amount of agriculture in the surrounding landscape, but likely the intensification of that agriculture, especially through increased pesticide use, that likely explains the trend (Hallmann *et al.* 2017). Agriculture is not only central to our efforts to curb biodiversity loss and environmental degradation, it may, in fact, be the signature of the Anthropocene. Agriculture and grazing land now occupy over 40% of the terrestrial surface of the Earth, much of which is increasingly being intensified (Foley *et al.* 2011).

If we are to reverse course and have some hope of transitioning to a sustainable future, we must begin to think about agriculture holistically, as both ecological and social systems (Kremen and Merenlender 2018; Vandermeer et al. 2018). While this imperative has received much attention over the last few decades as agroecology has developed as an academic field, recent calls for agroecological intensification (or regenerative agriculture) as an alternative to agricultural intensification have provided a roadmap for this sustainable future (Carlisle 2022; Kremen 2020; Mier y Terán Giménez Cacho *et al.* 2018). An essential part of this effort will be to increase our understanding of the ecology and management of natural pest control.

Understanding Natural Pest Control

Natural pest control (NPC) aims to promote pest regulation indirectly by conserving habitat in and around farms to bolster the populations of natural enemies and the broader agroecological networks that help to suppress pests (Barbosa 1998; Bianchi *et al.* 2006; Iuliano & Gratton 2020; Lewis *et al.* 1997). It has alternatively been called conservation biocontrol (Barbosa 1998; Begg *et al.* 2017) or autonomous pest control (Vandermeer *et al.* 2010), but the shared principle behind these concepts is to work with nature to suppress pest populations, rather than against it. This stands in contrast with classical biocontrol programs, which typically aim to introduce novel natural enemies to regulate pests, but risk species invasion and the disruption of ecological communities (Simberloff 2012). There are, of course, plenty of compelling examples of NPC in the literature, such as the Guatemalan milpa farmers whose traditional management of diverse maize-based agroecosystems keeps pest levels naturally low (Morales & Perfecto 2000). Or, for example, the more academic study of pest control “ecosystem services” mediated by the positive impact of shade trees and forest patches on bird communities in coffee agroforests, which help to reduce pest damage and increase yields (Karp *et al.* 2013).

Despite these compelling examples, a general framework for natural pest control has proved elusive due to important gaps in our understanding of the ecology and management of these systems (Karp *et al.* 2018; Tscharntke *et al.* 2016). Ecologically, NPC systems are complex systems, usually involving a diverse network of interacting populations that collectively work to keep pest communities in check (Perfecto *et al.* 2014; Vandermeer *et al.* 2010, 2019). This has raised key questions about how the diversity of natural enemies impacts pest control function (Crowder & Jabbour 2014; Straub *et al.* 2008), how higher-order and non-consumptive interactions contribute to pest regulation (Culshaw-Maurer *et al.* 2020), and especially how population dynamics operate in these systems (Iuliano & Gratton 2020; Settele & Settle 2018), to name a few. On the management side, there are important gaps in our understanding of how both local and landscape-level factors impact natural pest control in the agroecological matrix (Tscharntke *et al.* 2016). Recent work aimed at understanding how landscape composition (i.e., the amount of natural habitat surrounding farms) (Dainese *et al.* 2019; Karp *et al.* 2018) and crop field size (Rosenheim *et al.* 2022) impact natural enemies and pests, have drawn mixed conclusions. Other research focuses on the impact of local farm management factors on NPC, which can be just as important for less mobile natural enemies (Gonthier *et al.* 2014). In both cases, however, most research on natural pest control management has relied on correlative work, since conducting manipulative experiments at this scale is particularly challenging. Although some of this work draws from impressive "big" data sets (Karp *et al.* 2018; Rosenheim *et al.* 2022), to gain mechanistic understanding we may need other approaches. Importantly, these dual lines of investigation, on the ecology and management of NPC, also offer an opportunity to advance general ecological understanding by adding perspective on some of the central questions in community ecology. For example, studying diverse natural enemy communities in agricultural landscapes can provide insight into classic topics, such as the relationship between ecological complexity and stability (Landi *et al.* 2018; May 1972), the role of spatial structure in the dynamics of metacommunities and systems of coupled consumer-resource oscillators (Gross *et al.* 2020; Koelle & Vandermeer 2005), and how higher-order interactions contribute to community structure and dynamics (Bairey *et al.* 2016; Grilli *et al.* 2017).

My dissertation explores two central topics within this broader framework, touching on both the basic ecology and management of natural pest control: 1) The role of interaction complexity (i.e., non-consumptive effects) on NPC and 2) the impact of local farm management perturbations on NPC communities and pest control provision. I approach these topics both theoretically, with dynamical modeling, and empirically, with a mix of manipulative field and laboratory experiments and comparative surveys. Through my research, I aim for mechanistic understanding, prioritizing experimentation and systematic manipulation over correlation, when possible, to ideally gain novel insight into these subjects.

Ants & Coffee – A Model System

To ground these questions, my research is based on (or inspired by) the ecologically complex coffee agroecosystems of southern Mexico, where I focus mainly on ant-mediated natural pest control. Coffee agroecosystems are ideal model systems to better understand how natural pest control operates. Coffee is typically grown in the tropics, often in biodiversity hotspots, making them especially important for conservation (Jha *et al.* 2014; Moguel & Toledo 1999; Perfecto *et al.* 1996). They are also managed along a continuum of agricultural intensification, ranging from diverse agroforests with shade trees, low chemical inputs, and polyculture crop production, to intensive, high-input coffee monocultures (Moguel & Toledo 1999). This spectrum of management makes coffee the perfect testing ground for understanding both the impacts of agricultural management and the role of ecological complexity in NPC.

When managed as agroforests, coffee agroecosystems can house diverse communities of natural enemies and impressive levels of unplanned biodiversity (Perfecto *et al.* 1996).

A key part of that diversity, for natural pest control, is the community of ants, which is highly diverse and comprised mainly of generalist consumers that forage both on the ground, on shade trees, and directly on coffee plants, where they can interact with coffee herbivores and pests (Morris *et al.* 2018; Philpott & Armbrecht 2006). Generally, ants are known to form protective relationships with plants, both directly, when mediated by plant traits such as extrafloral nectaries, and indirectly when mediated through hemipteran insects (Rosumek *et al.* 2009; Styrsky & Eubanks 2007). While the latter of these relationships can sometimes be damaging, several reviews of general ant-plant interactions have shown that on the whole (roughly 70% of the time) these are beneficial for plants (Rosumek *et al.* 2009; Styrsky & Eubanks 2007). Ants are also known to be generally important for pest control in agriculture, where they help to regulate pest populations and reduce pest damage (Anjos *et al.* 2022; Offenberg 2015). Despite this, ants are often overlooked in biocontrol programs and ant populations may even be negatively impacted when farms use insecticides to control pests because they are usually broad-spectrum.

It's important to note that my research builds on a rich literature of work on coffee agroecology and ant-mediated pest control. Of particular importance is work that has documented the general impacts of coffee management on biodiversity (see references in Philpott et al. 2008; Perfecto and Vandermeer 2015) and research illuminating the role of ants in the regulation of the coffee berry borer, the most damaging insect pest of coffee around the world (see references in Philpott and Armbrecht 2006; Morris et al. 2018). With this dissertation, I aim to both synthesize and expand on this work, exploring both novel components and questions, but ultimately, to use this research as a case study that can inform our more general understanding of natural pest control.

Dissertation Framework & Overview

My dissertation research follows two avenues of investigation. Chapters 2 and 3 focus on interaction complexity, where I investigate the impact of non-consumptive effects (NCEs) in pest control. Specifically, I explore the impact of ants removing coffee pests from plants through an aggressive interaction and how this mediates multi-predator interactions, spatial dynamics, and overall pest regulation. This interaction is related to the classic case of predator non-consumptive effects and prey refuge seeking, but my research seeks to understand its implications for multi-predator interactions and dynamics in pest control specifically, on which there is less work (Culshaw-Maurer *et al.* 2020). In Chapter 2, I explore this interaction empirically with a mesocosm experiment and demonstrate how it can result in spatial complementarity between predators, improved overall pest damage reduction, and interestingly, how variability in alternative resources for the non-consumptive inducing ant may impact the strength of this interaction. In Chapter 3, I model the dynamics of this interaction more generally to understand how NCEs impact the spatial coupling of consumers-resource oscillators and the dynamics of pest control. My model results show that this non-consumptive effect interacts with spatial coupling to stabilize dynamical complexity and potentially result in more efficient pest regulation.

In Chapters 4-6 I explore the impacts of farm management perturbations on natural pest control. Although much work has been conducted exploring the general impacts of coffee farm management on ant communities, in these Chapters I expand on this work with several broad manipulative experiments, which generally have been difficult to conduct in these agriculturally managed landscapes. I look separately at the impacts of coffee cutting and replanting and the importance of coffee epiphytes and epiphyte removal, both on ant communities and ant-mediated pest control. In Chapter 4, I show how the local management perturbation from coffee clearing and replanting has broad impacts on ant communities by disturbing ground cover and coffee plant vegetative complexity. For this study, I conducted a five-year experiment, where the impacts of disturbance were followed both before and after coffee management, allowing for understanding of the longer-term implications on ant community recovery. In Chapter 5, I explore how general farm management and intensification impact the diversity and abundance of epiphyte plants growing on coffee, which are potential habitat for ants. I surveyed two neighboring farms of contrasting management – a shaded, low-input polyculture, and a low-shade, high-input monoculture. I show that epiphyte diversity and abundance are significantly higher on shaded, low-input farms, and that this trend may be partly explained by coffee pruning and the presence of nearby forest. In Chapter 6, I report the results from an experiment, where I show that management intensification through coffee epiphyte removal suppresses ant communities and ant-mediated pest control services. I also show how this management disturbance may interact with seasonal drought stress to worsen the negative impacts on ants – aligning with emerging research demonstrating the concerning interaction of agricultural intensification and climate change on global insect declines (Outhwaite *et al.* 2022).

In my final Chapter, I attempt to synthesize these findings and evaluate them in the context of the broader literature.

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Chapter 2 An Aggressive Non-Consumptive Effect Mediates Pest Control and Multi-Predator Interactions in a Coffee Agroecosystem[1]

2.1 Abstract

Natural pest control is an alternative to pesticide use in agriculture, which may help to curb insect declines and promote crop production. Non-consumptive interactions in natural pest control, which historically have received far less attention than consumptive interactions, may have distinct impacts on pest damage suppression and may also mediate positive multi-predator interactions. Additionally, when non-consumptive effects are driven by natural enemy aggression, variation in alternative resources for enemies may impact the strength of pest control. Here we study control of the coffee berry borer (CBB), *Hypothenemus hampei*, by a keystone arboreal ant species, *Azteca sericeasur*, which exhibits a non-consumptive effect on CBB by throwing them off coffee plants. We conducted two experiments to investigate: 1) if the strength of this behavior is driven by spatial or temporal variability in scale insect density (an alternative resource which *Azteca* tends for honeydew), 2) if this behavior mediates positive interactions between *Azteca* and other ground-foraging ants, and 3) the effect this behavior has on the overall suppression of CBB damage in multi-predator scenarios. Our behavioral experiment showed that nearly all interactions between *Azteca* and CBB are non-consumptive and that this behavior occurs more frequently in the dry season and with higher densities of scale insects on coffee

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branches. Our multi-predator experiment revealed that borers thrown off coffee plants by *Azteca* can survive and potentially damage other nearby plants but may be suppressed by ground-foraging ants. Although we found no non-additive effects between *Azteca* and ground-foraging ants on overall CBB damage, together, both species resulted in the lowest level of plant damage with the subsequent reduction in "spillover" damage caused by thrown CBB, indicating spatial complementarity between predators. These results present a unique case of natural pest control, where damage suppression is driven almost exclusively by non-consumptive natural enemy aggression, as opposed to consumption or prey behavioral changes. Furthermore, our results demonstrate the variability that may occur in non-consumptive pest control interactions when natural enemy aggressive behavior is impacted by alternative resources, and also show how these non-consumptive effects can mediate positive interactions between natural enemies to enhance overall crop damage reduction.

2.2 Introduction

With the increasing alarm surrounding global insect declines (Wagner 2020; Hallmann et al. 2017; van Klink et al. 2020), a prescription which continues to emerge is the need for drastic reductions in pesticide and insecticide use (Kremen and Merenlender 2018; Harvey et al. 2020), which appears to be one of the major drivers of the observed declines (Wagner 2020; Sánchez-Bayo and Wyckhuys 2019). Natural pest control, through the conservation of natural enemy habitat in agricultural landscapes, can serve as an alternative to promote the production of crops, the regulation of pests, and the conservation of biodiversity (Bianchi, Booij, and Tscharntke 2006; Dainese et al. 2019; Karp et al. 2013). One of the challenges to this approach is understanding how diverse communities of natural enemies impact the overall functioning of pest regulation (Straub, Finke, and Snyder 2008; Griffin, Byrnes, and Cardinale 2013; Letourneau et al. 2009; Casula, Wilby, and Thomas 2006). However, in focusing primarily on the relationship between predator richness and pest control, this research often overlooks the complexity of interactions amongst predators and pests which serve as the ultimate mechanisms for pest suppression (Crowder and Jabbour 2014). When interactions are studied in pest control, most often, direct, consumptive interactions receive the bulk of the attention (Eubanks and Finke 2014). This is despite the growing awareness of the ubiquity of trait-mediated and non-consumptive interactions in communities, which, more generally, have been shown to have impacts of equivalent magnitudes on prey regulation (Werner and Peacor 2003; Preisser, Bolnick, and Benard 2005) and plant communities through trophic cascades (Schmitz, Krivan, and Ovadia 2004).

In pest control, non-consumptive effects of natural enemies can increase pest risk and reduce pest damage by magnitudes comparable to consumptive interactions (Thaler and Griffin 2008; Eubanks and Finke 2014; Hermann and Landis 2017). These interactions can have effects on pest populations that are disproportionate to the density of natural enemies, potentially serving as mechanisms for the influence of keystone biocontrol agents (Meadows, Owen, and Snyder 2017). This may occur when the mere presence of natural enemies changes the behavior of prey to reduce pest feeding rates, while not necessarily impacting pest densities themselves (Eubanks and Finke 2014). Importantly, non-consumptive effects can also mediate the interactions between natural enemies (Davenport and Chalcraft 2013) and may potentially influence the impact of natural enemy diversity on pest control (Meadows, Owen, and Snyder 2017; Ingerslew and Finke 2018). When enemies compete directly over shared prey resources or space, multi-predator interactions often have negative impacts on prey regulation, but when predators are spatially separated or functionally distinct, non-consumptive effects can result in positive synergistic pest regulation (Ingerslew and Finke 2018; Meadows, Owen, and Snyder 2017).

A particularly interesting example of this was documented by Losey and Denno (1998), where they showed that the presence of a Coccinellid predator on plants caused aphids to drop to the ground making them more available to a ground foraging beetle (Losey and Denno 1998). This study demonstrated how the spatial separation of predators on plants and the ground, along with the non-consumptive effect of one of the predators on the pest, enhanced overall control and resulted in positive synergistic multi-predator effects (Losey and Denno 1998). Other research on aphid dropping in multiple wasp enemy communities has found more conflicting results, where consumptive effects between enemies resulted in interference and reduced overall prey suppression, but non-consumptive effects yielded positive additive prey suppression (Ingerslew and Finke 2018). Despite this research, few studies have explored the importance of non-consumptive interactions in multi-enemy pest control scenarios, where impacts on crop damage are explicitly tested (Hermann and Landis 2017). Furthermore, the dynamics of non-consumptive interactions are not well understood in pest control, and few studies have tested how non-consumptive enemy behavior changes across space or time (Hermann and Landis 2017; Sheriff et al. 2018), despite the long history of dynamical research on consumptive predator-prey interactions. This may be particularly important when non-consumptive effects are driven by predator aggression, as opposed to prey behavioral responses, and spatial or temporal variation in alternative resources for predators impacts the strength of non-consumptive effects, potentially resulting in variable or inconsistent pest damage suppression.

In shaded coffee agroforests, communities of natural enemies can be quite diverse, leading to a host of potential multi-predator interactions and non-consumptive effects (Perfecto, Vandermeer, and Philpott 2014; J. Vandermeer, Perfecto, and Philpott 2010; J. Vandermeer et al. 2019). Ants have received much attention in the coffee pest control literature, particularly in Latin America, where a number of species are known natural enemies of the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari 1867), (Morris et al. 2018; Philpott and Armbrecht 2006), a major global pest of coffee, which bores into fruits and significantly reduces yield. In southern Mexico, the ecology of the arboreal ant, *Azteca sericeasur* (Longino 2007), has been investigated extensively for its apparent keystone role in the interaction networks of coffee farms (J. Vandermeer et al. 2019; J. Vandermeer, Perfecto, and Philpott 2010). It is well documented that this ant suppresses the damage of CBB, in both the laboratory (Pardee and Philpott 2011; Philpott, Pardee, and Gonthier 2012), and in the field (Gonthier et al. 2013; Morris, Vandermeer, and Perfecto 2015; Jiménez-Soto et al. 2013). This species is commonly observed foraging on coffee bushes in this region where it tends hemipteran insects (scale) for honeydew. When *Azteca* ants participate in these mutualistic relationships with scale, they can indirectly benefit coffee by patrolling plants and preying on other herbivores which might threaten the supply of honeydew resources from their scale partners (Morris et al. 2018; Perfecto and Vandermeer 2006). However, this behavior may also drive non-consumptive interactions with coffee herbivores, including CBB. This has been observed previously, where *Azteca* ants will attack CBB individuals during their colonization of coffee plants, often throwing or pushing them off of plants to the ground (Jiménez-Soto et al. 2013). Unlike many other cases of non-consumptive enemy-prey interactions (Hermann and Landis 2017), this effect appears to be driven by the enemy's antagonistic but non-consumptive impact and not by a behavioral response of the prey.

Despite this aggressive behavior, it is still not well understood what the overall impact of this non-consumptive interaction is on coffee pest control. Previous work in this system has reported conflicting frequencies of this behavior compared to direct consumption of CBB (Perfecto and Vandermeer 2006; Jiménez-Soto et al. 2013). Variability in the intensity of this interaction or in the proportion of non-consumptive to consumptive interactions by ants may have important impacts on the dynamics and efficacy of pest control in this system. Interestingly, because this non-consumptive interaction is driven by enemy aggression, rather than prey defense, these dynamics may be governed by the availability of honeydew resources for ants from scale insects on coffee, which previous research suggests may influence *Azteca*'s reduction of CBB damage (Rivera-Salinas et al. 2018). While it is not always clear how honeydew availability (Clark and Singer 2018) or hemipteran insect density (Kaplan and Eubanks 2005) influences ant-plant defensive interactions more generally, research on other species of *Azteca* ants in this region has demonstrated that seasonal variability in plant carbon pools may impact scale insect honeydew and the strength of ant-plant defense (Pringle et al. 2013). Furthermore, it is not clear what impact this non-consumptive effect has on overall CBB damage and the broader control of coffee pests in the community, where the flux of resources from coffee plants to the ground may mediate interactions between *Azteca* and other ground foraging predators, potentially resulting in enhanced control of pests and positive multi-predator effects.

To assess these questions and gain understanding into the natural history of this complex pest control interaction, we conducted both a field behavioral experiment and a laboratory multi-predator experiment. With our behavior experiment, we aimed to test 1) if the strength of *Azteca's* aggressive non-consumptive effect on CBB varies positively across space (on coffee) or time (between seasons) with the density of scale insects (an alternative resource). With our laboratory experiment we aimed to understand 2) how this behavior impacts overall CBB damage in multi-predator communities and 3) if CBB throwing results in positive interactions or spatial complementarity between *Azteca* and ground foraging predators. Ultimately, we aimed to illuminate how this unique non-consumptive effect, driven by the aggressive behavior of a dominant keystone consumer, influences the overall regulation of this important agricultural pest.

2.3 Methods

To understand the impact of the non-consumptive interaction between *Azteca sericeasur* and coffee berry borers on pest control we conducted two experiments. First, we performed a behavioral experiment in the field to investigate variability in the strength of this non-consumptive interaction, regarding the availability of scale insect resources for *Azteca* across space on coffee bushes and time between seasons. Second, we conducted a laboratory experiment to assess the impact of this behavior on borer damage in multi-predator scenarios and to understand how this non-consumptive effect mediates interactions between *Azteca* and ground-foraging predators. All experiments were conducted at Finca Irlanda in Chiapas, Mexico. The farm is a 300-hectare, certified organic, shaded coffee polyculture situated at roughly 1000m elevation at 15°11′ N, 90°20′ W. Data for the behavioral experiment were collected during July 2019 and February 2020, while data for the multi-predator experiment were collected during October of 2016.

2.3.1 Field Behavior Experiment

To assess the variability of this non-consumptive behavior in the field we conducted a behavioral assay. We haphazardly selected 20 sites where *Azteca sericeasur* ants were active on coffee bushes. We chose sites that were separated by a minimum of 6.13 meters (between ant nest host trees) to increase the likelihood that ants from each site represented different colonies from different shade trees, however the average distance between sites was roughly 43m. We measured the activity of ants on coffee by counting the number of individuals crossing a fixed point on the central trunk of the coffee bushes for one minute. We selected one coffee bush at each site with at least five individual ants observed during the one-minute period. Then, we selected one branch on each coffee plant and measured the branch-level ant activity by visually scanning the branch and counting all individuals. We chose branches that had a minimum of three ant individuals for our experiment. Finally, we estimated the availability of resources for *Azteca* ants from scale insects by counting the number of adult scale insects on branches of a few common species, including the green coffee scale, *Coccus viridis*. On one coffee bush replicate some aphid individuals were also being tended by ants and were included in our count of scale, although this did not statistically alter our results.

To assess the variability of borer throwing by *Azteca*, we placed individual live adult borers on coffee plants and recorded the resulting behavior of *Azteca* ants. Adult borers were extracted from bored green coffee fruits collected in the field. For each behavioral trial, borer individuals were placed on a leaf of the chosen branch of coffee plant replicates and observed for up to three minutes. We recorded three possible outcomes from these trials. First, we recorded "consumption events" when *Azteca* ants encountered borers, captured them, and returned to the ant foraging trail on the trunk of the coffee plants (which we assumed indicated that the ants were bringing borers back to their nest). Second, we recorded the non-consumptive dropping behavior when ants interacted with borers by capturing them and dropping them from leaves or by pushing them off leaves, in either case resulting in the removal of the borer from the coffee plant. Finally, if ants encountered borers but did not remove them, or if ants failed to encounter borers, we recorded the result as a non-interaction. Since we were only interested in the overall rate of borer throwing in this study, and other studies have reported more on the details of ant-borer behavioral interactions (Jiménez-Soto et al. 2013), we chose to simplify our interaction outcomes into these three principal categories. The time of all interactions was recorded and if no interaction occurred, we recorded the end time of the trial at three minutes. We repeated behavioral trials five times per bush on the same branches to estimate the frequency of borer throwing per each site using new CBB individuals for each replicate.

To test for variability in the proportion of borers thrown by ants due to resource variability by season, we conducted this experiment in two different seasons. First, during the rainy season in July 2019, when scales insects are typically considered to be more abundant and then in February 2020, during the dry season, when there are typically fewer arthropods, including hemipterans (Williams-Guillén, Perfecto, and Vandermeer 2008). Local precipitation at the farm varied significantly between these sampling points, with 249mm rainfall measured during July 2019 and 43mm during February 2020, indicating that these sampling periods represent distinct seasons. In most cases, the trials were repeated at the same sites for both sampling periods and on the same coffee bushes. When this was not possible due to low ant activity during the second sampling in the dry season, we substituted another nearby bush with sufficient *Azteca* activity at the same site, or in four cases a new site was chosen. Only 19 site replicates were used during the dry season.

To test if season or scale insect density on branches drove differences in the frequency of non-consumptive behavior by *Azteca,* we conducted a generalized linear mixed model (GLMM). We included season as a categorical fixed effect, branch level scale density as a continuous fixed effect, and their interaction as a fixed effect, as well as site as a random effect (to control for spatial non-independence between repeated replicates on the same plant). We modeled behavioral outcomes using a binomial error distribution and logit link function (outcomes were reduced to two possible types: non-consumptive throwing or no interaction given the lack of observed consumption; see Results). To assess whether there were differences in scale insect abundance on coffee branches between seasons we conducted a generalized linear model (GLM). We included the sampling time (season) as a fixed effect and ran the GLM using a Poisson error distribution with a log link function, to account for count data.

2.3.2 Multi-predator Interaction Experiment

To better understand the impact of *Azteca*'s non-consumptive effect on CBB damage reduction in multi-predator communities we conducted an additive, fully factorial laboratory experiment with two predators. We designed mesocosms in the laboratory using coffee plant saplings (Fig. 2.1). Mesocosms included four different treatments: a control with no ants, an *Azteca* only treatment, a ground-foraging ant treatment, and a treatment with both ant species. We used the ant species *Wasmannia auropunctata* (Roger 1863), as the ground-foraging species, since it is known predator of coffee berry borer (Gonthier et al. 2013; Newson, Vandermeer, and Perfecto 2021) and can be easily collected and manipulated in the laboratory. Coffee plants were acquired from the nursery at Finca Irlanda and were all *Coffea arabica* individuals of the same variety and age. All plants were roughly 70 cm tall and were watered every 2-3 days in the lab.

Plants were placed in 70 cm diameter plastic washtubs and were kept in plastic containers to avoid water or dirt from spilling into the mesocosms. A coating of fluon was applied to the sides of tubs to keep ants and borers from escaping mesocosms (although some borers could potentially fly out). Additionally, fluon was applied to the outside of plant containers and tanglefoot to the base of coffee plants to avoid the direct interaction of the different ant species. We chose to limit direct ant interactions because of the artificial proximity of the ants in our mesocosms (on small coffee saplings) and the potential for this to unnaturally amplify ant aggression by reducing the amount of territory that multiple ants would typically share in the field. A total of ten tubs and 20 coffee saplings were used throughout the experiment. For treatments with *Wasmannia* ants, ants were placed on the floor of washtubs and kept in open plastic containers along with pieces of moss, plants, and twigs collected during ant collection in the field to provide temporary shelter and suitable microclimatic conditions (Fig. 2.1). For treatments with *Azteca* ants, ants were placed directly on coffee bushes. A small drop of honey was placed on five leaves of each coffee plant to simulate the honey dew resources provided by scale insects for *Azteca* ants. Roughly 40 individuals of *Azteca* were placed on bushes. For *Wasmannia* treatments, we filled ant containers with a minimum of 100 individuals, although this number likely varied substantially between replicates due to the difficulty of counting such small ants. These densities of ants were chosen given our observations of what is typical for these species in the field. We used the same number of ants for the treatment with both ant species as for individual treatments in order to conduct an additive experiment. We used this design to directly test for non-additive effects from the interaction of ants on borer control, assuming that the resulting borer damage measured from the treatment with both ants would differ from the sum of that of the individual ant treatments, if a synergistic or facilitative interaction occurs (Cardinale et al. 2003).

All ants were collected in Finca Irlanda and stored in plastic containers with perforated lids between trials. *Azteca* ants were collected from different nest trees separated by a minimum of five meters for different replicates. Both majors and minors of *Azteca* were collected along with fragments of carton nest material. *Wasmannia* ants and brood were collected by scraping epiphytes and bark from trees and from hollowed out branches collected from the ground. Although *Wasmannia* ants in the area of the study may exist in large "supercolonies" spread out across farms (Yitbarek, Vandermeer, and Perfecto 2017), we attempted to collect from different areas separated by at least two meters for each replicate. All ants were kept in containers in the lab until the morning of experimental trials, but no longer than one week. In two instances some ants were reused to supplement *Wasmannia* colonies with low activity.

Because coffee saplings were too young to produce fruits, we collected fruits from plants in the field to add to our mesocosms. We added branches with 20 unbored fruits to plants in the lab by positioning them across branches of the saplings and added 20 unbored individual green coffee fruits on the floor of mesocosms (Fig. 2.1). Fruits were placed both on coffee plants and on the ground to track individual borers and borer damage in both places. Branches that were added to coffee plants had all leaves removed and any additional fruits until each branch had exactly 20 unbored fruits. Fruits and coffee plants were arranged in experimental mesocosms at least two hours before beginning trials with ants and borers to allow plant volatiles to dissipate.

At the start of each experimental trial 40 individual coffee berry borers were placed on the coffee plants in our mesocosms. We chose this density to more easily track CBB damage given the possibility that some borers may die or escape in the laboratory, but this density is also frequently observed on individual plants in the field (Barrera 2008 and unpublished data). All borers were collected from bored fruits in the field by dissecting them. To prevent falling or thrown borers from being lost in plant containers, we fitted a small plastic skirt around each plant to deflect borers onto the ground arena of mesocosms (Fig. 2.1). 24 hours after placing borers in mesocosms we checked all coffee fruits for evidence of borer holes and counted the total number of CBB in fruits on plants and on the ground. Tracking fruit damage as a measure of pest suppression was preferred over measuring borer mortality, since it was sometimes difficult to assess when individual borers were dead and if the cause of death was due to ant attack or environmental exposure in mesocosms. This also allowed for a more direct assessment of the impact of this non-consumptive effect on crop damage suppression. Additionally, we counted all CBB individuals that were found outside of fruits at the end of trials to track CBB movement and ensure that few borers were escaping the mesocosms (Fig. S2.1). To minimize the impact of residual ant pheromones or plant volatile chemicals, all plastic tubs were cleaned with alcohol in between trials. Coffee plants were alternated such that at least 48 hours passed before being used again in experiments. To control for differences between mesocosms or coffee plants, treatments were assigned to each mesocosm randomly. A total of 59 trials were conducted (*N*=15 control, *N*=13 *Wasmannia* only, *N*=17 *Azteca* only, *N*=14 both ants) in blocks during the first two weeks of October 2016.

To assess whether the number of borers observed inside coffee fruits differed between treatments after 24 hours we conducted generalized linear mixed models (GLMMs). We ran individual GLMMs on the number of borers in fruits on plants and the ground separately, and on the combined outcome. We included *Azteca* and *Wasmannia* presence or absence as fixed effects in the models. Their interaction was also included as a fixed effect to determine statistical significance of the both-ant treatment, which would indicate non-additive predator effects (for the combined data). Due to the heavily non-consumptive nature of the Azteca-CBB interaction (see Results) we used additive models, as opposed to a multiplicative risk model (Sih, Englund, and Wooster 1998), which is based on prey depletion effects through consumption (McCoy, Stier, and Osenberg 2012). To account for count data, the models were run using a Poisson error distribution with a log link function. We corrected for observed overdispersion in our plant level model by running a Poisson-lognormal error distribution using an observation-level random effect (Elston et al. 2001). In all models, mesocosm number and trial date (block) were added as random effects to account for any impacts of inconsistency in our laboratory environment. For all GLMMs, fixed effect parameters and the variance of random effects were estimated by maximum likelihood with Laplace approximation. All GLMMs were run using the "glmer" function from the lme4 package while GLMs were run using the "glm" function, both in R version 4.0.2 (R Core Team 2021).

2.4 Results

Does the non-consumptive interaction vary across time or space with scale insect density?

Interestingly, in nearly 200 behavioral trials we observed only one occurrence of what appeared to be consumptive behavior by *Azteca* on the coffee berry borer. Overall, nearly 43% of cases resulted in the non-consumptive effect of *Azteca* throwing or dropping borers from plants. Otherwise, borers were either not removed by *Azteca* or not found during the three-minute trials (this includes five cases where borers fell or flew off plants on their own). Unexpectedly, we found no difference in the amount of adult scale on coffee branches between seasons (Fig. 2.2, Table 2.1), indicating that scale density available to *Azteca* did not vary across time. However, we did find a significant difference in the proportion of dropped borers by *Azteca* between the two sampling times (Fig. 2.3, Table 2.1), where more borers were thrown off plants during the dry season (removing the one case of consumption from the analysis). The proportion of borers that were thrown by *Azteca* was also positively influenced by the amount of scale that were present on individual coffee branches (Fig. 2.4, Table 2.1). Additionally, the results from our GLMM showed a significant interaction between branch scale and season on the non-consumptive behavior, where CBB throwing was more consistent across a range of scale densities during the dry season (Fig. 2.4, Table 2.1).

What is the impact of the non-consumptive interaction on pest damage and multi-enemy interactions?

Overall, in the plant and ground combined data, we did not observe a difference in the number of borers found in fruits between our control treatments and treatments with *Azteca* only. However, we did observe a significant decrease in borers in the *Wasmannia* and both ant treatments (Fig. S2.2, Table 2.2). We did not find a significant interaction between *Azteca* and *Wasmannia* presence in our treatments, indicating that non-additive multi-predator effects were not observed in the combined data set (Fig. S2.2, Table 2.2).

Separating plant and ground borer data, we observed opposite impacts of *Azteca* presence on borer infestation levels, which accounts for the lack of an effect for *Azteca* in the overall data. On the coffee plant, *Azteca* ants lowered the number of borers found in fruits by roughly 57% compared to controls (Fig 2.5a, Table 2.2), in line with previous evidence demonstrating the effectiveness of *Azteca* at reducing borer damage (Gonthier et al. 2013; Jiménez-Soto et al. 2013; Morris, Vandermeer, and Perfecto 2015). However, because *Wasmannia* were restricted to the ground in this experiment, they had no effect on borer damage on the plant (Fig 2.5a, Table 2.2). The treatment with both ants also resulted in significantly fewer CBB in fruits on plants than in the control, reflecting the positive effect of *Azteca* ants on coffee plants (Fig 2.5a, Table 2.2).

On the ground, we observed a base level of damage in fruits in our control treatments from borers that either fall during trials on their own or fly off plants to the ground (Fig 2.5b, Table 2.2). *Wasmannia* only ant treatments significantly reduced borer damage from these levels, however, *Azteca* only treatments significantly increased borer damage on the ground compared to the control (Fig 2.5b, Table 2.2), reflecting the non-consumptive throwing behavior of the ants. Although significantly less than the *Azteca* only treatment, ground borer damage in the treatment with both ants was not different than the control (Fig 2.5b, Table 2.2).

2.5 Discussion

Collectively, our results document a unique case-study in natural pest control where crop damage suppression is driven almost exclusively by the non-consumptive attack of a dominant natural enemy. Our behavioral data show that the strength of this aggressive non-consumptive interaction – throwing of coffee berry borers off plants by *Azteca sericeasur* – may be driven by variability in alternative resources for *Azteca*, potentially resulting in variable pest control efficiency. Our multi-predator laboratory experiment demonstrates how this non-consumptive effect mediates spatial complementarity between arboreal and ground foraging natural enemies and may result in enhanced reduction of borer damage, when ground foragers are present on farms.

Surprisingly, from our behavioral experiment, we show that this pest control interaction is almost exclusively non-consumptive, which is interesting considering the previously documented efficiency of *Azteca* in reducing borer damage on plants (Gonthier et al. 2013; Jiménez-Soto et al. 2013; Morris, Vandermeer, and Perfecto 2015). Although we set out to test the hypothesis that this behavior may vary due to seasonal variation in resources from scale insects, we did not find a significant difference in scale abundance on coffee plants between seasons. However, we did still find a significant effect of season on borer throwing, where slightly more borers were thrown during the dry season compared to the rainy season. We also found an overall significant positive effect of the density of scale insects on coffee branches on the frequency of *Azteca's* throwing behavior, regardless of season, which aligns with related research showing a negative relationship between scale density and CBB damage on coffee with *Azteca* (Perfecto and Vandermeer 2006; Rivera-Salinas et al. 2018). This suggests that the tendency for *Azteca* to exhibit this non-consumptive behavior may be explained both by spatial variation in scale resources at different sites across coffee farms, and by temporal variation across seasons.

Despite not finding a difference in scale abundance between seasons, it is possible that the effect of season on borer throwing that we observed was driven by the quality of honeydew resources from scale insects which may vary seasonally in the region of this study (Pringle et al. 2013). Research on ant-scale-plant interactions in Central America and Mexico has shown that water stress during dry seasons changes the concentration of carbohydrates within plants, which may cascade upward to affect scale honeydew quality and hemipteran-tending ant activity, ultimately impacting ant defense of plants (Pringle et al. 2013). A previous study in our system used exclosure experiments on coffee bushes to show that *Azteca's* suppression of borer damage also varies by time of year (Rivera-Salinas et al. 2018), which may be explained by variation in honeydew quality. While we did not test honeydew composition in this experiment, our results align with the findings from Pringle et al. (2013), in that ant defense of plants is more consistent during the dry season than the wet season, potentially implying that seasonal differences in honeydew quality are at play. This would also explain the greater consistency in throwing we observed across a range of scale densities during the dry season. Additionally, variation in ant throwing behavior may be driven by seasonal differences in the nutritional needs of ant colonies across time (Cook et al. 2011). An important caveat is that although we found these differences between two different sampling times with distinct precipitation levels, we are limited in our ability to infer long-term seasonal trends in non-consumptive effects from this data. Regardless of the precise mechanism, non-consumptive effects driven by predator aggression as opposed to prey behavior may result in distinct dynamics, when driven by variation in alternate resources for enemies, which may ultimately result in variable or unreliable pest suppression. To our knowledge, this phenomenon has not been previously explored in natural pest control, and should be investigated further to understand the general impact of spatial and long-term seasonal (Hermann and Landis 2017) variation in non-consumptive enemy aggression on pest damage suppression.

In addition to our behavioral experiment, our multi-predator experiment helped to illuminate some important questions about the community ecology of this interaction. First, it appears that when there are no other predators in the system, many of the borers that are thrown off coffee plants by *Azteca* survive those attacks, potentially remaining in the borer population pool as reproductive individuals. Furthermore, thrown CBB individuals that survive attacks could relocate to old fruits on the ground or other coffee bushes and damage new fruits if *Azteca* is not foraging on those bushes. Despite the potential negative consequences of this “spillover” effect of thrown CBB, we also confirmed the results of previous laboratory (Pardee and Philpott

2011; Philpott, Pardee, and Gonthier 2012) and field experiments (Gonthier et al. 2013; Jiménez-Soto et al. 2013; Morris, Vandermeer, and Perfecto 2015) which show that *Azteca* is a highly efficient pest control agent in reducing borer damage on coffee bushes where they forage. Additionally, we demonstrate that a ground predator, *Wasmannia auropunctata,* suppresses borer individuals while foraging on the ground. Although it is already known that these ants, which nest both on plants and the ground, are important predators of borers on coffee plants (Gonthier et al. 2013; Newson, Vandermeer, and Perfecto 2021), less research has focused on their potential to consume borers on the ground. This is despite their ability to enter borer holes where they potentially predate CBB larvae and pupae in old fruits that fall to the ground (Morris and Perfecto 2016).

Regarding the impact of this non-consumptive effect on multi-predator interactions, the results from both experiments suggest that *Azteca* may significantly increase resource availability on the ground for ground-foraging predators like other ants, which could potentially result in facilitation or synergistic predator effects (Morris et al. 2018). However, despite this clear potential benefit for borer control, we did not find evidence of non-additive effects from our multi-predator experiment. Although *Wasmannia* presence consistently reduced borer damage, and in treatments with both ants more borers were made available to *Wasmannia* on the ground by *Azteca*, their rate of damage reduction did not appear to increase under these circumstances. In fact, *Wasmannia* ants reduced borer damage in fruits on the ground by roughly the same quantity in the both-ant treatment as in the *Wasmannia* only treatment. One potential limitation is that we do not actually know what quantity of borers were being directly consumed by *Wasmannia* from our data, since we were only measuring the reduction in berry damage and not directly tracking ant behavior. However, we did observe parts of CBB individuals near

Wasmannia containers in several replicates, and ants carrying CBB in their mandibles, suggesting consumption does occur. Further research should investigate interactions between ants, ground foraging predators, and herbivores under more realistic conditions in the field (Hermann and Landis 2017), on mature coffee plants where predators can interact freely.

These contrasting results reveal the complexity of pest control interactions when non-consumptive interactions are involved. Although there may be instances when *Azteca* ants do consume borers in the field, our data suggest that this occurs very infrequently, despite the well-known importance of this ant for reducing borer damage. Interestingly, this behavior may actually explain the efficiency of *Azteca* as a keystone pest control agent. A previous field experiment conducted in this study system manipulated the densities of borers that *Azteca* were exposed to on coffee plants and found the same level of borer damage reduction across a range of pest densities (Morris, Vandermeer, and Perfecto 2015). Importantly, this study failed to find a satiating effect at high densities of borers, which, given our results here, is likely explained by the fact that *Azteca* are simply not consuming CBB. This behavior may then ultimately result in the most efficient reduction of borers on coffee plants with high levels of *Azteca* activity. Subsequently, in farms with sufficient ground-foraging predator abundance, the “spillover” of these borer individuals from bushes with *Azteca* can be regulated by other predators, like *Wasmannia*, reducing their survival and colonization of other coffee bushes. The both-ant treatment in our lab experiment resulted in the best overall control of CBB by first reducing borer damage on plants to its lowest levels and then minimizing the “spillover” from *Azteca*’s non-consumptive effect of throwing borers to the ground, demonstrating the potential for spatial complementarity between arboreal and ground-foraging natural enemies. Beyond ants, other predators, like certain species of web-building spiders which form associative relationships with

A. sericeasur in this region (Marín, Jackson, and Perfecto 2015), may act as filters to collect resources thrown by *Azteca* and potentially buffer the effect of thrown borers and other pests. Additionally, thrown borers may also experience increased mortality risk from the loss of energy spent searching for refuges in old fruits on the ground, from potentially shifting their diets to these inferior resources, or from searching for new coffee plants to colonize.

While non-consumptive effects have long been studied in community ecology (Werner and Peacor 2003; Schmitz, Krivan, and Ovadia 2004; Preisser, Bolnick, and Benard 2005), there are fewer examples of non-consumptive mediated pest control (Walzer and Schausberger 2009; Hermann and Landis 2017). This study provides an unique addition to existing literature, where non-consumptive natural enemy aggression, rather than prey defensive behavior (or direct predation), dominates pest damage suppression and drives spatial complementarity between natural enemies. While this interaction may enhance coffee pest suppression in diverse communities when ground-foraging predators are conserved, variation in other resources that mediate *Azteca*'s aggressive behavior, like scale insect abundance and potentially honeydew quality, could result in variable pest control efficacy. Future research exploring the impact of similar non-consumptive interactions on pest populations (Sheriff et al. 2018) in diverse agroecological communities and under more realistic field conditions across growing seasons (Hermann and Landis 2017) will help illuminate the broader importance of these interactions for natural pest control. Ultimately, this case-study demonstrates the complexity of natural pest control ecology and highlights the need to consider specific interaction mechanisms and spatial and temporal variability in those interactions for the management of this important ecosystem service (J. H. Vandermeer and Perfecto 2017).

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2.8 Tables & Figures

Table 2.1 – Summary of statistical model results for the field behavioral experiment. The generalized linear mixed model (GLMM) was run using a binomial error distribution (logit link). The generalized linear model (GLM) was run using a Poisson error distribution (log link). Parameter estimates (± SE), *z*-values, and *p*-values are provided. Asterisks represent interaction effects.

Parameter	**Estimate (± SE)**	***z* value**	***p*(>|*z*|)**
GLMM: Proportion of CBB Thrown ~			
Intercept	-1.544 ± 0.452	-3.413	<0.001
Season	1.113 ± 0.462	2.409	0.016
Scale	0.023 ± 0.008	3.026	0.002
Season*Scale	-0.017 ± 0.009	-1.973	0.048
GLM: Branch Scale ~			
Intercept	3.615 ± 0.037	98.537	<0.001
Season	<0.001 ± 0.053	0.004	0.997

Table 2.2 – Summary of generalized linear mixed model results for the multi-predator experiment. All models were run using Poisson error distributions (log link). Parameter estimates (± SE), *z*-values, and *p*-values are provided. The overall category shows the statistical results for the combined plant and ground CBB fruit damage. Asterisks represent interaction effects.

Parameter	**Estimate (± SE)**	***z* value**	***p*(>|*z*|)**
Overall CBB Damage ~			
Intercept	2.944 ± 0.063	46.477	<0.001
Azteca	-0.043 ± 0.087	-0.495	0.620
Wasmannia	-0.316 ± 0.099	-3.198	0.001
Azteca*Wasmannia	-0.019 ± 0.140	-0.136	0.891
Plant CBB Damage ~			
Intercept	2.182 ± 0.177	12.327	<0.001
Azteca	-0.950 ± 0.213	-4.456	<0.001
Wasmannia	0.045 ± 0.217	0.207	0.836
Azteca*Wasmannia	-0.025 ± 0.322	-0.076	0.939
Ground CBB Damage ~			
Intercept	2.213 ± 0.098	22.502	<0.001
Azteca	0.436 ± 0.111	3.924	<0.001
Wasmannia	-0.779 ± 0.167	-4.664	<0.001
Azteca*Wasmannia	0.352 ± 0.200	1.761	0.078

Figure 2.1 – Experimental setup of multi-predator interaction mesocosms.

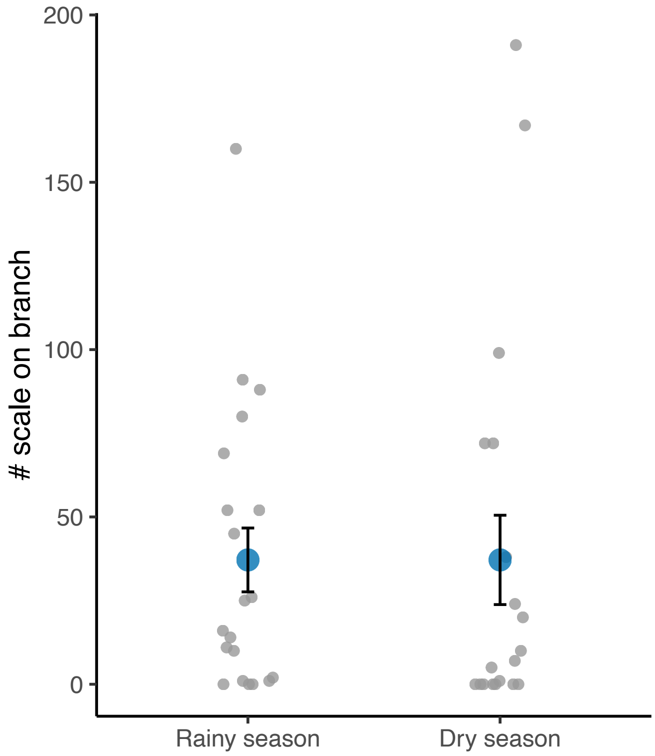


Figure 2.2 – Number of adult scale insects on coffee branches by season. Raw data and means (± SE) are shown (means shown in blue). Data from the rainy season were sampled in July 2019, whereas data from the dry season were sampled in February 2020. Data are pooled across several species of scale that are typically tended by *Azteca sericeasur* in this system.

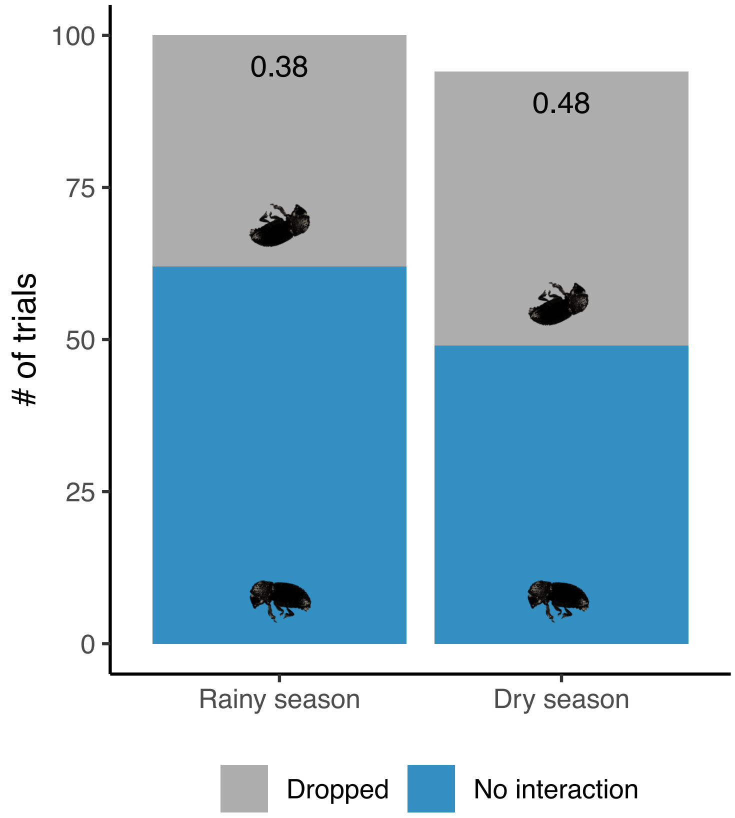


Figure 2.3 – Number of coffee berry borers (CBB) thrown off plants by *Azteca sericeasur* by season. Total counts from behavioral trials are tallied for the different sampling times. Data from the rainy season were sampled in July 2019, whereas data from the dry season were sampled in February 2020. Overall proportion of CBB thrown by season is shown in bold at the top of columns.

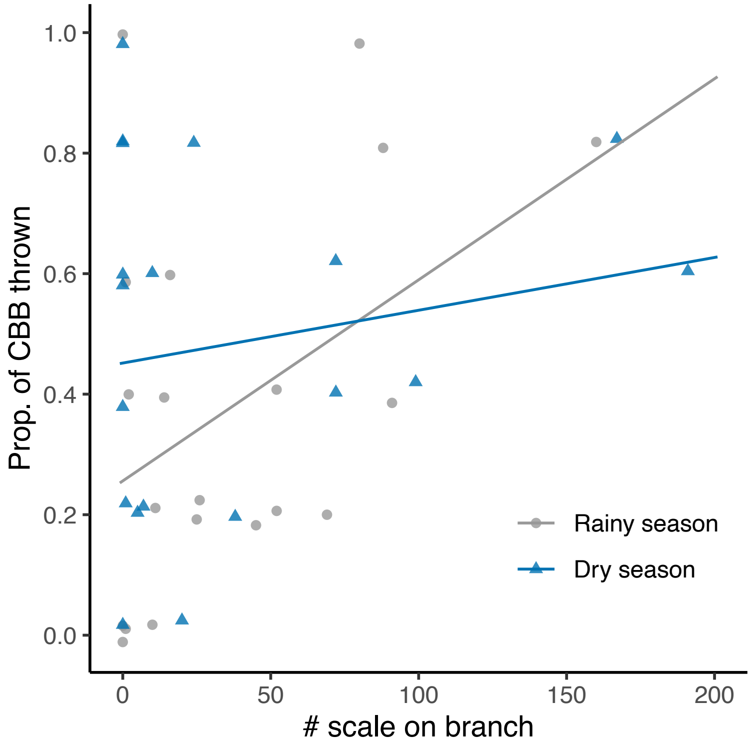


Figure 2.4 – Proportion of coffee berry borers (CBB) thrown per plant across scale insect density. Shows the relationship between the number of adult scale insects on coffee branches and the proportional result of *Azteca* behavioral trials for each coffee plant. Proportions are calculated from the five behavioral trial replicates conducted per coffee plant. Trend lines show the relative effect of season by scale density.

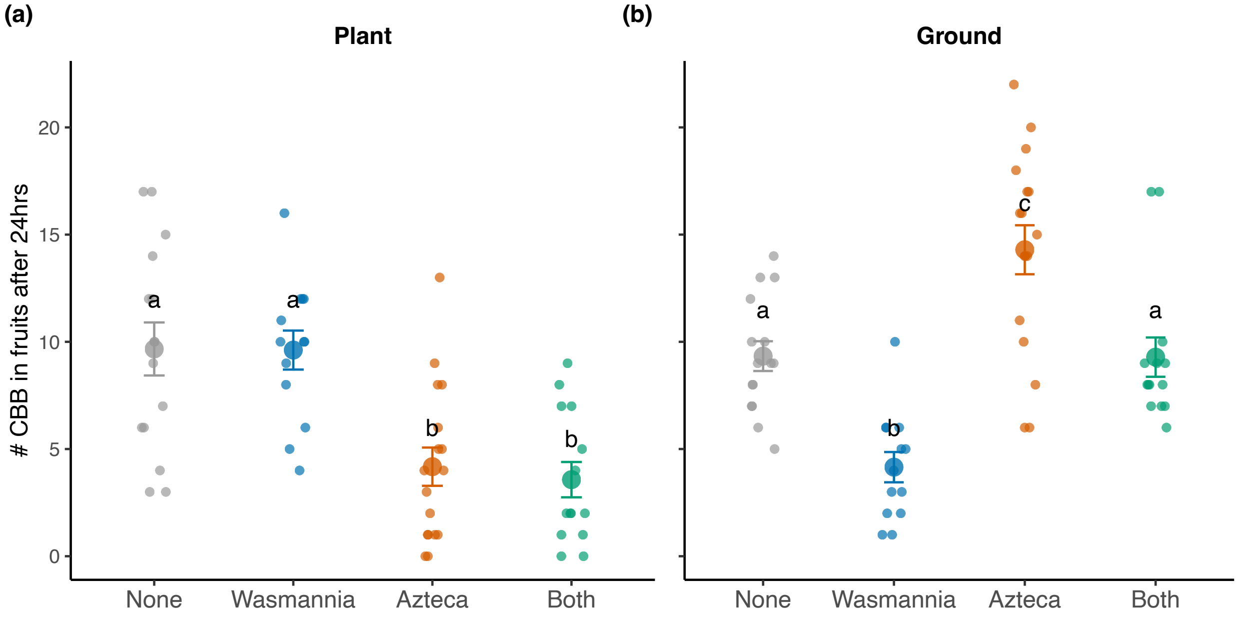
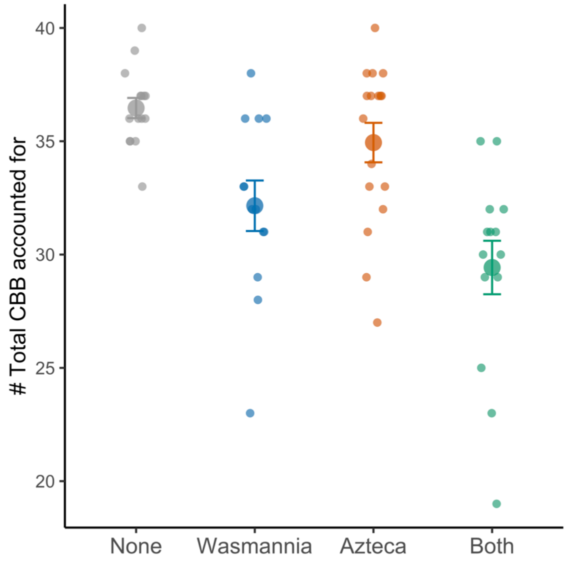


Figure 2.5 – Number of coffee berry borers (CBB) in fruits on the plant and ground in mesocosms. Shows the total number of CBB found bored into fruits on the plant (a) and the ground (b) at the end of the lab experiment after 24 hrs. Raw data are shown for each treatment along with the mean (± SE). Significant differences in means (from generalized linear mixed models with Poisson error distribution and log link) for each figure are indicated with different letters.

2.9 Supplementary Material

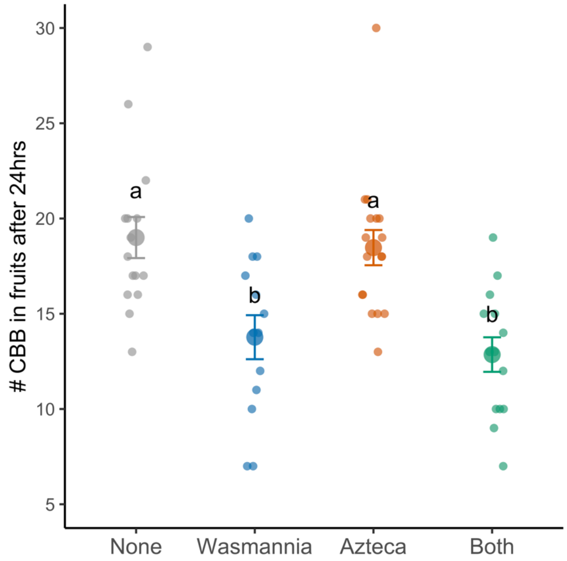
Tracking borer movement in experimental mesocosms

Independent borer movement, either falling or flying, was possible in all laboratory mesocosm treatments, including our control. Because mesocosms were open, it was possible for flying CBB to escape. To assess this possibility, we tracked all borers after the trials were finished and counted all individuals that were observed in fruits (one borer per hole) and outside of fruits on plants and the ground in mesocosm tubs. These data indicate that there is no difference in borers accounted for between the no ant treatment and the *Azteca* treatment, as expected given the near-0 consumption rate by *Azteca* that we found in the field. In both the control and *Azteca* treatments the number of accounted for borers is ~35 which is fairly close to the 40 used in our treatments, indicating that few borer individuals are actually leaving the mesocosms. In the *Wasmannia* only and both ant treatments, accounted for borers are slightly less than the control which is likely due to consumption by *Wasmannia*.



Supplementary Figure 2.1 - Overall number of coffee berry borers (CBB) accounted for. Shows total borer individuals accounted for at the end of experimental trials. Means (+- SE) shown with larger points and error bars.

Supplementary results from multi-predator experiment



Supplementary Figure 2.2 – Overall number of coffee berry borers (CBB) in fruits. Shows the total number of CBB found bored into fruits at the end of the lab experiment after 24 hrs. Raw data are shown for each treatment along with the mean (± SE). Significant differences in means (from a generalized linear mixed model with Poisson error distribution and log link) are indicated with different letters.

Chapter 3 A Non-Consumptive Effect and Prey Spatial Structure Mediate Multi-Predator Interactions and Dynamics in Pest Control[2]

3.1 Abstract

Non-consumptive effects (NCEs) have been demonstrated to be just as important as consumptive interactions for determining the structure and stability of ecological communities. Yet there is limited work exploring their dynamical impact in empirical communities, especially in natural pest control systems where they are likely ubiquitous and play a central role in regulating pests. A particularly interesting scenario occurs when the NCE of one predator drives spatial structure in a prey population, where it is then consumed by an alternative predator in another spatial pool. We modeled this general scenario using an empirical example of coffee pest control, where two natural enemies consume a coffee pest, on plants and the ground, and the NCE of the plant enemy drives spatial coupling between the pest pools. We developed a modified Rosenzweig-MacArthur predator-prey model with bi-directional migration where the NCE amplifies downward movement of the pest. Initial model simulation demonstrated how forced coupling, with downward migration only, results in chaotic, complex, and asynchronous dynamical behavior. Fully coupling the model, with both upward migration and the NCE eliminated much of these complex dynamics, returning the system to stable limit cycle behavior and synchronizing dynamics. The NCE also resulted in spatial complementarity between the

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predators and one-way facilitation of the ground ant by increasing its resource pool. Pertinent to pest control, we found that increasing the strength of the NCE in our model largely reduced pest densities and predator variability across simulations, both on the plant and the ground. Overall, our results show the importance of considering the general dynamics of non-consumptive effects and how these interactions can result in non-intuitive, but potentially positive impacts in natural pest control.

3.2 Introduction

Higher-order and trait-mediated interactions shape the structure of ecological communities and play an important role in determining their stability (Bairey *et al.* 2016; Grilli *et al.* 2017). In food-webs, they can have cascading impacts on communities that are equivalent to direct, density-mediated effects (Preisser *et al.* 2005; Schmitz *et al.* 2004; Werner & Peacor 2003). Of particular interest are non-consumptive effects (NCEs), where a predator (or natural enemy antagonist) impacts a prey (or resource) negatively without directly consuming it, often resulting in indirect effects on other species (Wirsing *et al.* 2021). These interactions often impact prey behavior or development, causing shifts in diet and habitat use that can influence food-web structure and dynamics (Bolker *et al.* 2003; Schmitz 2008). Non-consumptive effects can mediate multi-enemy interactions where the NCE of one natural enemy can indirectly benefit others if prey resources are made more available (Davenport & Chalcraft 2013). NCEs may also explain the keystone role of certain natural enemies if their presence results in outsized impacts on their respective communities through prey behavioral shifts (Meadows *et al.* 2017; Preisser & Bolnick 2008). Despite their central role in ecosystems, and some important efforts to generalize theory (Larsen 2012; Terry *et al.* 2017), there is less understanding of the dynamical impact of non-consumptive effects in empirical systems (Bolker *et al.* 2003), especially in natural pest control, where these interactions are likely ubiquitous (Culshaw-Maurer *et al.* 2020).

While natural pest control (the promotion of natural enemy communities to regulate pests in agroecosystems) holds much promise as a sustainable alternative to pesticide use (Bianchi *et al.* 2006), a general framework for its management has remained elusive (Karp *et al.* 2018; Rosenheim *et al.* 2022; Tscharntke *et al.* 2016). This is in part, because natural pest control communities are complex ecological systems that require study of both interaction complexity and dynamics (Eubanks & Finke 2014; Vandermeer *et al.* 2010, 2019). Even relatively simple natural pest control communities can host an array of interactions giving rise to complex and sometimes unexpected dynamics (Vandermeer 2023). To better understand how these systems function, and to better inform their management for more effective, sustainable pest control, increased focus on the interaction mechanisms driving their dynamics is essential (Crowder & Jabbour 2014; Eubanks & Finke 2014; Hermann & Landis 2017). Because pest control is typically considered from a consumptive lens, its ecological study has only recently begun to embrace the importance of non-consumptive effects for regulating pests (Culshaw-Maurer *et al.* 2020). In particular, there is a need to better understand how NCEs impact pest population dynamics and stability, and the cascading impact of these interactions on crop yields (Culshaw-Maurer *et al.* 2020; Sheriff *et al.* 2018). However, these questions have proved difficult to study empirically, due to the longer time scales required (Culshaw-Maurer *et al.* 2020; Sheriff *et al.* 2018).

A particularly interesting, and classic, non-consumptive effect scenario occurs when natural enemies scare or antagonize prey, causing them to seek refuge or shift their habitat (Bolker *et al.* 2003; Wirsing *et al.* 2021). The spatial structure that is generated or amplified by this interaction results in multiple spatial pools of the prey population, where prey can be consumed by other predators in the alternative pool. This scenario probably occurs frequently in natural pest control systems, as natural enemies cause pests to move up and down plants, to the ground, or between crop fields (Culshaw-Maurer *et al.* 2020). This type of NCE can mediate multi-predator interactions (see Losey & Denno 1998 for a classic example), suppress prey behavior (Thaler & Griffin 2008), and reduce crop damage (Culshaw-Maurer *et al.* 2020). From a dynamical standpoint, this interaction drives the spatial coupling of predator-prey oscillatory systems, which is likely to have important consequences for population dynamics and stability. More general theoretical work has extensively explored the dynamics of spatially coupled consumer-resource systems (Gramlich *et al.* 2016; Jansen 2001; Koelle & Vandermeer 2005). There is also some theoretical work investigating non-consumptive effects on altered prey behavior (Ives & Dobson 1987) and refuge seeking (Abrams 2008; Sih 1987). However, there is very little theory addressing the dynamics of NCEs in multi-enemy natural pest control scenarios, which is surprising, considering that these systems are inherently dynamical (Terry *et al.* 2017).

Here we seek to combine these perspectives, from natural enemy ecology, non-consumptive effects, and spatially coupled oscillators, and explore their dynamics in natural pest control. We model a general scenario of an NCE driving spatial coupling between predator-prey systems which is inspired by an empirical case study of natural pest control in coffee. In our example system, two natural enemy populations consume a coffee pest in distinct spatial pools, on plants and the ground, and the NCE of the plant natural enemy amplifies the movement of the pest from the plant to the ground, creating a potentially interesting dynamic, distinct from direct consumer-resource scenarios. While we use this empirical system to guide our exploration of the phenomenon, our modeling approach is general and should apply qualitatively to other systems of NCE-spatial dynamics, both in natural and agro-ecosystems. We focused our model analysis on three central questions: 1) What is the impact of pest spatial structure and the non-consumptive effect on system dynamics and stability? 2) Does the NCE mediate positive interactions between predators? 3) How does the NCE impact overall pest control function and efficiency?

3.3 Modeling approach

3.3.1 Modeling Framework & Case Study Natural History

Here we model a general three-species system, with two predators consuming the same prey species in two distinct spatial pools. Prey migrate between the spatial populations in both directions and the non-consumptive effect of one predator amplifies the outward migration rate of its prey (Fig. 3.1a). For the purposes of understanding the impact of these interactions in pest control, we ground our general modeling framework in a real-world example (Fig. 3.1b) from a coffee agroecosystem in Chiapas, Mexico, which we have studied extensively in the field (Morris & Perfecto 2022). The prey is a major insect pest of coffee, the coffee berry borer (*Hypothemeus hampei*), and the two predator species are the arboreal ant *Azteca sericeasur* and the ground-foraging ant *Wasmannia auropunctata.* For simplicity, we refer to these species as the borer, arboreal ant, and ground ant throughout the rest of this article. The borer is a specialist pest of coffee, using only coffee fruits for consumption and reproduction (Damon 2000). The arboreal ant species interacts with borers on coffee plants while they are colonizing fruits and either consumes them or throws them off the plant while they tend hemipterans (scale insects) for honeydew resources (Jiménez-Soto *et al.* 2013; Morris & Perfecto 2022). To keep the model tractable, we focus only on the arboreal ant's interactions with borers in our model (and not their alternative resources). The ground-foraging ant interacts with and consumes borers while they are on the ground (Morris & Perfecto 2022). Borers move off plants during dispersal to colonize other plants or when bored fruits senesce and fall from plants (Damon 2000). This creates a potential pool of borers on the ground. Borers also disperse from these fruits and move back onto plants, resulting in two-way movement, downward from the focal plant and upward from the ground. For generality sake, we refer to borer movement as upward and downward migration (where this could also represent the movement of pests between crop fields or prey between habitats). Our understanding of these interactions is based on years of study and observation of the natural history of this pest and the community ecology of its regulation on coffee farms in southern Mexico (Jiménez-Soto *et al.* 2013; Morris & Perfecto 2022; Vandermeer *et al.* 2010, 2019).

3.3.2 Model

We use a modified Lotka-Volterra approach, resembling the well-known Rosenzweig-MacArthur model, with bi-directional prey migration and the non-consumptive effect of one of the predators, all modeled with ordinary differential equations (ODEs). The pest grows in both spatial populations independently (B_p and B_g), following a standard logistic growth model with intrinsic growth (r_i) and carrying capacity (K_i) (Eqs. 1 and 3). Both predators (A and W) consume borers in their respective pools (Eqs. 2 and 4) following a type-II Holling functional response (f_{ij}, Eq. 5), assimilate prey resources at a rate of b_i (conversion efficiency), and have their own independent loss terms, where m_i represents the per capita mortality rate. Upward (d_u)

and downward (d_d) pest migration is modeled diffusively in both borer pools by altering the overall growth of the population at a rate proportional to the number of borers present and is lossless and instantaneous (see Gramlich et al. 2016 for further discussion of modeling similar spatial dynamics). The downward migration term is then modified by the arboreal ant's non-consumptive effect (c) on the plant borer population, where this term increases linearly with respect to the arboreal ant population. This approach to modeling non-consumptive effects is somewhat unique, in that more general approaches typically incorporate NCEs into consumer functional response terms (Larsen 2012; Terry *et al.* 2017), while there are fewer examples of modeling NCEs explicitly in spatial dynamics.

$$\frac{dB_p}{dt} = r_{B_p}B_p\left(1 - \frac{B_p}{K_{B_p}}\right) \; - \; f_{B_pA}B_pA \quad - \quad d_d(1+cA)B_p \quad + \quad d_uB_g$$

Eq. 1

$$\frac{dA}{dt} = b_Af_{B_pA}B_pA \quad - \quad m_AA$$

Eq. 2

$$\frac{dB_g}{dt} = r_{B_g}B_g\left(1 - \frac{B_g}{K_{B_g}}\right) \; - \; f_{B_gW}B_gW \quad + \quad d_d(1+cA)B_p \quad - \quad d_uB_g$$

Eq. 3

$$\frac{dW}{dt} = b_Wf_{B_gW}B_gW \quad - \quad m_AW$$

Eq. 4

Where,

$$f_{ij} = \left(\frac{a_j}{1 + h_ja_ji}\right)$$

Eq. 5

is the functional response of predator j on prey i, and a_j represents the predator attack rate and h_j the predator handling time. For ease of reference, all parameters are also listed and defined in table 3.1 and model interactions are diagramed in Figure 3.1a.

3.3.3 Simulation Approach

For this study, we relied on numerical analysis to understand model dynamics, since systems with several populations make local stability analysis challenging, and because the complex dynamics which arise in multi-dimensional systems often require numerical integration to assess broader global stability (Hastings & Powell 1991)**.** All numerical analysis of our model was performed using simulations primarily under different conditions of the three novel parameters of interest – the upward (d_u) and downward (d_d) migration rates, and the non-consumptive effect (c). We focused on these parameters to understand how prey spatial structure interacts with the non-consumptive effect (NCE) to mediate multi-predator interactions and pest dynamics. In particular, we manipulated NCE and not the consumptive parameters in our model to isolate its impact, which is often challenging in empirical studies, especially when measuring dynamical behavior (Culshaw-Maurer *et al.* 2020).

We took an iterative approach to numerical analysis, starting with the simplest version of the model, then increasing model complexity in a step-by-step fashion. Numerical analysis was initiated with all three of our novel parameters (upward (d_u) and downward (d_d) migration, and the non-consumptive effect (c)) set to 0, reducing the model to two separate predator-prey oscillators with no interaction, where all non-focal parameters were set to produce limit cycle behavior. We used this as a starting point for model analysis, as it is common practice for understanding the dynamics of coupled consumer-resource oscillatory systems (Hastings &

Powell 1991; Jansen 2001; Koelle & Vandermeer 2005) and because limit cycle behavior in the non-coupled Rosenzweig-MacArthur (which this system represents when the novel parameters are zeroed) has been well studied (Jansen 2001).

From this starting point, we then explored the forced coupling formulation of the model, where downward migration (d_d) of the borer couples both predator-prey systems by linking the prey spatial pools, while the upward migration (d_u) and non-consumptive effect (c) parameters were maintained at 0. Under these conditions, we ran systematic parameter sweeps across a range of downward migration rates. Bifurcation, Lyapunov exponent estimation, and phase plane analysis (elaborated below) were used to understand the dynamical behavior of our model under these conditions. Subsequently, we performed similar numerical analyses, with non-0 values of upward migration, and with non-0 values of the non-consumptive effect, to model the fully coupled system, with various analyses to summarize dynamical trends across three-dimensional parameter space (described below). All simulations were run for 1000 time steps with data output at every half time step.

3.3.4 Parameterization & Initial Conditions

Prey carrying capacity and initial population densities were informed partly by the natural history literature available and partly by personal field observation, for our case-study focal system. The coffee berry borer is known to reach particularly high densities on plants, and since coffee plants are sometimes observed with near 100% infestation, infested fruits can have many borer individuals after reproduction takes place, and plants typically produce hundreds of fruits (Barrera 2008), we set the borer carrying capacity to $K = 500$. Initial starting densities for borers were set to $B_p\ (0) = 100$ individuals on plants and $B_g\ (0) = 50$ on the ground since ground populations in senesced fruits near plants are likely less than those on that actual focal plant. Ant predator initial conditions were set to fewer individuals ($A\ (0) = 5$, $W\ (0) = 5$), since there are likely fewer ant workers on any individual coffee plant or point on the ground than borers. These numbers are more difficult to base in natural history given the fact that the reproductive unit of ants is at the colony level which is comprised of many thousands of individuals. However, we did not find a significant qualitative impact of initial conditions on our simulation results, and generally, it is typical to see much greater abundances of prey than predators in ecological communities.

All other non-focal parameters were set to produce limit cycle behavior in the uncoupled base model when the novel parameters of interest were set to 0, as described above. Unless specifically noted, we used the following values for our non-focal parameters throughout our analysis: $r_i = 2,\ K_i = 500,\ a_j = .1,\ b_j = .1,\ h_j = .2,\ m_j = .3$, where i refers to either prey population and j to either predator.

3.3.5 Analysis

Our general analytical approach in developing theory around this non-consumptive interaction focused on measuring the population densities, variance, and elements of stability from dynamics (Bolker *et al.* 2003). With numerical simulation, our aim was to understand broader trends in global stability but constrained mainly to the novel parameters of interest in our model – the downward (d_d) and upward (d_u) migration terms, and the non-consumptive effect (c). Although there are many measures of stability, as has been thoroughly discussed in the ecological literature (Grimm & Wissel 1997), we focused particularly on the variability or constancy of dynamics across time. This was for practical reasons, given our modeling and simulation approach, but was also driven by our questions in the context of pest control, where dynamical variability or unpredictability can have significant consequences.

3.3.5.1 Bifurcation Plots, Lyapunov Exponents, Phase Planes, and Poincare Sections

Bifurcation analysis was performed to understand the impact of individual parameters on the model's dynamical behavior. For this analysis, maxima and minima of simulation time series data were recorded after transient dynamics were removed (the first half of time series). These data were then plotted for a given parameter sweep to identify dynamical trends and zones of distinct behavior (e.g., chaos, limit cycles, equilibria). To augment this analysis, we calculated estimates of Lyapunov exponents to identify potential areas of chaotic behavior. This was done by comparing the time series of individual simulations to corresponding simulations with slightly altered initial conditions. We calculated the divergence of the two-time series by taking the log of the absolute value of their difference at each time step and extracting a linear regression coefficient from this data across time. Throughout the study, all bifurcation and Lyapunov exponent analyses correspond to the ground borer population (B_g), which we observed to exhibit complex dynamics upon initial simulation exploration, due to the directionality of one-way forcing from downward migration (d_a) (see Results).

In addition to bifurcations, we also plotted phase planes of dynamics and Poincare sections, particularly when complex, potentially chaotic, dynamics were observed. For phase planes, we combined the population densities of the two borer populations on the ground (B_g) and the plant (B_p) to visualize all population dynamics together in three-dimensional space and explore potential chaotic attractors (Glaum 2017). Exploratory strobic Poincare sections were also constructed for these areas, where we plotted the log of population densities from the ground oscillator (both the ant and borer) for all maxima of one of the populations from the plant oscillator (Vandermeer 2023), particularly in the zone where the ground oscillator exhibited chaos like dynamics (see Results).

3.3.5.2 Qualitative Dynamics & System Synchrony

To explore broader dynamical trends and stability across multi-dimensional parameter space in our model, we developed an algorithm to assess the qualitative dynamics of individual simulations. We first analyzed simulation output for equilibria by calculating the standard deviation (SD) of time series after removing transient dynamics. When the SD was 0 or very close to 0 we recorded this as equilibrium dynamics. If not at equilibrium, we used the output from our bifurcation analysis to identify local maxima and minima in each time series. From this, we measured the number of unique maxima and minima (Glaum 2014) to assess if simulations resulted in two-point cycles, four-point cycles, or multi-point cycles, which could include chaos or quasiperiodic behavior. In all cases, we removed transient dynamics from our analysis (the first half of time series) and rounded maxima and minima values to deal with slight differences in values from numerical simulation impression. We limited this analysis to the ground borer population (B_p) which was observed to exhibit more complex dynamics under forced coupling of the two oscillators with downward migration only (see Results).

We also sought to understand the patterns of synchrony in our system, which can play an important role in the dynamics of coupled oscillators, and has been explored extensively in consumer-resource spatial models (Gramlich *et al.* 2016; Jansen 2001; Koelle & Vandermeer 2005). While there are a number of potential approaches to measure synchrony in dynamics (Koelle & Vandermeer 2005), we limited our approach to the estimation of the Pearson correlation coefficient from time series outputs between both predator populations and both prey populations, since this was a secondary focus of our study.

3.3.5.3 Non-Consumptive Effect Trends

To augment our other analyses, and to gain insight specifically into the impact of the non-consumptive effect (c) (NCE) on system dynamics, we conducted systematic sweeps of this parameter across a range of upward (d_u) and downward (d_d) migration rates. From the time series output of these simulations, we calculated a few standard metrics to quantify aspects of their dynamical behavior, including mean population density, maximum density, and the coefficient of variation of the dynamics (CV). We then plotted these metrics across the NCE parameter range and ran regressions on this data to estimate the qualitative impact of increasing the NCE on these metrics for each population in the model. We ran generalized linear mixed models with gamma error distributions and log link functions (to account for strictly positive data) and extracted regression coefficients to estimate if increasing the NCE has an overall negative or positive effect on the given metric. We then plotted these coefficients across the multi-dimensional migration parameter space to understand the impact of the non-consumptive effect (NCE) across our focal parameter space. For this analysis any NCE sweep coefficients below 0 represent an overall reduction in the specific dynamics metric for a given population as NCE strength increases, allowing us to test the effect of this focal parameter both on general system stability and to approximate its qualitative impact on pest regulation. This analysis is visualized and explained more thoroughly in our supplementary material (Fig. S3.1).

3.4 Results

3.4.1 Forced coupling through downward migration

Model analysis was conducted from the reference point of the decoupled system of two predator-prey oscillators in simple limit cycle behavior (Fig. 3.2a). To understand the impact of spatial separation of the prey population into two distinct pools coupled by migration and a non-consumptive effect, we began with the forced-coupled system with downward migration (d_d) of the prey, while keeping upward migration (d_u) and the non-consumptive effect (c) at 0. A parameter sweep of the downward migration term (d_d) revealed zones of chaotic, quasiperiodic, and complex cyclic dynamics (Figs. 3.2, 3.3a, and 3.4), appearing to destabilize the simple limit cycle behavior of the uncoupled system (Fig. 3.2a). The chaotic zone appears suddenly at minimal levels of downward migration (Fig. 3.2b) (d_d~.05), moving abruptly from limit cycle dynamics into chaotic behavior (Fig. 3.4b), which resembles a "canard explosion" that has been observed in other forced coupled pairs of oscillators (Awal *et al.* 2019). The chaotic zone persists across a range of d_d with occasional reverse period doubling observed until roughly $d_d = .3$ (Fig. 3.4b). The chaotic nature of this zone is confirmed by the positive value of the estimated Lyapunov exponent across this range (Fig. 3.3a). Three-dimensional phase plane analysis also shows the quintessential strange attractor dynamics typical of chaos (Fig. 3.3b), which was further supported by a stroboscopic Poincare section taken from this zone (Fig. S3.2). Interestingly, this behavior was observed in both populations of the ground forced oscillator (the borer (B_g) and ant (W)), which receives the migrating borer individuals, but not in the plant forcing oscillator, which maintained limit cycle behavior across the observed chaotic zone – behavior which has been described as chimeric in other coupled oscillator systems (Awal *et al.* 2019). For all bifurcations and chaos-related analyses we assessed data from the ground borer

(B_g) population, although similar dynamics were consistently observed in its corresponding predator, the ground ant (W).

Upon increasing d_d beyond the chaotic area, a large zone of 4-point oscillatory dynamics was observed (Fig. 3.2c & 3.3a), which was followed by an apparent quasiperiodic zone (Fig. 3.2d & 3.4c), from roughly $d_d = 1.17$ to $d_d = 1.2$. Although the dynamics for this area were complex, Lyapunov exponent approximations did not suggest chaos (Fig. 3.3a). Finally, at higher levels of d_d the system returned to basic limit cycles (Fig. 3.2e & Fig. 3.3a), while the corresponding forcing oscillator populations went to equilibrium, with the arboreal ant reaching near 0 levels.

While we focused our analysis in this study on our three novel focal parameters and left all other parameters symmetrical between the two oscillators for our principal analysis, we also tested the impact of forced coupled dynamics for several asymmetrical parameter conditions to ensure that our results were robust and not just a unique result of system symmetry. We ran similar parameter sweeps of the downward migration term (d_d) in our forced coupled model (with $d_u = 0$ & $c = 0$) when the forcing oscillator predator had altered attack rate (a_A) and handling time (h_A) values (Fig. S3.3). Across nearly all instances, we observed the same general qualitative behavior in the forced coupled model, where chaotic, multi-point, and quasiperiodic dynamics were observed (Fig. S3.3), confirming that these trends are also found under asymmetric parameter conditions.

3.4.2 Full spatial coupling with the non-consumptive effect

Fully coupling the system with upward migration and activating the non-consumptive effect generally stabilized model dynamics by reducing variability and dynamical complexity, in most cases, returning the system to simple limit cycle behavior. For this analysis, we reran parameter sweeps of the downward migration (d_d) parameter with upward migration (d_u) and the non-consumptive effect (c) set to non-0 values, both separately and together. Fully coupling the system with upward migration alone eliminated the chaotic zone and complex cycles observed in the forced coupled system, resulting in two-point limit cycle behavior across the range of downward migration tested (Fig. S3.4). The forced-coupled system with the non-consumptive effect activated nearly eliminated the chaotic zone across the same range of downward migration and appears to mostly shift its behavior to quasiperiodic, given our Lyapunov exponent (LE) estimates. Initial quasiperiodic and complex cycles found with forced coupling only were also eliminated across their original zones with the NCE (Fig. S3.4). Fully coupling the system with the non-consumptive effect activated resulted in simple two-point cycles across the full range of downward migration tested (Fig. S3.4). LE analysis of three-dimensional parameter space confirmed the elimination of chaotic dynamics when the system was fully coupled with the NCE (Fig. 3.5a), although there was a small band of elevated LEs at intermediate levels of d_d and lower values of c, when $d_u = 0$ (Fig. 3.5b). Analysis of qualitative dynamics across the three-dimensional parameter space agreed with our other analyses in suggesting that the fully coupled system with both d_u and c activated reduced the complexity of dynamics and return the system to simple limit cycle behavior (Fig. 3.6a). However, when $d_u = 0$, we again saw some remnants of the complex multi-point dynamics observed in the forced coupled system (where $d_d > 0$, & $d_u = 0$ & $c = 0$), but mostly reduced dynamical complexity across a large range of positive d_d and c (Fig. 3.6b). Most of this behavior was either concentrated near the forced coupled zone where $d_u = 0$ and c was close to 0, or was observed in a curved band that spanned intermediate values of the additive sum of d_d and c (Fig. 3.6b). Beyond these values, as shown in the upper right part of the Figure 3.6b, the system returned to two-point limit cycle dynamics. Interestingly, for most of the curved band of multi-point dynamics observed in our qualitative dynamics analysis, the estimated LE was not significantly above 0 (Fig. 3.5b), implying that dynamics in this zone are likely quasiperiodic cycles.

Our synchrony analysis revealed that most asynchronous dynamics in our coupled oscillator system occurred while the model was either forced coupled when $d_d > 0$, & $d_u = 0$ & $c = 0$, or across certain zones when $d_d > 0$ & $d_u = 0$ but $c > 0$ (Fig. 3.7a). While increasing c from 0, when $d_d > 0$ & $d_u = 0$, initially pushed the forced coupled system from asynchrony into synchrony at lower levels of d_d and c, increasing beyond this range led to a curved band of strongly asynchronous dynamics (Fig. 3.7b) that aligned with the zone of multi-point, complex dynamics in our qualitative dynamics analysis (Fig. 3.6b). Beyond this zone, in the upper right of Figure 3.6b, dynamics were by default asynchronous, since both the predator and prey population in the plant forcing oscillator were at equilibrium (Pearson's correlation coefficient was undefined since the SD was 0, essentially equivalent to 0 correlation). Otherwise, positive values for all three of our focal parameters resulted in fully synchronous dynamics (Fig. 3.6a). While these results and our figures refer to the synchrony of the two predator populations, the prey populations displayed nearly identical behavior.

3.4.3 Non-consumptive effect general impacts

To further understand the impact of the non-consumptive effect on model dynamics, and specifically on pest control, we conducted a systematic analysis of the impact of increasing non-consumptive effect (c) strength across a range of downward (d_d) and upward (d_u) migration. Our non-consumptive effect trend analysis revealed that increasing NCE strength generally decreased the mean population densities observed in simulations of both populations of borers, with some exceptions in the case of the ground borer (Fig. 3.8 a & b). Importantly, this decreasing trend in mean borer densities with increased non-consumptive effect strength was held across the full range of downward and upward migration for the plant borer (B_p), the focal population in pest control (Fig. 3.8a). Interestingly, for the ground borer population (B_g), this trend was also observed across most migration conditions, except at low d_d and high d_u (Fig. 3.8b). For the same analysis, the two predator populations (A and W) exhibited distinct trends in mean density, where arboreal ant (A) densities decreased, and ground ant (W) densities increased, consistently with increased NCE strength across all bi-directional migration parameter space (Fig. S3.5).

Interestingly, increasing the strength of the non-consumptive effect also resulted in reduced variability of both predator populations (Fig. 3.8 c & d), as assessed by the trend in the population coefficient of variation from simulations across sweeps of the NCE. This was consistent across nearly all migration values tested, except at very low values of downward migration and high values of upward migration. Borer populations exhibited less pronounced trends in variability with increased NCE, where arboreal borer variability was mostly unchanged or decreased (when upward migration was low), and ground borer variability increased slightly across most migration parameter values (Fig. S3.5).

3.5 Discussion

This work explores the impact of non-consumptive effects on spatially coupled predator-prey systems and highlights their potential role in natural pest control dynamics. The results of our model analysis augment empirical research demonstrating the importance of non-consumptive effects in multi-predator systems and as indirect mechanisms of pest regulation (Culshaw-Maurer *et al.* 2020).

Initial model exploration sought to understand how both prey spatial structure and the non-consumptive effect impact system dynamics and stability. We approached this by focusing on the novel model parameters (downward migration (d_d), upward migration (d_u), and the non-consumptive effect (c)) and scaling up the complexity of the model by changing the value of these parameters. We found that forced coupling of the model, with only downward migration, generated complex, asynchronous dynamics and reduced the stability of the base model's limit cycle behavior (when all novel parameters were zeroed, equivalent to the well-known Rosenzweig-McArthur model). Forced coupling resulted in a chaotic zone, as well as multi-point and quasiperiodic cycles in the dynamics of the forced ground oscillator. Complex behavior is well-known in systems of forced oscillators and results from the imbalanced flow between oscillators (Awal *et al.* 2019). Contrastingly, fully coupling the model with bidirectional migration synchronized the system and reduced the dynamic complexity observed with forced coupling. This was observed consistently across several metrics, including our analysis of qualitative dynamics, bifurcation analysis and Lyapunov exponent estimates, and population correlations. Putting these results in an ecological context, two-way migration or movement is likely important for stabilizing the dynamics of coupled consumer-resource systems, especially when mediated by a non-consumptive effect. One can imagine that in most ecosystems, where predators are not omnipresent or always active, some return migration would be likely, allowing for increased stability in coupled systems (Wirsing *et al.* 2021).

Interestingly, activating the non-consumptive term in our model also reduced the complexity of forced-coupled dynamics and stabilized the system, both with, and independent of upward migration. This was true for most metrics and across most of the explored multi-dimensional parameter space, however, we did find a zone of intermediate values of d_d & c where more complex multi-point and quasiperiodic dynamics were observed when d_u was set to 0. We also found that the non-consumptive effect only partially resulted in synchrony between oscillators when there was no upward migration, where larger values of downward migration and the NCE pushed the plant oscillator into equilibrium and the ground oscillator into limit cycle dynamics, resulting in asynchrony. Regarding the reduction of complex dynamics and increased stability seen with the NCE, it is not immediately obvious why this occurs. One possibility is that the non-consumptive effect works as a kind of predator density dependence for the plant ant population, which we explore further, below in our discussion.

Beyond general system dynamics, we also sought to understand how the non-consumptive effect in our model mediates multi-predator interactions. Empirical studies of this question have revealed that NCEs can often promote facilitation between spatially separated predators by making prey more available, but sometimes also result in negative interactions (Culshaw-Maurer *et al.* 2020; Davenport & Chalcraft 2013). Our simulation results showed the NCE had a mostly positive effect on the ground ant, by making resources more available in the ground borer pool resulting in increased ground ant densities. However, intuitively, we found the opposite effect for the plant ant population. In the context of this simple model, because the plant ant has no other resources, the result of the NCE on its own resource pool should only reduce its growth rate (Culshaw-Maurer *et al.* 2020), however, it could have other benefits for the plant ant by reducing its variability, which is what we observed. Indeed, one reason we believe the NCE works to stabilize the overall system is by functioning as a kind of predator density dependence, where the plant ant regulates itself as it grows, keeping it from overexploiting its resource and displaying more variable population swings. Given our model results, it seems clear that the NCE drives spatial complementarity and at least one-way facilitation between predators, which aligns with previous empirical work on this specific study system (Morris & Perfecto 2022). Furthermore, in nature, the plant ant has a host of other resources available (Vandermeer *et al.* 2010), which would buffer the negative impact of the NCE reducing its resource supply.

Our final area of investigation sought to understand the overall role of this non-consumptive effect in this system of natural pest control. Our simulation results collectively suggest that the NCE has a positive impact on pest control dynamics. The non-consumptive effect promoted overall system stability by mostly eliminating complex chaotic and multi-point dynamics. We also saw reduced overall densities of the plant borer across nearly all migration parameter conditions and, interestingly, the ground borer across much of the range of bi-directional migration parameters tested. This suggests that the dynamical impact of the non-consumptive effect increased both the stability and efficiency of pest control. This is a significant result and is particularly interesting in the case of the ground borer. One potential explanation for this non-intuitive finding is that the increased flow of borers to the ground from the NCE helps to buffer population fluctuations in the ground ant, keeping it from crashing to lower values, which prevents the ground borer from exploding and reduces its mean density. This is also supported by the positive effect of ground ant density and reduction of ground ant variability that we observed when increasing the NCE. For the limited range of migration parameters where the NCE increased ground borer densities, this occurred only when $d_u > d_d$, however, this scenario is potentially less likely to occur in our system in nature, given that the greater population of borers is usually on the plant where the bulk of their resources are. Perhaps early in the growing season, when fruits are just beginning to develop on coffee plants, colonization of plants (upward migration) from borer movement out of berries on the ground might temporarily be higher than outward movement off plants. This would be an interesting modeling scenario to explore considering this seasonal dispersal dynamic in the system. In most other cases, we expect downward (or off-plant) migration rates to typically be higher, which given our findings may actually result in reduced pools of the ground borer. Additionally, it should be noted that the overall reduction in predator variability, for both populations, is also beneficial in making natural pest control more reliable across time.

The general qualitative findings from this study also align with previous empirical investigation of this specific pest control system in coffee, which shows that multiple spatially separated ant predators can result in more effective pest control when mediated by the non-consumptive effect of the arboreal ant (Morris & Perfecto 2022). However, empirical study of this system also suggests that this may be a somewhat unique NCE case, where actual consumption by the arboreal ant is quite low in the field (Jiménez-Soto *et al.* 2013; Morris & Perfecto 2022). A follow-up study could model the dynamical impact of alternative resources for the arboreal ant (such as honeydew from hemipteran insects), which may mediate seasonal variability in NCE strength (Morris & Perfecto 2022). That being said, this investigation was aimed at more general understanding of NCE dynamics in spatially coupled predator-prey (pest) systems and was not meant to precisely confirm empirical findings or to provide any prescriptive analysis. Indeed, we believe this modeling framing is general and should apply to a variety of non-consumptive dynamics, both in and outside of the pest control framework. While our approach was not an exhaustive exploration of dynamics, but rather a starting point, further research could seek to understand how other parameters in our model, in particular exploring more asymmetrical coupling, affect the overall dynamical findings we observed.

Natural pest control communities are complex ecological systems that require understanding of processes and system dynamics (Vandermeer *et al.* 2010, 2019). More research is needed on the dynamics of interaction mechanisms (Eubanks & Finke 2014), including non-consumptive effects, spatial coupling, and multi-predator interactions. Despite recent interest in trait-mediated and non-consumptive effects in the field of community ecology, there is still much less work attempting to link theory to empirical investigation (Bolker *et al.* 2003; Terry *et al.* 2017), especially in pest control (Culshaw-Maurer et al. 2020). Dynamical modeling approaches can augment empirical research of non-consumptive effects in pest control, which can be difficult to conduct over longer timescales and across a range of interaction conditions (Culshaw-Maurer *et al.* 2020; Hermann & Landis 2017; Sheriff *et al.* 2018). Studying the dynamics of these complex interaction mechanisms in natural pest control, and consumer-resource systems more broadly, will be crucial for improving the management of agroecosystems and for better understanding the stability of ecological communities.

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We thank Paul Glaum for invaluable input on model analysis and code development. Additionally, we thank Annette Ostling and Mark Novak for feedback on analysis, and Zachary Hajian-Forooshani for assistance with code development and analysis. We thank John Thiels at

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3.8 Tables & Figures

Table 3.1 – List of model parameters. Predator and prey parameters were fixed in most instances, while focal parameters were manipulated for systematic numerical analysis and model understanding. General predator and prey parameters listed here apply to both individual predator (arboreal ant (A) and ground ant (W)) and prey (plant borer (B_p) and ground borer (B_g)) populations.

Parameter	**Definition**
Predator parameters (for A and W)	
a_i	Attack rate
h_i	Handling time
b_i	Conversion efficiency
m_i	Mortality
Prey (pest) parameters (for B_p and B_g)	
r_i	Intrinsic growth rate
K_i	Carrying capacity
General focal parameters	
d_d	Downward migration of the pest
d_u	Upward migration of the pest
c	Non-consumptive effect (arboreal ant throwing pest off plant)

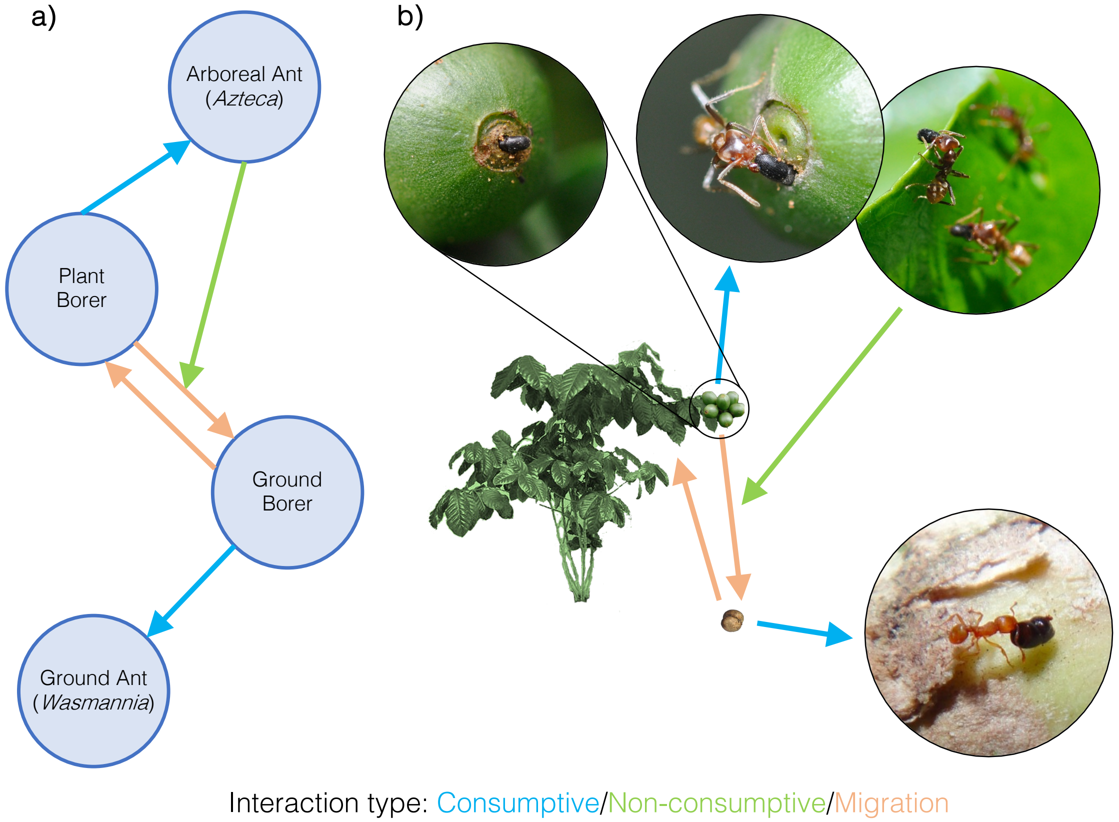


Figure 3.1– Diagram of the full model and case-study natural history. In **a & b)** arrows indicate the direction of consumption, movement, or the impact of the non-consumptive effect. Blue arrows show consumptive interactions, orange arrows show migration, and green arrows show the non-consumptive effect. In **b)** photos of the pest (the coffee berry borer), the arboreal ant (*Azteca sericeasur,* shown manipulating the borer), and the ground ant (*Wasmannia auropunctata,* also with the borer) are depicted.

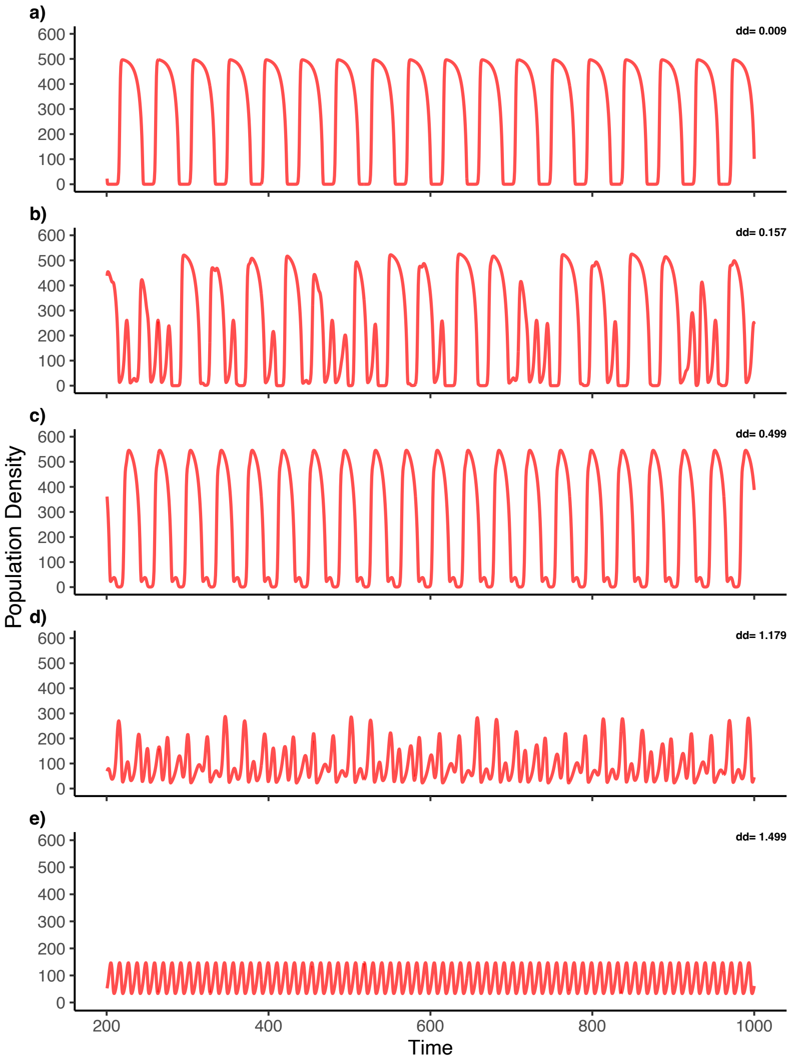


Figure 3.2 – Example time series. Shows example time series plots from simulations for the ground borer population (B_g) across a range of downward migration (d_d) values. In all cases, both upward migration (d_u) and the non-consumptive effect (c) are set to 0, eliciting forced-coupled oscillatory dynamics. **a)** Shows the base oscillatory limit cycle dynamics when the value of d_d is close to 0. In **b)**, as d_d increases we see chaotic dynamics in B_g, which is receiving the migrating individuals. In **c)** four-point cyclic dynamics are observed, while in **d)** we see quasiperiodic multi-point dynamics. Finally, in **e)** we observed a return to two-point cycles with high values of d_d. Specific values of d_d for each simulation are displayed in the top right of each figure.

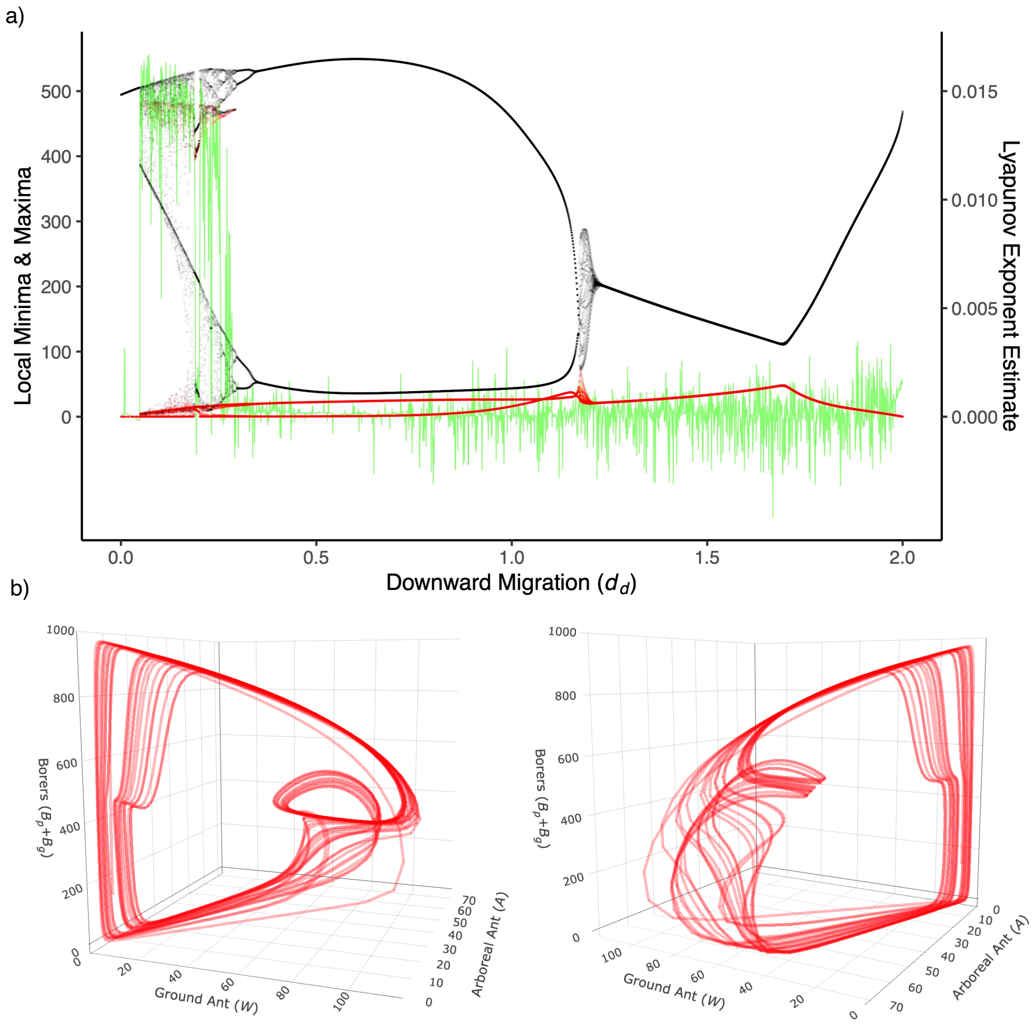


Figure 3.3 – Bifurcation plot and chaotic attractor. In **a)** the full bifurcation plot of the downward migration term (d_d) is shown for the forced coupled dynamics (when both d_u and c are set to 0) with the corresponding Lyapunov exponent estimate (green line). Black points represent local maxima and red points local minima. All data are assessed from the ground borer population (B_g). In **b)** a three-dimensional phase plane of the chaotic attractor is shown for $d_d = .229$, which is within the chaotic zone in a) where the Lyapunov exponent estimate is significantly positive. To display all four populations from the model, the total borer population from the plant (B_p) and ground (B_g) is summed on the vertical axis. Two angles of the same three-dimensional plot are shown for perspective.

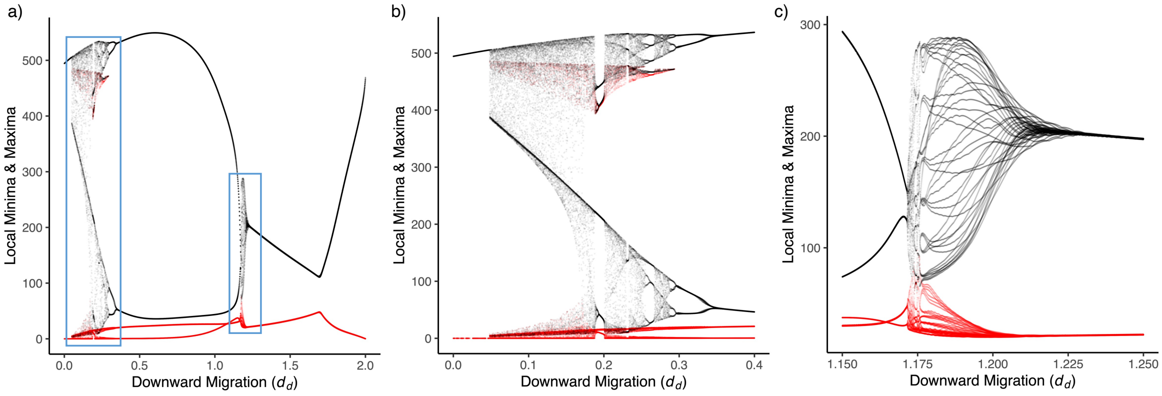


Figure 3.4 – High-resolution bifurcation plots. All plots show bifurcation diagrams of the downward migration parameter (d_d) when the model is forced coupled, with d_u and c set to 0. Points are the local maxima (black) and minima (red) of the ground borer (B_g) population. The full parameter sweep from Fig. 3.3a is shown again here in **a)** to illustrate the two focal zones depicted in b) and c) with blue boxes. **b)** Shows an expanded bifurcation plot of the chaotic zone from the left blue box in a). **c)** Shows an expanded bifurcation plot of the apparent quasiperiodic zone from the right blue box in a).

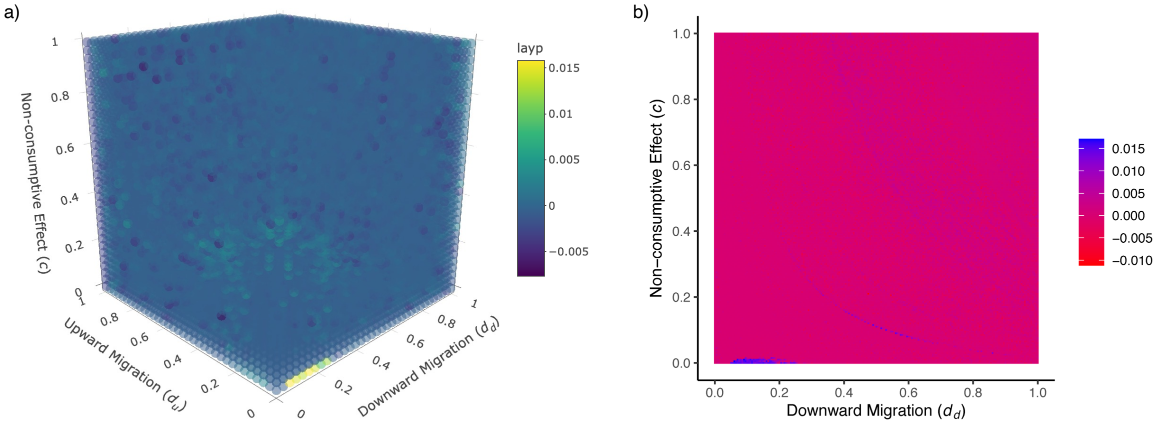


Figure 3.5 – Lyapunov exponent estimates. Figures show Lyapunov exponent estimates (LE) from numerical simulations across multi-dimensional parameter space. In **a)** LEs are shown in three-dimensional parameter space for a range of values across both migration parameters (d_d and d_u) and the non-consumptive effect (c). In **b)** LEs are shown for higher resolution parameter space across d_d and c. Points that are more yellow or green in a) and bluer in b) indicate more positive LE values associated with chaotic behavior. All LEs are assessed in the ground borer (B_g) population.

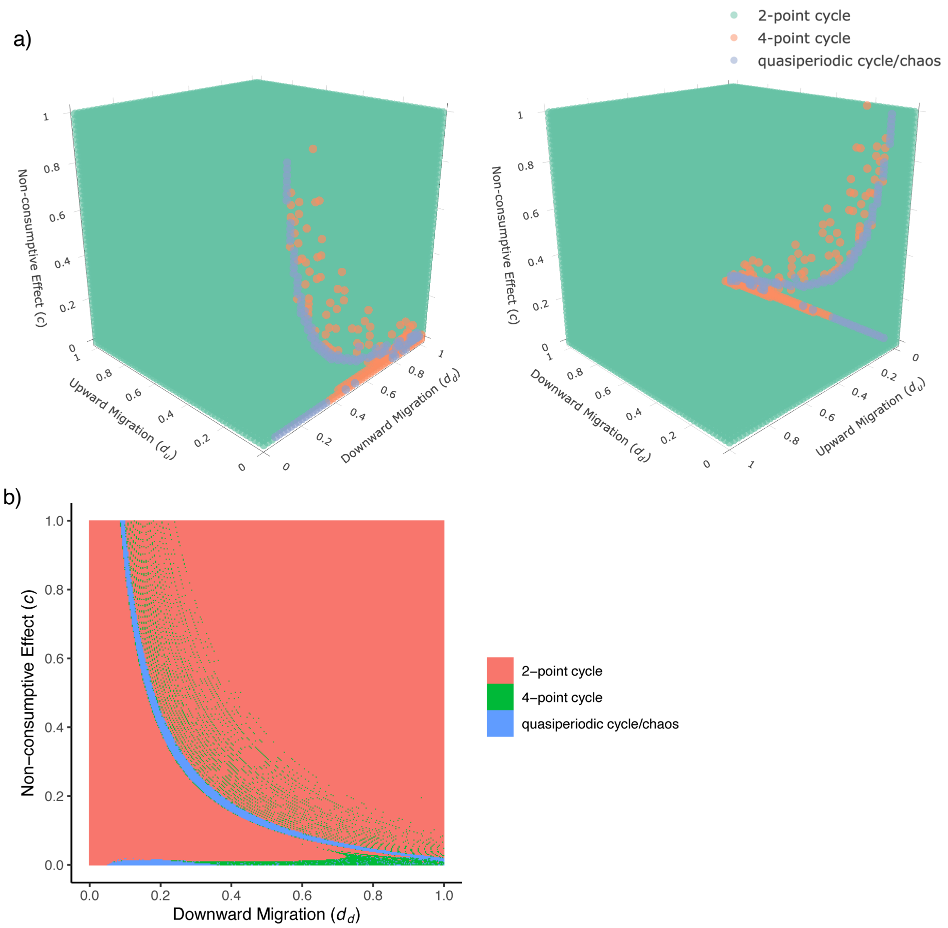


Figure 3.6 – Qualitative dynamics. Figures show general qualitative dynamics from numerical simulations. In all figures, colors represent three different qualitative states: 2-point cycles, 4-point cycles, and multipoint cycles, which may represent either quasiperiodic or chaotic dynamics. In **a)** qualitative dynamics are shown in three-dimensional parameter space for a range of values across both migration parameters (d_d and d_u) and the non-consumptive effect (c). The two images show different visual perspectives of the same three-dimensional figure. In **b)** qualitative dynamics are shown for higher resolution parameter space across c and d_d. All dynamics are assessed in the ground borer (B_g) population.

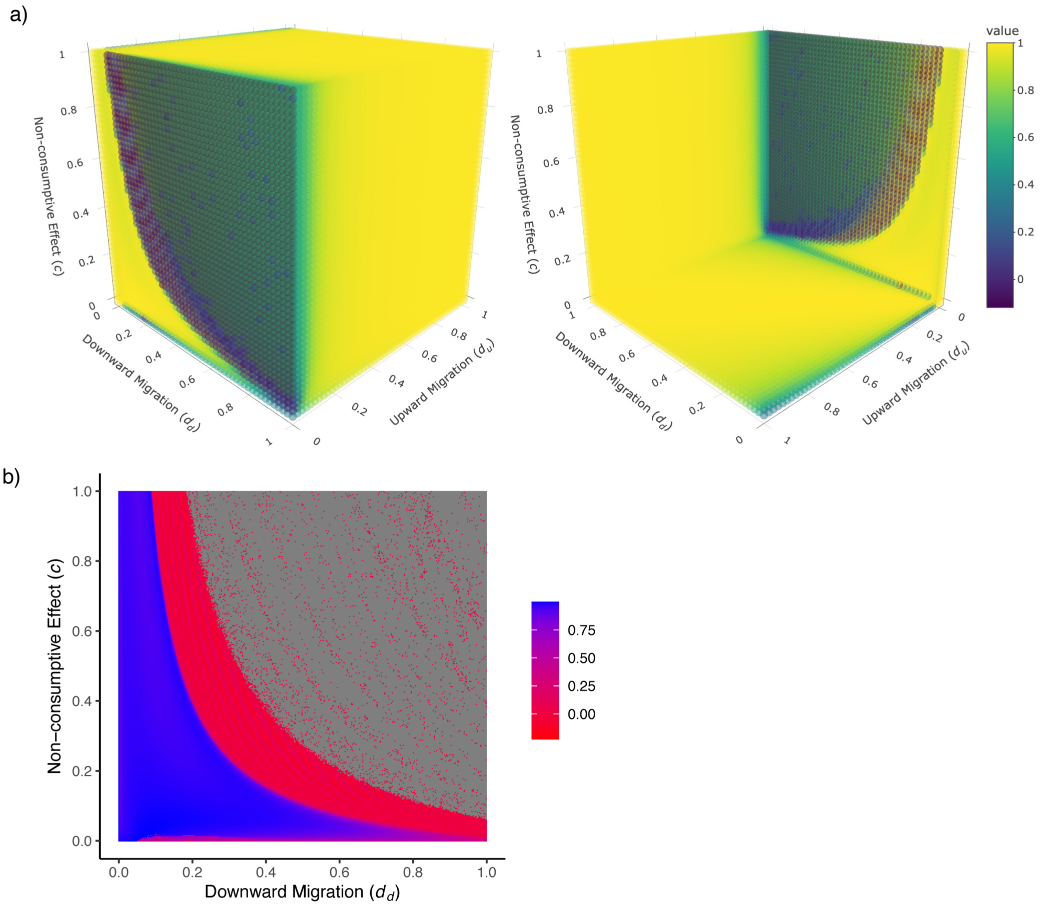


Figure 3.7 – Assessing synchrony with population correlations. All figures show the Pearson correlation coefficients of simulations between the two predator populations, the arboreal (A) and ground (W) ants. In all cases, correlations were assessed after removing transient dynamics. In **a)** correlations are shown in three-dimensional parameter space for a range of values across both migration parameters (d_d and d_u) and the non-consumptive effect (c). The two images show different visual perspectives of the same three-dimensional figure. In **b)** correlations are shown for higher resolution parameter space across c and d_d. The grey area in b) represents correlations that are by default 0 when A was at equilibrium, and thus had a standard deviation of 0.

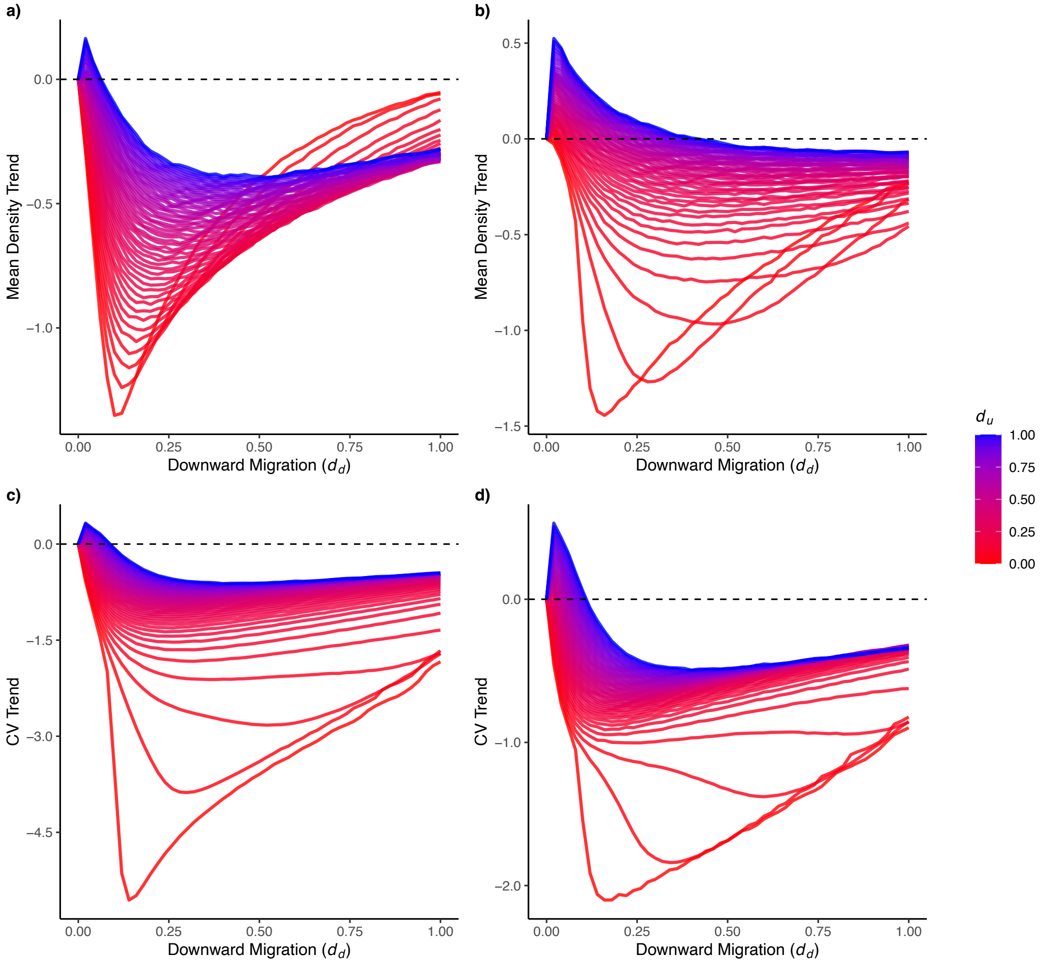
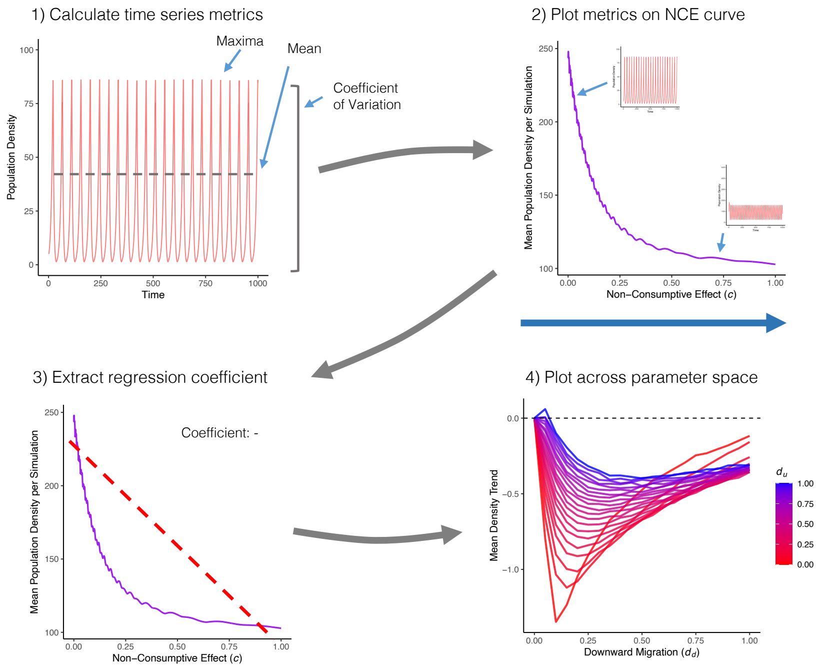
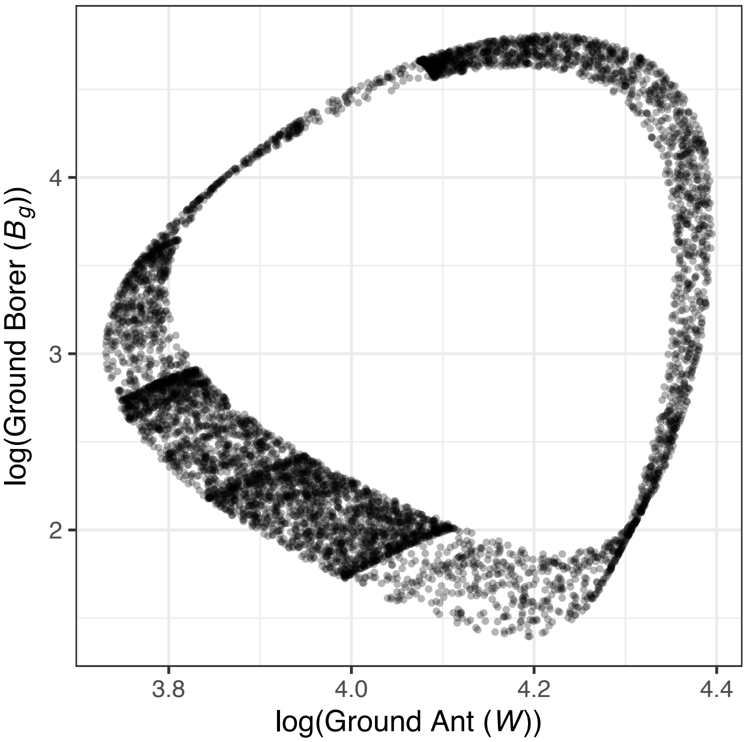


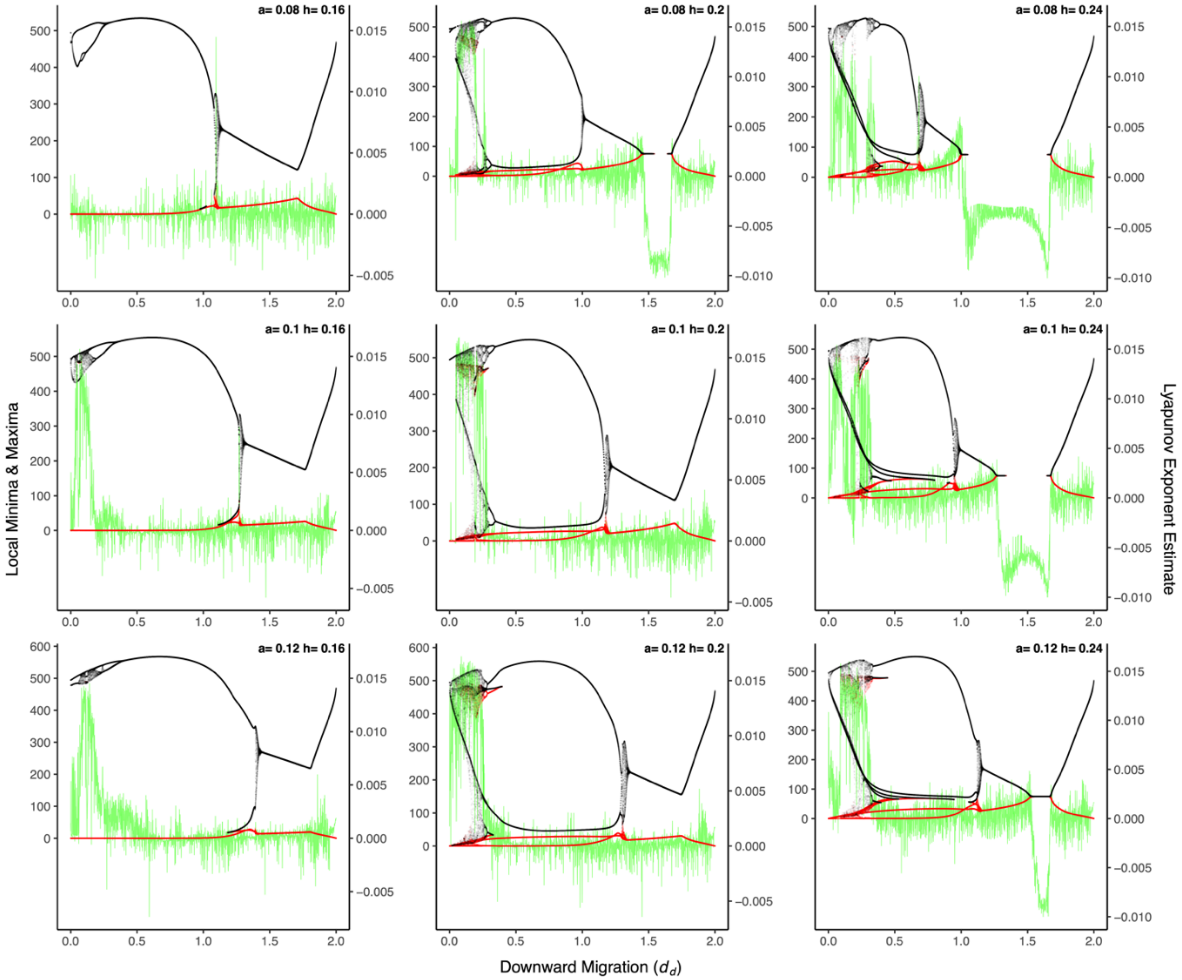
Figure 3.8 – Non-consumptive effect trend analysis. Plots show the qualitative impact of increasing the non-consumptive effect (c) (NCE) on various dynamical metrics for different populations. Line points represent coefficients from regressions (generalized linear models with gamma error distributions and log link functions) of the NCE parameter space against either **a & b)** mean prey density or **c & d)** predator population variation (coefficient of variation). These values are plotted across a range of downward (d_d) and upward (d_u) migration parameter values. Values above or below the dashed zero line in plots indicate a general positive or negative impact of increasing NCE strength on the given metric. In **a & b)** the mean population density trend is plotted for the plant borer (B_p) and ground borer (B_g), respectively. In **c & d)** the coefficient of variation trend is plotted for the arboreal ant (A) and ground ant (W), respectively.



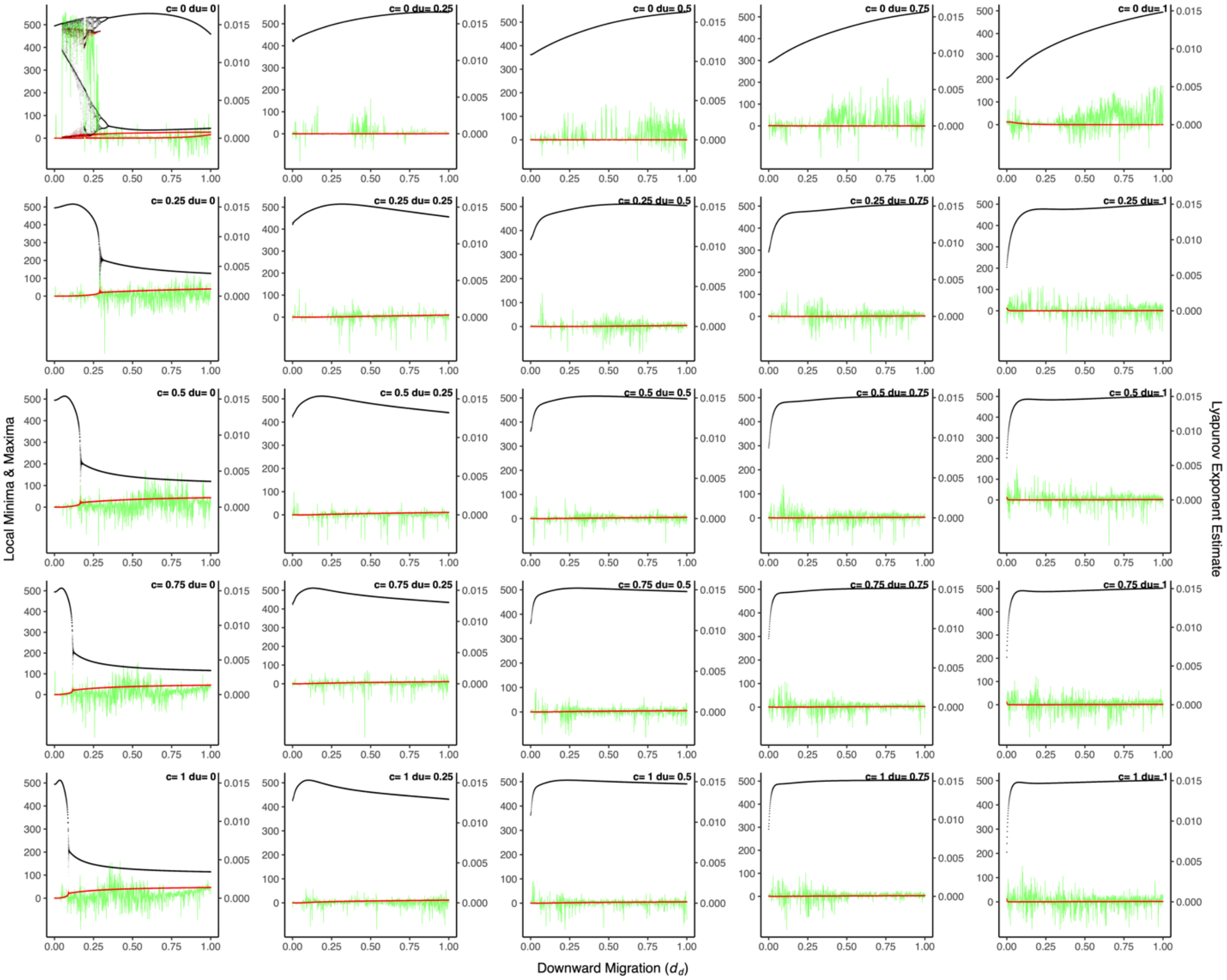
Supplementary Figure 3.1 – Non-consumptive effect (c) trend assessment. This figure illustrates our approach to assessing the qualitative impacts of activating and increasing the non-consumptive effect (c) (NCE) term in our model. We measured its impact on several metrics of the simulated dynamics for all populations in our model and used this approach to generalize those impacts across the multi-dimensional parameter space of our two migration terms (d_d and d_u). In **1)** we calculate basic summary statistics and metrics for the time series of individual simulations. We assess the mean, maxima, and coefficient of variation of each time series after removing transient dynamics. In **2)** we plot these metrics, independently, across the NCE parameter space to understand the general trends observed while increasing the NCE for each population. In **3)** we run a regression analysis (generalized linear mixed models with gamma error distributions and a log-link function) of the NCE curve and extracted the coefficient to estimate trends in our summary metrics from increasing the NCE while holding other parameters fixed. Finally, in **4)** we plot the regression coefficients across the two migration parameters to assess the general qualitative impact of increasing the strength of the NCE in our model. When the plotted curves fall below the horizontal 0 line, this indicates that increasing the NCE generally reduces the focal metric across the given parameter range.



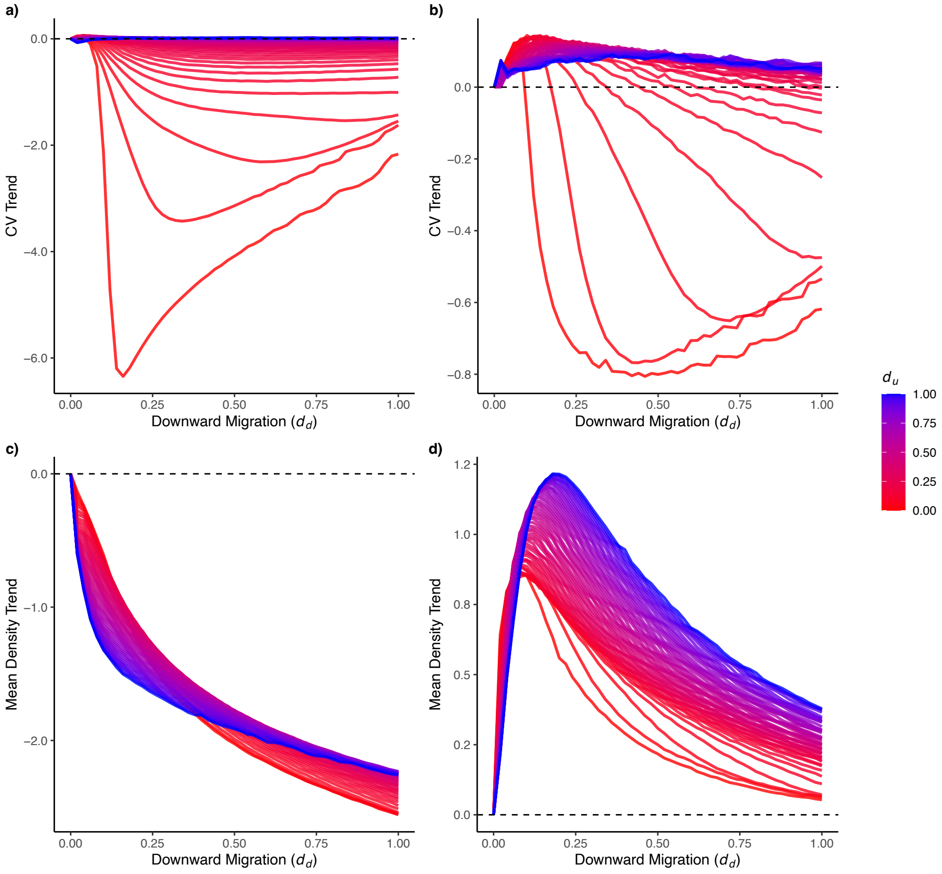
Supplementary Figure 3.2 – Example Poincare section for the dynamics of the chaotic oscillator of the ground borer (B_g) and ground ant (W) populations. The densities of the two populations are plotted stroboscopically for all maxima in the arboreal ant (A) dynamics. Downward migration was set to $d_d = 0.22$ for this example, within the observed chaotic zone when both upward migration (d_u) and the non-consumptive effect (c) were set to 0.



Supplementary Figure 3.3 – Exploratory bifurcation plots of downward migration (d_d) with symmetric and asymmetric forcing oscillator (arboreal ant (A)) parameters. In all cases, upward migration (d_u) and the non-consumptive effect (c) are set to 0, thus, increasing downward migration increases the strength of forced coupling. The central plot shows the bifurcation plot for symmetrical parameter conditions (other than d_d, d_u , c) between both predators and both prey populations. All other plots show alterations in the attack rate (a_a)) and handling time (h_a) of the arboreal ant from the forcing oscillator. All bifurcation plots are assessed for the ground borer population (B_g) from the chaotic oscillator. Black points represent local maxima while red points represent local minima. Green lines show the estimated Lyapunov exponents from numerical simulations. Specific values of a_a and h_a used for bifurcations are displayed in the top right corner of each plot.



Supplementary Figure 3.4 – Exploratory bifurcation plots of downward migration (d_d) across a range of upward migration (d_u) and non-consumptive effect (c) values. All bifurcation plots are assessed for the ground borer population (B_g) from the chaotic oscillator. Black points represent local maxima while red points represent local minima. Green lines depict Lyapunov exponent estimates from numerical simulations. Specific values of d_u and c used for bifurcations are displayed in the top right corner of each plot.



Supplementary Figure 3.5 – Non-consumptive effect trend analysis. Plots show the qualitative impact of increasing the non-consumptive effect (c) (NCE) on prey variability and mean predator density. Line points represent coefficients from regressions (generalized linear models with gamma error distributions and log link functions) of the NCE parameter space against either **a & b)** prey population variation (coefficient of variation) or **c & d)** mean predator density. These values are plotted across a range of downward (d_d) and upward (d_u) migration parameter values. Values above or below the dashed zero line in plots indicate a general positive or negative impact of increasing NCE strength on the given metric. In **a & b)** the coefficient of variation trend is plotted for the plant borer (B_p) and ground borer (B_g), respectively. In **c & d)** the mean population density trend is plotted for the arboreal ant (A) and ground ant (W), respectively. This is the reciprocal of Figure 3.8 in the main text (with CV and mean density reversed for the respective populations).

Chapter 4 Local Coffee Farm Disturbance Disrupts Both Arboreal and Ground Ant Communities in a Five-Year Experimental Manipulation[3]

4.1 Abstract

Agricultural intensification, with increased chemical pesticide application, is a primary driver of global insect declines. Natural pest control (NPC) is a sustainable alternative which relies on the conservation of habitat in and around agriculture to promote natural enemy populations and pest regulation. While recent studies have focused on the role of natural habitat surrounding farms, understanding the impacts of local farm management, and especially intensification, is crucial to better promote NPC. Ants are indicator species and important natural enemies that play a key role in natural pest control in agriculture, especially in coffee systems. A number of studies have investigated the relationship between ants and coffee farm management, which spans a spectrum from pesticide-heavy monoculture to diverse shaded polyculture with low inputs. However, there are almost no broad experimental manipulations that test the effects of coffee intensification on whole ant communities. We conducted a "natural" experiment, tracking a period of intense local disturbance on a coffee farm to better understand how specific management practices impact ants. We measured several environmental variables and surveyed ground and arboreal ants in a plot before and after disturbance (coffee plant renewal), annually, over a five-year period. The management disturbance resulted in significant shifts in ground

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vegetation cover and exposed soil, as well as the extensive cutting (and replanting) of old coffee plants. In the ant community, we saw reductions in overall species richness in the plot for both ground and arboreal ants, as well as reductions in mean species richness per bait and ant abundance, and observed high rates of community turnover immediately after disturbance. We also saw marked shifts in the abundance of several focal pest control agents, although these trends were less clear. We found several significant relationships with environmental variables (metrics of disturbance) and ants, including a positive relationship between coffee plant height and total richness in the plot, as well as positive relationships between ground vegetation cover and mean ant diversity and activity (and corresponding negative relationships with leaf litter). Importantly, ant species richness mostly recovered five years after the disturbance, as coffee plants matured (increased vegetation complexity), however, community composition did not return to the original state. This study provides key mechanistic insight, through a manipulative experiment, into the management of ant communities and associated natural pest control in coffee. Future studies should expand on this approach to test the broader impacts of whole farm transformation and coffee intensification in agricultural landscapes.

4.2 Introduction

Agricultural intensification is arguably the most important driver of terrestrial insect declines around the world (Outhwaite *et al.* 2022; Raven & Wagner 2021; Sánchez-Bayo & Wyckhuys 2019; Wagner 2020; Wagner *et al.* 2021). This reality has prompted repeated calls to drastically reduce global pesticide use and promote more sustainable, agroecological practices for regulating pests (Kremen & Merenlender 2018; Forister et al. 2019; Sánchez-Bayo & Wyckhuys 2019; Harvey et al. 2020). Natural pest control, through the conservation of habitat in and around farms, can boost natural enemy diversity and reduce pest damage (Bianchi *et al.* 2006; Dainese *et al.* 2019; Karp *et al.* 2013), thus, offering a potential win-win strategy. Recent literature has focused on understanding how general landscape-level factors, especially the amount of natural habitat surrounding farms, impact natural pest control (Chaplin-Kramer *et al.* 2011; Dainese *et al.* 2019; Karp *et al.* 2018; Rusch *et al.* 2016), however, a general approach to management remains elusive (Karp *et al.* 2018; Tscharntke *et al.* 2016). Because different natural enemies respond to different scales of agricultural management (Gonthier *et al.* 2014), it is equally important to improve our understanding of the impact of local farm management practices and intensification.

Ants are excellent indicator species for assessing the impacts of disturbance, both generally (Andersen 2019) and in agriculture (Ibarra-Isassi *et al.* 2021). They also serve as important natural enemies in agriculture, helping to reduce pest damage and enhance yields across a range of crops (Anjos *et al.* 2022; Offenberg 2015). Coffee systems are often situated in tropical biodiversity hotspots (Moguel & Toledo 1999; Perfecto *et al.* 1996) and offer a unique potential to benefit from the conservation of diverse natural enemy communities and subsequent pest control (Philpott & Armbrecht 2006; Vandermeer *et al.* 2019). In coffee, ants have proven to be especially important in regulating insect pests, like the notorious coffee berry borer, but their response to local management factors is less clear (Morris *et al.* 2018). Coffee is traditionally grown under a shade canopy, but management spans a spectrum of intensification ranging from diverse, low-input, shaded polycultures with high levels of vegetation complexity to high-input monocultures, with low vegetation complexity (Jha *et al.* 2014; Moguel & Toledo 1999).

A number of studies have been conducted to understand the impact of coffee farm management on ant communities, investigating different functional groups of ants (e.g., ground and arboreal), across a range of management practices (see numerous citations in Morris et al. 2018, as well as more recent work by Escobar-Ramírez et al. 2020 and Ibarra-Isassi et al. 2021). While important contributions, the vast majority of these studies rely on correlational surveys, making it difficult to assess cause and effect and to pinpoint the mechanisms driving management effects on ants. A few studies have conducted experimental manipulations to understand more specific drivers. For example, Perfecto & Vandeermer (1996) reduced shade and leaf litter in coffee plots, potentially simulating shade-tree pruning and farm intensification, and showed that the disturbance-tolerant fire ant *Solenopsis invicta* increased in abundance, which corresponded to reduced diversity of other ant species (Perfecto & Vandermeer 1996). Other studies have added artificial nesting resources (bamboo) to coffee plants and shown an increase in arboreal twig-nesting ant diversity (Jiménez-Soto & Philpott 2015; Philpott & Foster 2005). While a similar experiment showed no effect of artificial nests on ground ants (Murnen *et al.* 2013), increasing the diversity of natural twig resources has also been shown to increase ground ant diversity (Armbrecht *et al.* 2004).

Despite these examples, few studies have tracked coffee ant communities before and after farm management disturbance. One study surveyed ant communities before and after shade tree pruning and found significant shifts in the abundance of a few dominant ant species, but no changes in arboreal ant diversity immediately following disturbance (Philpott 2005). However, shade at the site was only reduced by roughly 20% and ants were only censused one time after disturbance. To our knowledge, there are almost no studies which use broad perturbation manipulations to experimentally test the long-term impacts of local coffee management disturbance and intensification on ants. This is likely due to the difficultly, for researchers, of performing significant manipulations on farms, but the Philpott (2005) study is an excellent example of how shifts in farm management can opportunistically be used as a kind of "natural" experiment to mechanistically investigate management impacts on ant communities.

We set out to do just that, following the long-term impacts of a local management disturbance of coffee renovation (i.e., soil disruption, ground vegetation clearing, coffee pruning, and replanting). Anticipating the disturbance at the site where we work (see Methods below for description), we surveyed arboreal and ground ant communities and tracked environmental variables in a plot before and after the manipulation. We aimed to understand how disturbance impacted the general diversity, abundance, and composition of ant communities. We also investigated how abundant focal species of ants and specific pest control agents were impacted, and if any changes in ants could be explained by specific environmental changes from the disturbance within the plot. Finally, we repeated surveys annually for five years after the initial manipulation to understand both the immediate and long-term impacts of coffee management disturbance on ant communities.

4.3 Methods

4.3.1 Study Site & Plot Establishment

This study was conducted at Finca Irlanda, a 300ha, organic, commercial polyculture coffee farm in the Soconusco region of Chiapas, Mexico. The farm is located at approximately 1050m elevation at roughly 15°11´N, 92°20´W. As a certified organic, shaded-grown farm, local management does not rely on chemical pesticides and maintains a mix of nitrogen-fixing and other shade tree species which help to support a diverse community of natural enemies and unplanned biodiversity. However, like any coffee farm, local management occasionally involves the cutting and replanting of coffee after individual plants pass peak maturity and fruit production declines. We designed an experiment relying on this process of coffee renovation and regular ant surveys to test the impact of local farm perturbations on ant diversity and community composition.

Beginning in September 2016, we tracked ants over a five-year period in a 48x48m plot before and after a coffee renovation management disturbance. The plot was previously established in 2009 as part of a study on ant competition (Perfecto & Vandermeer 2013). A grid was established in the plot with flags placed every four meters to mark ground ant survey points, for a total of 169 points. The nearest coffee plant or tree at each point was marked with flagging tape for arboreal ant surveys. Coffee plants were prioritized, but when none were found nearby, we used shade trees and occasionally other non-herbaceous plants for arboreal surveys. We attempted to repeat arboreal surveys on these same plants over the five-year period, but with the initial disturbance and the passage of time, many plants were cut, pruned, or destroyed. When this occurred, we occasionally continued with sampling on cut coffee stumps or dead coffee, but in most cases, we shifted to the closest nearby live coffee plant. Throughout the study, we used this established grid to survey both arboreal and ground ant communities and track environmental conditions before and after management disturbance.

4.3.2 Farm Management Disturbance

In September 2016, coffee renovation took place across several sections of the farm. This occurs regularly at our site as coffee plants mature and fruit production begins to decline. However, many sections of the farm also required coffee renovation after the coffee rust epidemic of 2011-2012 destroyed many plants. In particular, older plants of susceptible varieties were replaced by newer rust-tolerant hybrid varieties (mostly Sarchimor and Marsellea), as occurred in coffee-growing regions throughout the world (Li *et al.* 2022). With coffee replanting and renovation comes a suite of other local management disturbances, which we observed at our study site and in other sections throughout the farm. The most significant impact is that many of the older, larger coffee plants are heavily pruned or cut down altogether to make room for the new samplings. During this process, farm workers engaged in heavy manual weeding, removing a significant portion of live herbaceous vegetation cover. To prepare sites for coffee planting and promote more efficient harvest in the future, narrow terraces were excavated to flatten the ground. This resulted in a significant visual disturbance to the soil substrate and an increase in exposed soil. In some parts of the farm, workers also pruned shade trees, presumably to promote the growth of the new coffee saplings. Finally, small coffee samplings of about 20-30cm were planted systematically in the terraces roughly every 1-2 meters. During and after coffee planting, agricultural lime was applied to the soil in and around the holes where coffee saplings were planted. In sum, the coffee renovation process resulted in a significant disturbance to soil, ground vegetation, and coffee vegetation, and the addition of hundreds of new coffee saplings at each site, as depicted in Figure 4.1.

We used this disturbance as a “natural” experimental manipulation for this study. Many of the observed impacts of the coffee renovation are known to affect ant communities and at least temporarily reduce their activity, such as soil disturbance (Andersen 2019) and the application of lime (Offenberg 2015). We measured several environmental metrics in our study plot to quantify the disturbance and surveyed ants before and after to assess the overall impact of local coffee farm management on ant communities. Ant surveys and environmental data collection took place over a five-year period, beginning in September 2016 immediately before the disturbance, immediately after in October 2016, nine months later in June 2017, and roughly each year after in May 2018, June 2019, and August 2021 (sampling was not performed in 2020 due to the COVID-19 pandemic). Although sampling dates varied, all surveys were conducted well within the typical rainy season at our study site, which occurs roughly from mid-April to November (Li *et al.* 2022).

4.3.3 Measuring Local Disturbance

To assess the extent of local farm management disturbance we quantified several environmental variables in our study plot. At each sampling point in the grid, we measured shade canopy cover, and the proportion of ground vegetation, leaf litter, and exposed soil cover. We recorded percent canopy cover with a densiometer at chest height at each sampling point, facing the same direction for all recordings. Any coffee plant branches around chest height were pushed out of view of the densiometer to record only the shade canopy, but higher coffee branches were left unmanipulated. Ground cover measurements were estimated visually using a 45cm^2 quadrant. At each sampling point in the grid, we tossed the quadrant in a random direction within a meter distance. Ground vegetation cover included live herbaceous vegetation and leaf litter also included fallen woody plant material. We also recorded the identity of the arboreal plant sampled and visually estimated each plant's height. For coffee plants, we noted if the plant appeared dead or significantly pruned.

4.3.4 Ant Community Surveys

To measure changes in the ant community, we conducted tuna bait surveys at each grid point in the plot on the ground and arboreally. Tuna fish baits (in oil, roughly the size of a thumbnail) were placed directly on the ground at each flag marker where a small patch was cleared of vegetation (during plot setup at least 24h before baiting) to make baits more visible. Arboreal tuna baits were placed in marked plants (mostly coffee and occasionally other woody plants) at each point, typically at the intersection of a branch and the central trunk of the plant. At each point, a single tuna bait was used on the ground and another on the plant. Tuna baits were surveyed systematically along grid lines, where baits were left on one line at a time and were checked no sooner than 25 minutes after initial placement, but typically 30-40 minutes after. For each time point, surveys were conducted over the course of 3-5 days between 8:30am and 1:30pm local time to avoid the effects of colder early morning temperatures and afternoon rain, which is typical during the rainy season at the site. Surveys were not conducted during rainstorms to avoid temporary reductions in ant activity. Upon checking baits, we identified ants to species or morphospecies to the best of our ability in the field. Ant activity for each species at each bait was also estimated by counting the number of workers present (up to 20 individuals). After checking individual baits, tuna was collected and removed from the plot to avoid any lingering effects on the ant community.

When ants could not be confidently identified in the field, samples were collected and stored in alcohol for later identification with a dissecting microscope in the laboratory. Although the ant communities in this region are quite diverse, ants have been thoroughly documented over two decades of active ecological research at our study site. Previous identification materials developed from this work (Stacy Philpott unpublished) facilitated ant identification both in the field and the laboratory. We also relied on resources from AntWeb (AntWeb 2023) to assist identification. Despite this, we acknowledge the inherent difficulty of ant identification in this tropical region and the potential for some limitations in our identifications, however, we feel confident that morphospecies were identified consistently across survey sampling times. In the case that occasional species clumping occurred, when individuals were difficult to distinguish morphologically (e.g., genera such as *Brachymyrmex*), this should only make our results and estimates of diversity impacts more conservative.

Lastly, it should be noted that tuna bait surveys are known to only capture a subset of the total ant species present in an area, since some species do not recruit to baits (Gotelli *et al.* 2011; Perfecto & Vandermeer 2013). We used this approach for its convenience and to precisely repeat our sampling effort across the plot at each grid point over the course of time, which is more challenging with visual and manual surveys (Gotelli *et al.* 2011). Despite this limitation, we believe the repetition and standardization of this survey approach serves as a reliable estimation of ant community compositional change over time for this study.

4.3.5 Data Analysis

4.3.5.1 Farm Management Disturbance

To quantitatively assess the extent of local disturbance in the plot we ran a series of regression models of our environmental metrics against time. To test for temporal differences in the proportion of canopy cover, ground vegetation, leaf litter, and exposed soil we conducted independent generalized linear mixed models (GLMMs), with each of those variables as response variables run against time as a continuous fixed effect. To assess how coffee plants changed through time we ran a GLMM with plant height (m) as the response variable against time as a continuous fixed effect. To account for random variation within the plot and spatial non-independence of individual bait points, which were used repeatedly in surveys across time, we included bait identity as a random effect in all models. Models for proportional environmental variables were run with binomial error distributions (logit link), while the model for plant height was run with a gamma error distribution (log link) to accommodate positive, exponentially distributed data. To account for observed overdispersion in binomial GLMMs, we included an observation-level random effect (Elston *et al.* 2001).

4.3.5.2 Ant Species Richness & Abundance

To assess changes in the ant community before and after disturbance and across time in our study, we first conducted species rarefaction curves on our bait census data to ensure that ant communities were adequately and evenly sampled at each time point across the study. Species accumulation curve means and standard deviations were estimated from resampling using the "Mao Tau" estimator for site-based rarefaction (Colwell *et al.* 2012). Because sampling effort (number of baits) and spatial coverage (plot area) were fixed across time during the study, we assumed that asymptotic rarefaction curves could be reasonably compared to assess differences in species richness in the plot through time. In addition to plot level species richness data, we also assessed differences in mean ant richness per bait across time. We conducted a GLMM with species richness per bait as the response variable and time (continuous), strata (arboreal or ground), and their interaction as fixed effects. We ran the model using a Poisson error distribution (log link) to accommodate species richness count data. Bait identity was again included as a random effect (as described above).

To assess differences in overall ant abundance in the plot through time, we conducted a GLMM on ant presence-absence data at baits (unoccupied or occupied by one or more ant species). We ran the model using a binomial error distribution (logit link) to account for binary data, again with time, strata, and their interaction as fixed effects, and bait identity as a random effect.

4.3.5.3 Ant Community Composition & Turnover

With our ant survey data, we also investigated changes in the composition of ant communities through time. We did this in several ways. First, using the summed abundance data for all individual ant species, we assessed differences in composition (species identity and abundance) between the two sampled strata (ground vs. arboreal) and across time for each census time point. We calculated dissimilarity metrics between all groupings of strata (ground-arboreal) by time (six time points) using Bray-Curtis distance. We then used this dissimilarity matrix to create a non-metric multidimensional scaling (NMDS) plot to visualize relative compositional differences and ran a PERMANOVA on the data to test for statistical differences in composition by sampling strata (difference in group centroids). We then used the Bray-Curtis dissimilarity matrix to assess compositional turnover (temporal beta-diversity) in the plot, comparing dissimilarity estimates between each community through time compared to the first community census before disturbance in September 2016 (Dissimilarity Matrix [community T1~ community T2, community T1~ community Ti,…]). The above procedures for community composition were then repeated using the Jaccard distance metric, with only the identity of species sampled in communities (presence-absence data).

To further assess changes in community composition, we also created rank abundance plots to understand how the relative abundance and identity of both common and rare species changed in communities through time. We also plotted species identity and worker count (as a measure of activity) spatially, using bait grid coordinates in the plot to understand where different species were most common in space and how this arrangement changed through time. These analyses, together, were used to track specific species, including the most abundant focal species in both the ground and arboreal strata, and natural enemy species that are known to be important pest control agents of common coffee pests, like the coffee berry borer.

4.3.5.4 Ant-Environment Relationships

Finally, to understand the potential mechanisms of farm disturbance impacts on ant communities, we assessed plot-level relationships between our environmental variables and ants. We explored the impact of mean shade, plant height, and our ground cover variables on total ant richness, mean ant richness, and mean ant activity (number of workers) across our surveys. These relationships were investigated for the entire ant community, with ground and arboreal surveys combined, and with strata separated, to understand potential variable impacts of disturbance on these functional ant groups. In all cases, ant-environment relationships were explored using simple linear regressions.

For all data analysis, GLMMs were conducted with time centered and scaled to improve model fit and to facilitate coefficient interpretation. All GLMMs were run using ‘glmer’ from the lme4 package in R with fixed effect coefficients and random effect variance estimated using maximum likelihood through Laplace approximation. LMs were run using the base R function ‘lm’. PERMANOVAs were performed with 999 permutations using the ‘adonis2’ function from the vegan package in R, which was also used to calculate dissimilarity matrices and create NMDS plots. Species rarefactions were performed with site-based rarefaction using the Mao-Tau estimate with the vegan package in R. All analysis was conducted in R version 4.3.1 (R Core Team 2023).

4.4 Results

4.4.1 Farm Management Disturbance

Analysis of all environmental variables revealed a significant change across time following plot disturbance (Table 4.1). Confirming visual observation of the changes (Fig. 4.1), mean percent ground vegetation cover in the plot decreased immediately after disturbance by 34.4% and mean percent exposed soil increased significantly by nearly 700% (Fig. 4.2a & Fig. S4.1). After roughly nine months, ground vegetation and exposed soil returned approximately to previous levels and remained at those levels for the rest of the experiment. Following the pruning of old coffee plants and the planting of new saplings, mean coffee plant height at bait points changed significantly with time. Although coffee height only decreased slightly immediately after plot disturbance, nine months after disturbance, mean coffee height at bait points had dropped nearly 54% after many previously pruned older plants had died and arboreal baiting was focused more on recently planted saplings closer to grid bait points (Fig. 4.2b & Fig. S4.2). However, after this significant shift, mean coffee height increased at each time point over the course of the experiment, returning to the initial observed level at the end of the study, roughly five years after the disturbance. Although canopy cover changed significantly with time in our GLMM, the difference in means between time points was much smaller than other environmental variables, and the initial change immediately after disturbance was slightly positive, suggesting that shade trees were not affected in a meaningful way by farm management (Fig. S4.3).

4.4.2 Ant Species Richness & Abundance

Over the course of the experiment, we documented 54 different ant species in the plot (Table S4.1). Of those, 40 species were found during arboreal surveys, and 35 species were found in ground surveys, with 21 species in common between strata. Species rarefaction of the entire community suggested that ants were relatively well sampled – accumulation curves were near asymptotic across all survey dates (Fig. 4.3a), allowing for reasonable comparison of species richness between surveys, given the fixed sampling effort and spatial coverage of our study. This initial analysis revealed what appeared to be a pattern of gradual species richness reduction after disturbance, which remained significantly lower than pre-perturbation richness for several years, and then eventually increased with the final plot survey, nearly five years after beginning the experiment. Overall, species richness declined approximately 15% immediately after the disturbance and 34% from the initial level nine months after disturbance. Species accumulation curves of ant communities separated by strata revealed a similar, but more nuanced pattern (Fig. 4.3). In arboreal communities, species richness followed the same clear pattern of decline and gradual recovery over time, with an immediate decrease of 22% and overall decrease of 44% nine months after disturbance (Fig. 4.3b & Fig. 4.4a). The pattern in the ground community of ants was slightly different, however, with a slight increase in species richness immediately after disturbance before eventually declining 17% from initial levels (Fig. 4.3c & Fig. 4.4a). Although species richness in the ground community followed the same pattern of eventual decline, low levels for a number of years, and then gradual recovery, the differences in accumulation curves were less pronounced than for the arboreal community and the combined data. Interestingly, ground species richness appeared to recover fully with the final survey in July

2021 producing an accumulation curve that fully overlapped the initial sampling curve from September 2016 (Fig. 4.3c).

When assessing species richness on a per bait level between sampling strata and across time, we found a similar pattern with one noticeable difference in the ground community. Immediately following disturbance, mean richness at individual baits decreased in both the arboreal (~13% reduction) and ground (~10% reduction) communities (Fig. 4.4b), however, time was only a significant factor for the arboreal strata in our GLMM on species richness (Table 4.2). After the initial reduction, mean species richness at baits gradually increased with time in both strata, reaching initial levels roughly two years after the initial disturbance. In the arboreal community, species richness remained at this level in June 2019 and increased again slightly in July 2021. However, in the ground community, richness appeared more variable, increasing beyond initial levels in June 2019 and then declining again significantly two years later in July 2021, unlike the total richness data in the plot for ground ants. We also observed a significant overall difference in mean richness between strata, with more species per bait in the ground community (Table 4.2).

Trends in overall ant abundance in the plot were slightly more complex than for species richness. In both ground and arboreal communities there was an immediate decrease in the proportion of baits occupied during surveys, however, this decline was much more pronounced in the arboreal community and was only slight in the ground community (Fig. 4.5). Indeed, the effect of time in our GLMM was only significant for arboreal abundance (Table 4.2), suggesting that the proportion of ground baits occupied by ants did not change significantly across the study. Eventually, ant abundance in the arboreal community recovered with time, similar to the trends in species richness, and actually increased beyond the levels seen in the initial survey before disturbance.

4.4.3 Ant Community Composition & Turnover

Analysis of community composition revealed that ant communities were distinct, both between sampling strata and across time (Fig. 4.6 & Fig. S4.4). Upon visual inspection, our NMDS plots illustrated that the overall difference in community composition was greater between ground and arboreal groups than between time sampling points within strata, with certain species of ants clearly occurring only in one stratum or the other (Fig. 4.6a & Fig. S4.4). PERMANOVA tests of community dissimilarity confirmed a significant difference in composition between strata both using Bray-Curtis (Fig. 4.6a, permutations=999, R^2=0.689, F=22.189, p=0.003) and Jaccard dissimilarity (Fig. S4.4, permutations=999, R^2=0.491, F=9.636, p=0.001). This was confirmed visually in NMDS plots by the absence of overlap between polygons surrounding sampling points for each stratum (Stress level: Bray-Curtis=0.042, Jaccard=0.035).

Ant community composition also changed with time when compared to the initial community surveyed before disturbance in September 2016 (Fig 4.6 b & c). Both arboreal and ground communities shifted significantly immediately after disturbance, with the most significant compositional shifts occurring between the first two sampling points before and after disturbance for both dissimilarity metrics (Fig 4.6 b & c). With Jaccard dissimilarity, which uses only species identity, ant communities then stayed mostly the same across the time after the initial disturbance (Fig. 4.6c). However, with Bray-Curtis dissimilarity, which accounts for species identity and relative abundance, we did see a slight trend toward increased divergence from initial community composition, with greater overall dissimilarity through time in the ground community (Fig. 4.6b).

We also observed shifts in the relative abundance and identity of the dominant species in communities through time. While species rank abundance (number or baits occupied) plots revealed the typical distribution of a few common and many rare species (Fig. S4.5), the identity of more abundant species changed through time (Fig. S4.6 & Fig. S4.7), particularly in the ground community. These patterns were also reflected in spatial plots of the ant community, which illustrated marked changes in the abundance and spatial distribution of species through time (Fig. S4.8 & Fig. S4.9), particularly with focal and more abundant species (Figs. S4.10-S4.12).

These trends were also reflected in the abundances of focal pest control agents (species that are known to play a role in coffee pest control) through time (Fig. 4.7). While we did not see a consistent decrease in the abundances of these species after disturbance, we did observe marked variability in their abundances. Two species, *Solenopsis geminata* and *Crematogaster nigropilosa*, exhibited general reductions in abundance with time, where *S. geminata* was mostly eliminated from the ground community over time (Fig. 4.7c), while *C. nigropilosa* abundance eventually recovered on coffee plants (Fig. 4.7d). However, two other dominant and important pest control agents (Morris & Perfecto 2016), *Solenopsis picea* and *Wasmannia auropunctata*, showed much more variable trends over time (Fig. 4.7 a & b). *W. auropunctata*, a dominant arboreal and ground-foraging ant, and the most abundant species overall in our study, showed an initial decrease in abundance following disturbance in both strata, but then continued to increase in abundance over time, dominating the younger coffee samplings that were planted during the management disturbance, and occupying nearly 75% of all arboreal baits during the 2019 census

(Fig. 4.7b). *W. auropunctata* densities then dropped off significantly in our final census in 2021 after a two-year gap, accompanied by an increase in *S. picea* and the non-focal ant community on coffee plants (Fig. 4.7a & S4.11b).

4.4.4 Ant-Environment Relationships

Analysis of plot-level relationships between environmental variables (metrics of disturbance) and the ant community revealed several significant interactions. Mean plant height was positively related to the total richness of ants in the community, both overall (Fig. 4.8a, R^2=0.66, p=0.001), and individually for both the ground and arboreal ant communities (Fig. 4.8b, Arboreal: R^2=0.82, p=0.013, Ground: R^2=0.76, p=0.024). Interestingly, mean shade in the plot led to a reduction in mean ant species in the ground ant community (Fig. 4.8c, R^2=0.71, p=0.034), but not in the arboreal community (although mean shade only varied from roughly 73-81% cover). Ground vegetation and leaf litter also appeared to explain some of the variation in our ant community metrics (Fig. 4.9). Ground vegetation cover was positively related to mean ant species richness in the arboreal ant community (Fig. 4.9a, R^2=0.66, p=0.049), but not in the ground community. Ground vegetation was also positively related to mean ant activity (number of workers) in the arboreal ant community (Fig. 4.9b: R^2=0.69, p=0.040) and in the ant community overall (Fig. 4.9c, R^2=0.36, p=0.040), but not in the ground community individually. Correspondingly, leaf litter was negatively related to overall mean ant activity in the plot in both the arboreal and ground communities separately (Fig. 4.9d, Arboreal: R^2=0.81, p=0.015, Ground: R^2=0.71, p=0.034), and in the combined data (Fig. 4.9e, R^2=0.45, p=0.016).

4.5 Discussion

While a number of studies have investigated correlations between the diversity of ant communities and certain coffee farm management practices, we still lack a comprehensive mechanistic understanding of the management drivers of ants in coffee. Here we demonstrate the effects of local coffee farm management disturbance on both arboreal and ground ant communities over time, relying on an experimental manipulation and long-term (five-year) census data.

The data collected on various environmental factors over time confirmed what was evident to the eye at our site – that coffee renewal and replanting was indeed a significant perturbation. The most immediate change was observed in our ground cover data, where we saw a significant increase in exposed soil and a corresponding decrease in ground vegetation cover. These measures helped to quantify the extent of soil disturbance and occasional "tilling" due to the creation of level terraces at the site. While both of these measures recovered roughly nine months after the disturbance, their significant initial shift likely played a major role in the resulting shift in the ant community. In addition to ground cover, we observed a significant reduction in coffee plant height in the plot nine months after the disturbance and a gradual recovery as plants grew over time. This metric partially reflects the cutting of old coffee plants and replanting of saplings at the site during management renovation, but also reflects our focus on baiting coffee plants closest to our grid sampling points. In the initial sampling before disturbance, many bait sites at the plot did not have nearby live coffee plants. Because of this, we baited a number of nearby trees and other herbaceous plants, or in some cases pruned and dead coffee. Immediately after coffee replanting, we switched over to a few new coffee saplings when these were closer to our bait sites, but because the new plants were so small, we did not consider them fair measures of the arboreal ant community and decided to wait until nine months after disturbance, when plants were established to bait the new plants. We switched to these plants to prioritize coffee and only when they were closer to our grid bait points. At the end of our study, five years after the initial disturbance, mean coffee plant height was actually higher than when we first started, likely reflecting the rejuvenation of the plot with new plants. This and the observed increased density of coffee, which we did not directly measure, likely resulted in an increase in vegetative niche space over time (which we discuss later).

Overall, the ant community at our study site was significantly impacted by the disturbance in the plot. Ant diversity, in the form of total species richness, was significantly reduced in the plot immediately after the disturbance. When separating the data into ground and arboreal communities, we saw a more immediate and more pronounced decline in total species richness in the arboreal community, followed by lull in richness for several years. The reduction in ground ant richness was less pronounced and delayed until nine months after the initial disturbance, but followed the same qualitative pattern as the arboreal community. The slight initial increase in ground richness may have resulted from a kind of “extirpation debt” in the plot and temporary boost in diversity from the initial disturbance, but we do not have the data to address this speculation any further. What was clear is that eventually ground richness declined and remained lower than the initial richness for several years. Due to our standardized and repeated sampling effort over time, and the near asymptotic nature of our rarefaction curves, we feel that these richness data can be reasonably compared.

Mean species richness per bait, as an indirect measure of the overall richness and the evenness of species in the plot, showed a similar decline immediately following disturbance. Nine months after the disturbance mean richness remained low, but then began to recover through time. This trend held in the arboreal community actually reaching higher levels five years after disturbance compared to the initial survey of ants. Contrastingly, we saw the opposite in the ground community during our final baiting, where mean richness levels declined again after recovering from the initial disturbance (explaining the non-significant effect of time in our statistical model). One explanation for this result centers on the growth of coffee plants in the plot through time. As mentioned previously, the overall density and height of coffee plants in the plot was greater at the end of the study than before the disturbance. Plants were also observed with increased moss and epiphytes growing on their trunks over time. This expanded niche space and vegetation complexity could have provided arboreal ants with greater opportunities for colonization and increased habitat on individual plants, potentially allowing more species on average to coexist on coffee, explaining the increasing trend in mean arboreal richness with time after the disturbance. This possibility was also supported by the positive relationship between mean plant height and total ant species richness in the plot. The increase in coffee vegetative density may also explain the unexpected reduction of ground bait richness at the end of our study, as ants likely experienced much higher levels of shade on the ground as plants grew threw time. Eventually, when plants reached maturity, roughly around the end of the study, this high level of mid-story shade may have reduced microclimate temperatures such that certain ground species no longer found the plot favorable to nest in. For example, this may explain the near complete loss of *Solenopsis geminata* in the plot by the end of the survey, which is known to be a sun-adapted species (Perfecto & Vandermeer 1996). The difference in trends between ground mean bait richness and total ground richness may then be explained by variation across the plot, if there were still some light gaps and places in the plot where those shade-sensitive species could persist, keeping total ground richness high, but mean richness per bait lower. Interestingly, while we did not assess mid-story shade from coffee plants directly, we did find a significant negative relationship between plot level mean canopy cover (upper-story shade) and mean species richness in the ground ant community, supporting our potential explanation for the drop-off in mean ground ant richness. However, it is important to note that mean shade levels observed in our plot varied over a narrow range from 73-81% cover, where the contrast between sun and shade coffee farms is typically much more pronounced, with shaded farms generally supporting higher ant diversity (Morris *et al.* 2018).

Interestingly, species abundance (bait occupancy) in the plot was less clear when separated by strata, where we found an initial decline in arboreal abundance, but no significant change in ground abundance through time. The lack of change on the ground may be due to the fact that more disturbance-tolerant species simply fill in the gaps left by the species that were lost. This may be less likely to occur on arboreal baits as plants function more like habitat islands for arboreal specialists, where colonization of plants is a slower process than the movement of individual ants on the ground, making it less likely for arboreal species to immediately fill the opened niche space left behind by arboreal colonies that were lost. Eventually, however, arboreal ant abundance in the plot exceeded the original levels observed before the disturbance, possibly reflecting the increase in coffee vegetation density in the plot over time.

In addition to these metrics, we also observed changes in the composition of ant communities in the plot with disturbance. Our community composition analysis showed that ground and arboreal communities are indeed distinct and that these differences appear to be greater than the overall difference in communities through time. However, we also saw an increase in community dissimilarity with time when comparing community composition to the initial community surveyed in September 2016. Importantly, our data suggested that communities changed most rapidly immediately after the initial disturbance and then stayed mostly the same over the near five-year timeframe of the study. Community compositional changes were also observed in the spatial distribution and abundance of focal ant species over time, including with several pest control agents, however, these trends were not consistent across time. While we did observe a reduction in the abundance of two focal pest control species after disturbance, we saw variation of equal magnitude with two other species, with no consistent declines. In particular, the apparent (albeit, course) oscillations in *Wasmannia auropunctata* and *Solenopsis picea* densities through time suggest that other factors, such as direct ant interactions and competition are also playing a role in these communities (Cerdá *et al.* 2013). This is well known to occur in coffee (Perfecto & Vandermeer 2013), but interestingly, these dynamics may also be driven by the environmental impacts of disturbance in the plot. While we saw an initial reduction of *W. auropunctata* with initial disturbance, its abundance increased significantly on coffee saplings during the first few years after disturbance, with a corresponding drop-off of *S. picea*. However, after a two-year gap in sampling and at the end of the survey when coffee plants were full size, we observed a major reduction in *W. auropunctata* and a corresponding increase in *S. picea* and the rest of the non-focal ant community. One potential explanation is that the increase in coffee vegetative niche space and the growth of epiphytes and moss on coffee after several years promoted colonization of other ant species that eventually were able to outcompete *W. auropunctata*, reflecting a potential resource discovery-dominance dynamic over time (Cerdá *et al.* 2013). Regardless, it is difficult to say with our data whether there was a general negative impact of the plot disturbance on the focal pest control agents. Nevertheless, the general negative impact of the management perturbation on the ant community as a whole is likely to have spill-over consequences for pest control (Anjos *et al.* 2022), since it is known that a diverse array of species, and ant communities more broadly, are important in coffee pest regulation (Morris *et al.* 2018).

Beyond some of the environment-ant relationships already explored, our results suggest that disturbance of ground cover may explain some of the negative trends observed in ants. At the plot level, we found positive relationships between mean ground vegetation (live herbaceous cover) and mean ant species richness and activity, for arboreal ants, and negative relationships between mean leaf litter and mean ant activity for the whole ant community. These contrasting trends suggest that intact herbaceous ground cover is important for ants, while increased leaf litter, which may be a proxy for disturbance, suppresses ants. When ground vegetation was reduced to its lowest point immediately after the disturbance, we saw the lowest levels of mean ant richness per bait. Interestingly, ground vegetation only had a significant effect on arboreal ant communities, which is less intuitive than any effects on ground ants. While it is not clear why ground ants would not equally be affected by ground vegetation cover, arboreal communities may benefit from increased cover indirectly, potentially through increased resource availability or more favorable microclimate conditions.

An interesting finding in our study is that for nearly all environmental and ant community metrics, we saw a least some level of recovery in the long run. Ground cover and coffee plant height were most similar to initial levels five years after the disturbance. Initial declines in ant total richness, mean richness, and abundance, all similarly recovered over the course of the study, with the one exception of ground mean richness discussed previously. This general finding is promising and may reflect that ant communities can recover from local farm disturbance and potential coffee intensification, if the perturbation is short-term and nearby habitat is preserved to allow for recolonization. This was likely the case in the farm where we conducted this study, as it is a diverse, shaded polyculture and this disturbance took place only in particular patches and not across the entire farm simultaneously. When entire farms are transformed with truly intensive practices, such as the clearing of shade trees and the application of pesticides, ant community recovery may be less likely; however, we need more long-term sampling in agricultural areas to directly test this and to understand the broader impacts of intensification on insect declines (Wagner *et al.* 2021). Furthermore, while we observed recovery of richness and abundance, ant community composition both on the ground and arboreally never returned to its initial state. In fact, community dissimilarity increased with time, suggesting that disturbance may drive long-term shifts in species composition. However, due to the lack of repeated sampling data at our site, it is impossible for us to say what the typical background rate of turnover is in these communities.

Our manipulative study complements previous correlative research addressing the impact of coffee farm management on ant communities and diversity. Our results align with previous studies that show negative impacts of attributes of coffee intensification on varying aspects of ant communities (Armbrecht *et al.* 2005; Armbrecht & Perfecto 2003; Escobar-Ramírez *et al.* 2020; Ibarra-Isassi *et al.* 2021; De la Mora *et al.* 2013; Perfecto *et al.* 1997; Perfecto & Snelling 1995; Perfecto & Vandermeer 2002; Philpott *et al.* 2006). Importantly, our study offers more mechanistic insight into those changes by relying on a coffee management experimental manipulation. We also expand on the one previous "natural" experiment in coffee management that we encountered (Philpott 2005) by taking advantage of a more extensive perturbation, assessing both ground and arboreal communities, and following changes over a much longer time frame. This last component is essential to pick up on less obvious changes, such as increases in population variability and dynamics through time, despite what may sometimes appear as no change in overall ant richness or abundance. While we acknowledge that our conclusions would be stronger if the study was repeated across multiple plots and coffee farms, we believe that replication across individual baits and through time, over a five-year period provides a strong starting point to experimentally assess the general impacts of local coffee management impacts on ants. The agricultural management disturbance that we tracked provides useful insight into the potentially more pronounced negative impacts of broad-scale coffee intensification on ant and insect communities. Similar investigations will be essential if we are to better predict the impacts of agricultural intensification on biodiversity and, alternatively, better manage agroecosystems for natural pest control and the conservation of biodiversity.

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4.8 Tables & Figures

Table 4.1 – Model Outputs for Plot Disturbance Analysis. Results from generalized linear mixed models (GLMMs) testing various environmental metrics of plot disturbance across time. Output includes coefficient estimates (±SE), z-scores, and p-values. Model response variables are listed in the parameter column in bold with independent variables below for each model. GLMMs for shade, ground vegetation, leaf litter, and exposed soil were run with a binomial error distribution (logit link) to account for proportional data, while the GLMM for coffee height was run with a gamma error distribution (log link). Significant effects are bolded in the rightmost column.

Parameter	**Estimate**	**SE**	**z value**	**Pr(>|z|)**
Shade~				
Intercept	1.710	0.086	19.968	**<0.001**
Time	-0.068	0.031	-2.182	**0.029**
Ground Vegetation~				
Intercept	1.356	0.070	19.356	**<0.001**
Time	0.549	0.047	11.614	**<0.001**
Leaf Litter~				
Intercept	-1.726	0.061	-28.207	**<0.001**
Time	-0.298	0.039	-7.577	**<0.001**
Exposed Soil~				
Intercept	-5.097	0.195	-26.079	**<0.001**
Time	-1.034	0.138	-7.495	**<0.001**
Coffee Height~				
Intercept	0.272	0.033	8.314	**<0.001**
Time	0.144	0.015	9.353	**<0.001**

Table 4.2 – Model Outputs for Ant Community Analysis. Results from generalized linear mixed models (GLMMs) testing ant species richness and abundance across time and by strata (arboreal and ground ants). Output includes coefficient estimates (±SE), z-scores, and p-values. Model response variables are listed in the parameter column in bold with independent variables below for each model. The GLMM for ant species richness was run with a Poisson error distribution (log link) to account for count data, while the GLMM for ant abundance was run with a binomial error distribution (logit link) to account for presence-absence data. Significant effects are bolded in the rightmost column.

Parameter	Estimate	SE	z value	Pr(>|z|)
Species Richness (Strata Reference: Arboreal)~				
Intercept	0.003	0.031	0.089	0.929
Time	0.065	0.031	2.115	**0.034**
Strata	0.340	0.041	8.270	**<0.001**
Time*Strata	-0.057	0.040	-1.417	0.156
Species Richness (Strata Reference: Ground)~				
Intercept	0.342	0.026	12.936	**<0.001**
Time	0.007	0.026	0.283	0.777
Strata	-0.340	0.041	-8.270	**<0.001**
Time*Strata	0.057	0.040	1.417	0.156
Ant Abundance (Strata Reference: Arboreal)~				
Intercept	2.294	0.153	14.986	**<0.001**
Time	0.565	0.121	4.664	**<0.001**
Strata	1.729	0.229	7.565	**<0.001**
Time*Strata	-0.735	0.224	-3.287	**0.001**
Ant Abundance (Strata Reference: Ground)~				
Intercept	4.023	0.241	16.727	**<0.001**
Time	-0.171	0.188	-0.909	0.363
Strata	-1.729	0.228	-7.566	**<0.001**
Time*Strata	0.735	0.224	3.287	**0.001**

Supplementary Table 4.1 – Ant Species List and Overall Abundance by Strata.

Species Code	***Scientific Name***	**Arboreal**	**Ground**
AZNE	*Azteca sp.*	1	0
AZSE	*Azteca sericeasur*	1	0
BRAM	*Brachymyrmex sp.*	16	2
BRBL	*Brachymyrmex sp.*	27	16
BRBR	*Brachymyrmex sp.*	1	0
CAAT	*Camponotus atriceps*	1	0
CABR	*Camponotus bretesi*	13	1
CANO	*Camponotus novogranadensis*	2	0
CASE	*Camponotus sericeiventris*	31	8
CAST	*Camponotus striatus*	4	0
CATE	*Camponotus textor*	6	0
CRCA	*Crematogaster carinata*	1	0
CRCR	*Crematogaster crinosa*	2	0
CRCU	*Crematogaster curvispinosa*	2	0
CRNI	*Crematogaster nigripilosa*	53	5
CRSU	*Crematogaster sumichrasti*	9	0
EUSE	*Eurhopalothrix sepultura*	0	1
GNRE	*Gnamptogenys regularis*	2	3
GNST	*Gnamptogenys striatula*	3	195
GNSU	*Gnamptogenys sulcata*	2	3
HYNI	*Hypoponera nitidula*	0	3
HYOP	*Hypoponera opaciar*	0	1
LAPR	*Labidus praedator*	1	2
LEEC	*Nesomyrmex echanatinodis*	16	0
NYCR	*Nylanderia sp.*	10	14
ODLA	*Odontomachus laticeps*	0	14
ODME	*Odontomachus meinerti*	0	3
PAHA	*Pachycondyla harpax*	0	13
PHAZ	*Pheidole sp.*	3	28
PHCH	*Pheidole punctatissima*	1	0
PHFH	*Pheidole tisiphone*	0	48
PHL8	*Pheidole sp.*	2	0
PHPR	*Pheidole protensa*	4	331
PHPS	*Pheidole sp.*	5	14
PHRH	*Pheidole sp.*	12	64
PHRS	*Pheidole sp.*	0	22
PHSB	*Pheidole sp.*	0	58
PHSI	*Pheidole simonsi*	0	61
PHWA	*Pheidole sp.*	5	1
PRHY	*Procryptocerus hylaeus*	2	0
PSCH	*Pseudomyrmex championi*	1	0
PSEJ	*Pseudomyrmex ejectus*	21	0

PSSI	*Pseudomyrmex simplex*	1	0
ROTO	*Rogeria tonduzi*	2	4
SOGE	*Solenopsis geminata*	3	183
SOGR	*Solenopsis geminata* RED morph	0	5
SOMI	*Solenopsis sp.*	0	1
SOPI	*Solenopsis picea*	216	26
SOTE	*Solenopsis tericola*	3	7
SOZE	*Solenopsis zeteki*	0	6
SOZG	*Solenopsis sp.*	0	4
TACA	*Tapinoma sp.*	49	3
TECH	*Technomyrmex sp.*	2	0
WAAU	*Wasmannia auropunctata*	483	278

Figure 4.1 – Local Coffee Farm Management Disturbance. A visual example of the impacts of local coffee replanting and plot renewal. The increase in exposed soil and reduction of vegetation is apparent, as well as the removal of older mature coffee, planting of new coffee saplings, application of agricultural lime (at the base of saplings), and the formation of terraces to facilitate the growth and eventual harvest of planted coffee. (Note: photo taken from a nearby area of the farm, but the visual impact at the study site was similar.)

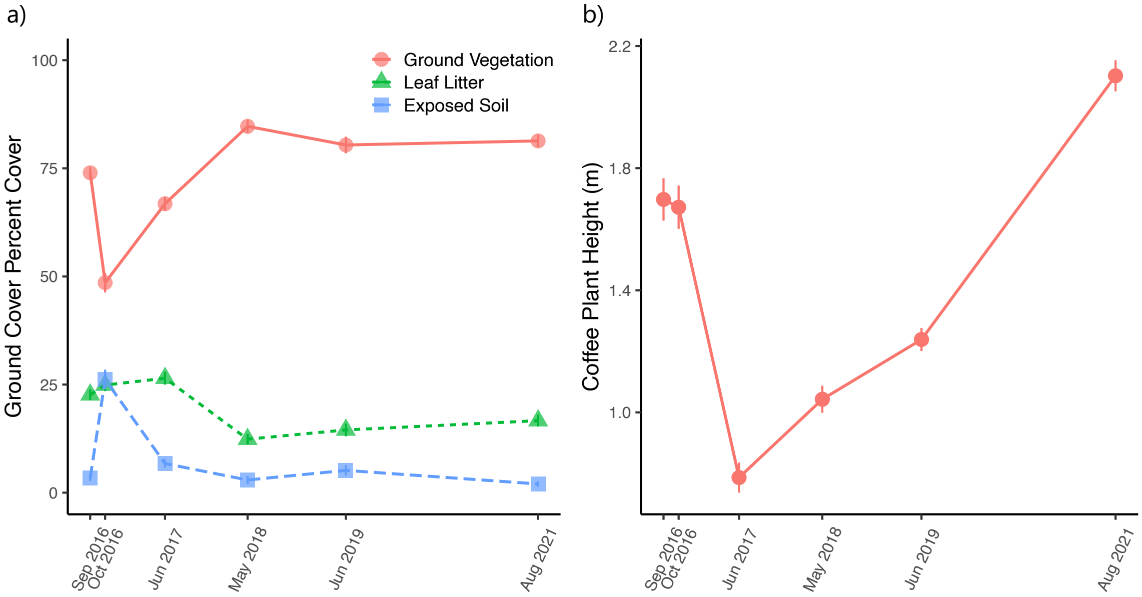


Figure 4.2 – Measuring Management Disturbance. Both figures show changes in the local environmental variables before and after farm management disturbance across time. **a)** Shows the mean (±SE) percent cover for ground vegetation, leaf litter, and exposed soil across all bait points in the plot for the duration of our study. **b)** Shows the mean (±SE) height (m) of coffee plants baited before and after disturbance for the duration of the study.

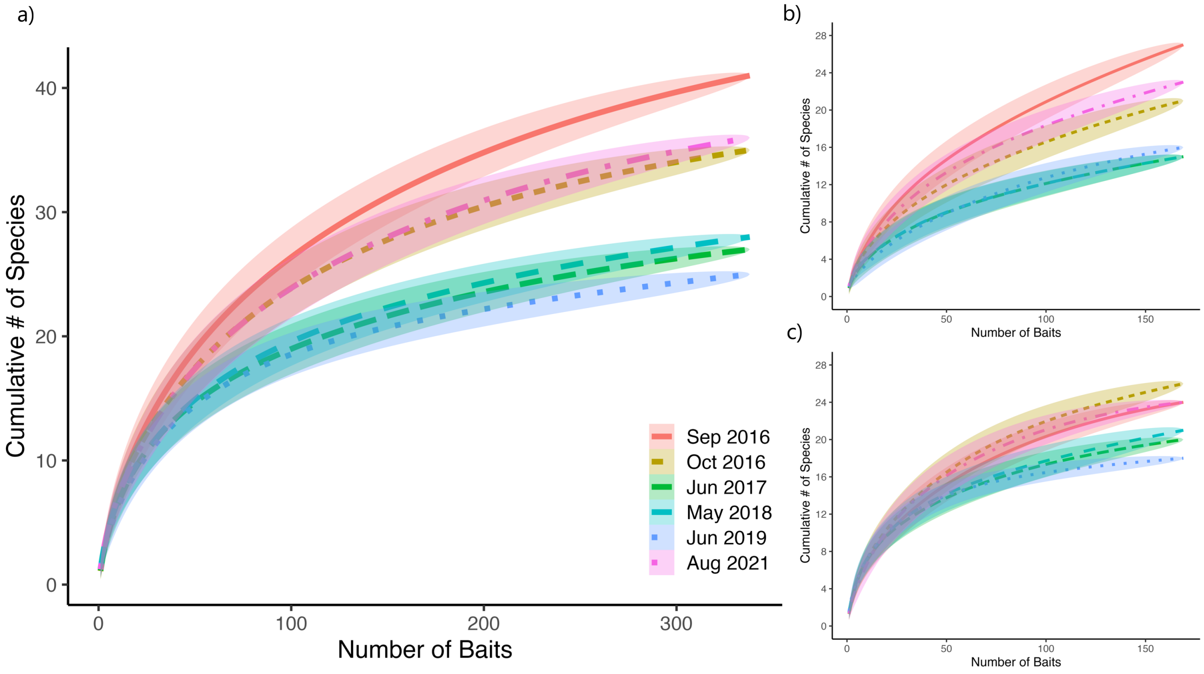


Figure 4.3 – Species Rarefaction. All figures show species rarefaction curves from surveys of the arboreal and ground ant communities in the plot across time. **a)** Displays species rarefaction for all ants in the community combined, while **b)** shows curves for the arboreal and **c)** ground ant surveys separately. In all plots, darker lines represent mean values from the resampling output, while the shaded area around lines shows the standard deviation from resampling. All curves were generated using the Mao Tau estimate (Colwell et al. 2012).

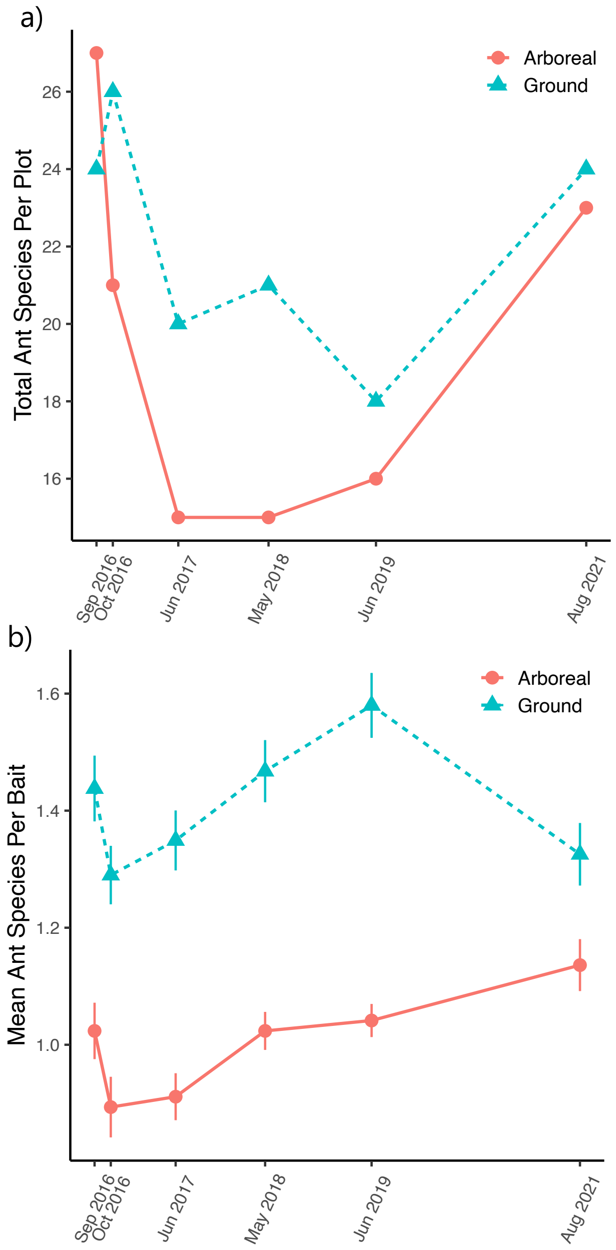


Figure 4.4 – Ant Species Richness. a) Shows total ant richness in the plot across time and **b)** mean (±SE) ant species richness per bait across time, both for arboreal and ground ant communities separately.

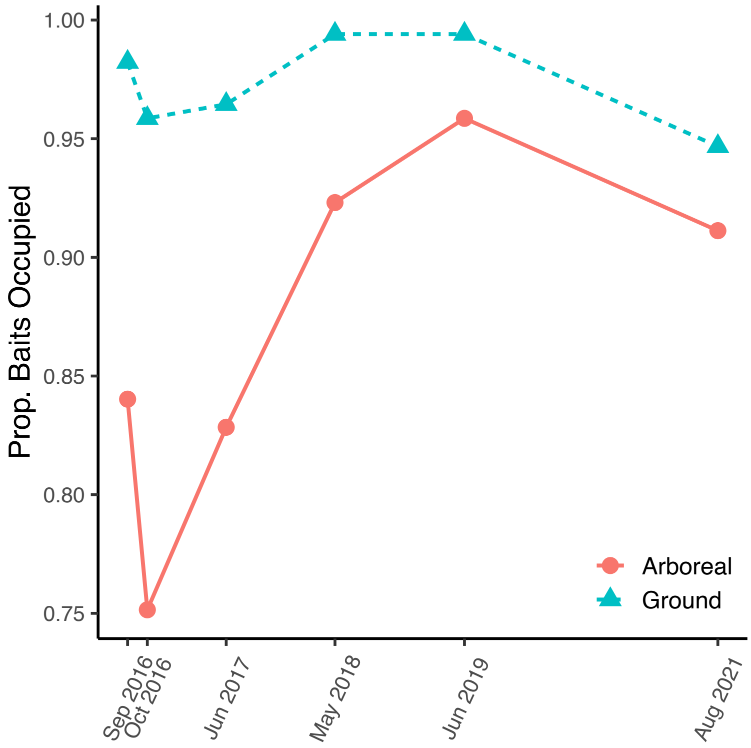


Figure 4.5 – Ant Abundance. Shows ant abundance measured as the proportion of total baits occupied at each sampling time point for the arboreal and ground ant communities separately.

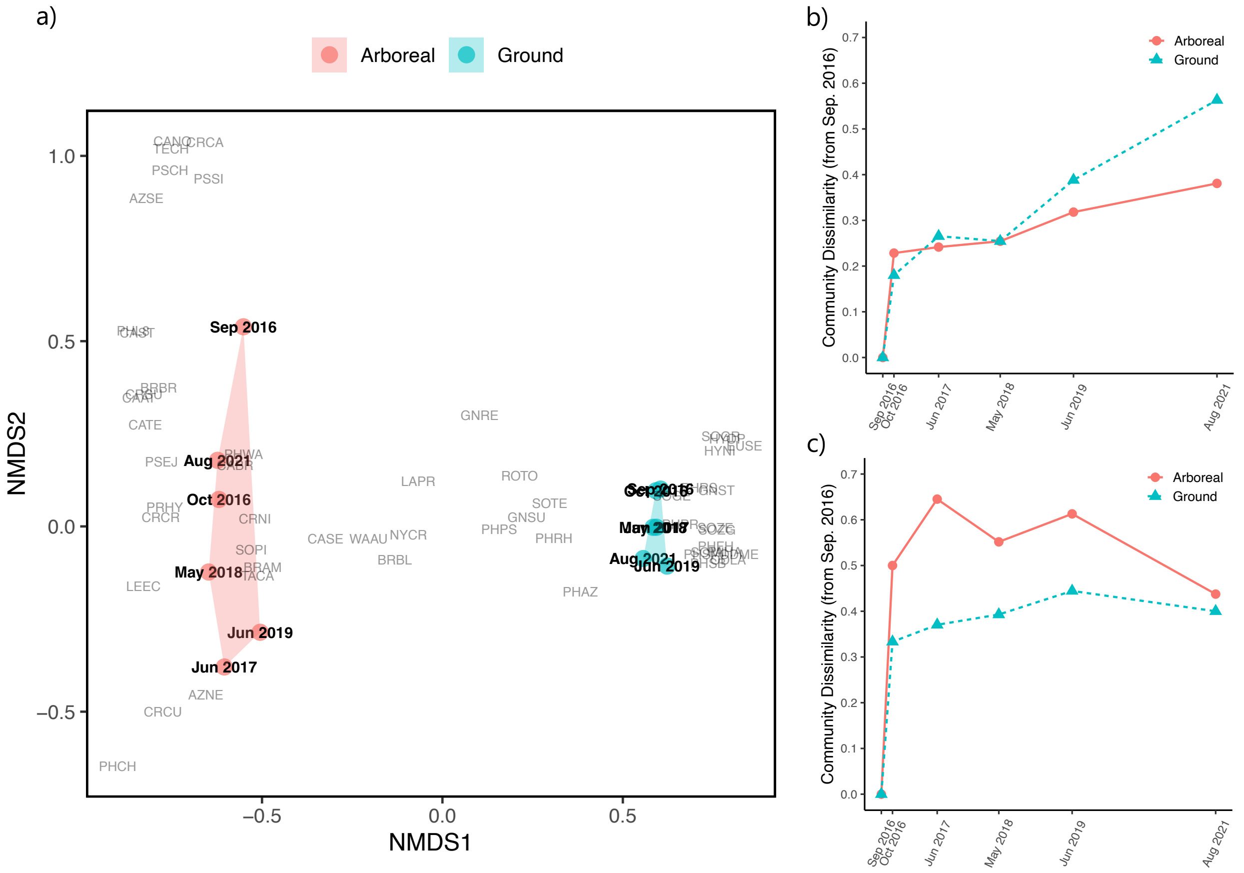


Figure 4.6 – Ant Community Composition and Turnover. a) Shows a non-metric multidimensional analysis (NMDS) of arboreal and ground ant communities with survey time points as temporal "sites". The relative abundance of individual ant species in NMDS space (between the two sampling strata) is displayed in grey text (species abbreviation codes). NMDS coordinates and dissimilarity measures were calculated using Bray-Curtis distance. Additionally, community turnover is assessed with pairwise dissimilarity through time compared to the original community in September 2016 using **b)** Bray-Curtis and **c)** Jaccard distance.

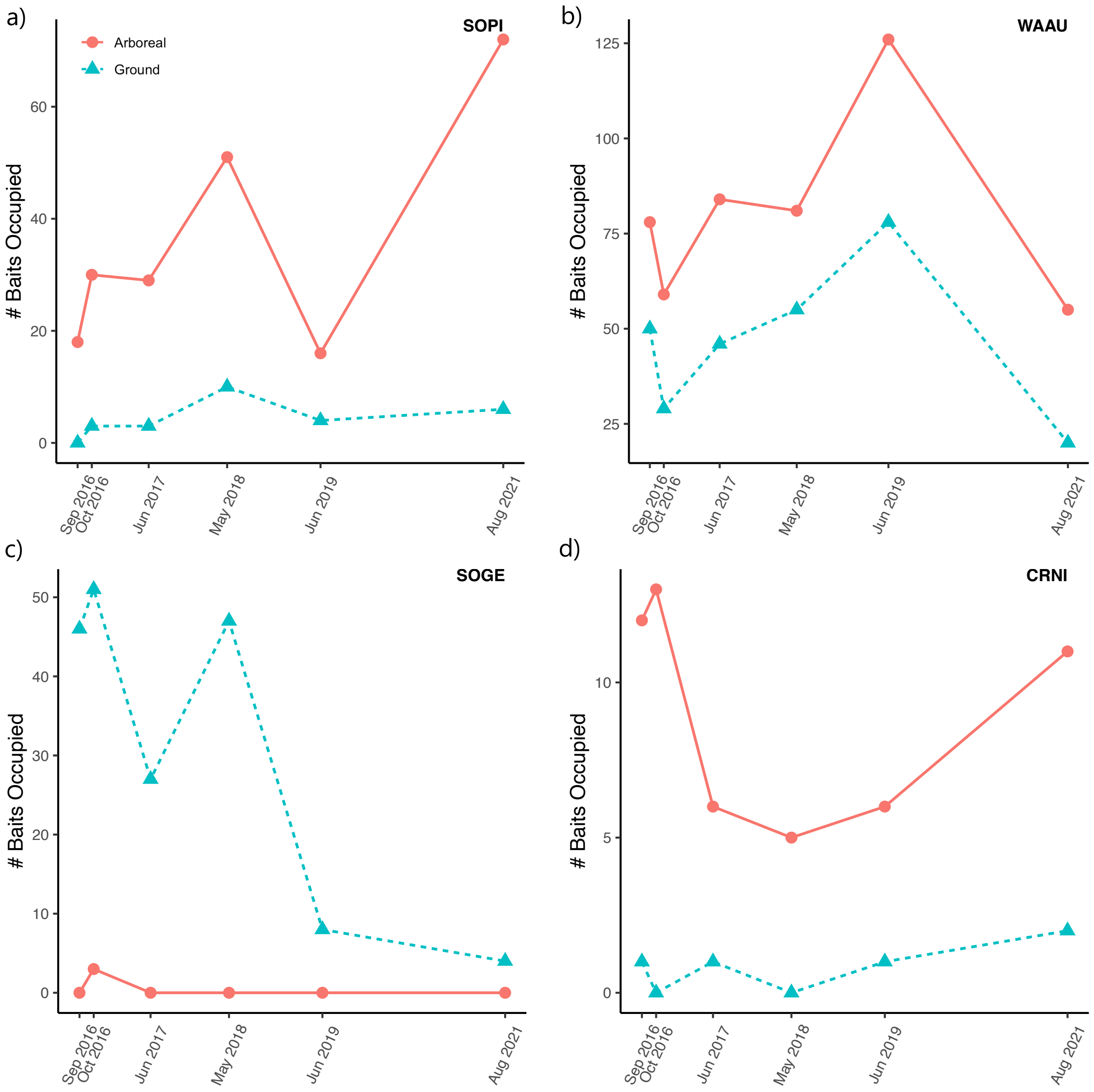


Figure 4.7 – Focal Pest Control Agents. Shows abundance (bait occupancy) through time of several ant species which are known natural enemies of the coffee berry borer: **a)** *Solenopsis picea*, **b)** *Wasmannia auropunctata*, **c)** *Solenopsis geminata*, and **d)** *Crematogaster nigropilosa*. Species abbreviations are displayed in the upper right corner of individual plots.

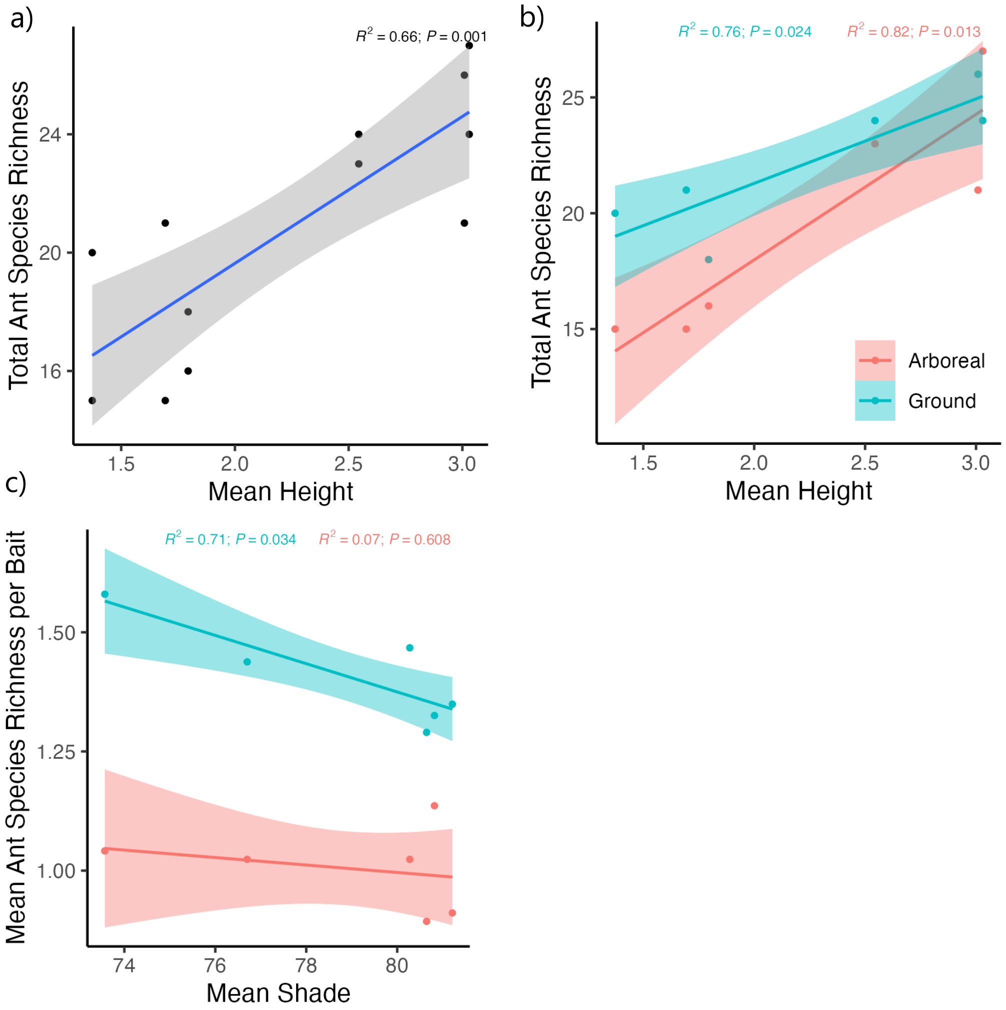


Figure 4.8 – Environment-Ant Relationships: Plant Height & Canopy Cover to Ant Richness. Both **a & b)** show the relationship between mean plant height and total species richness per plot sampling time. **a)** Shows the data combined while **b)** shows the data separated by strata. In **c)** the relationship between mean canopy cover in the plot and mean ant species richness per bait is displayed, with the data separated by strata. In all figures, model lines were fitted with simple linear regressions, where the R^2 and *p-values* displayed in figures correspond to those models.

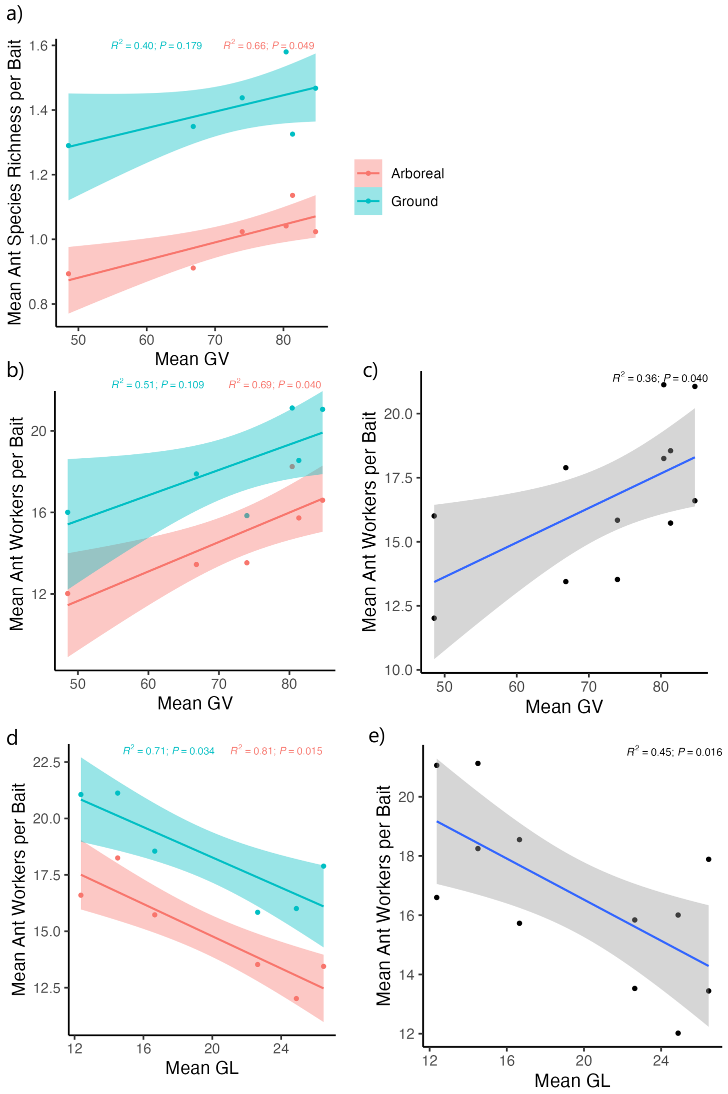
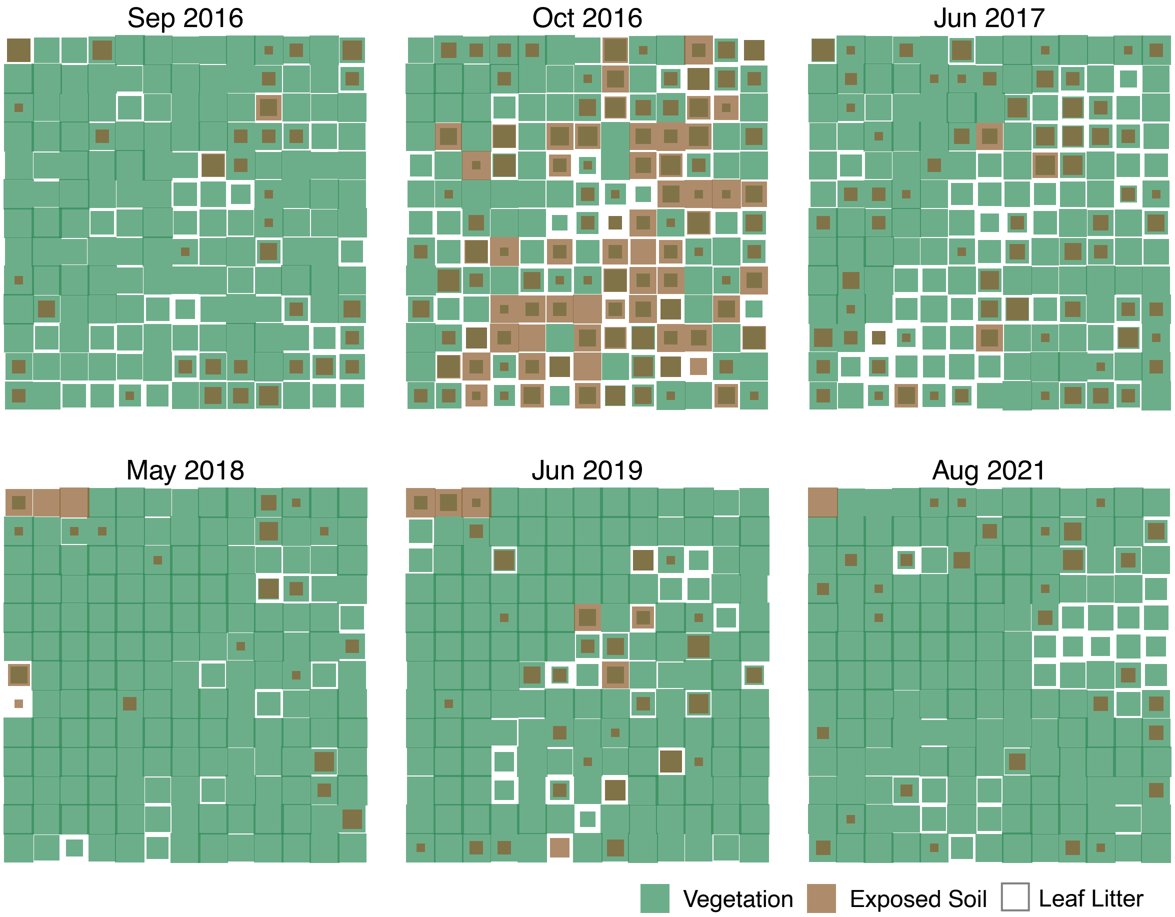
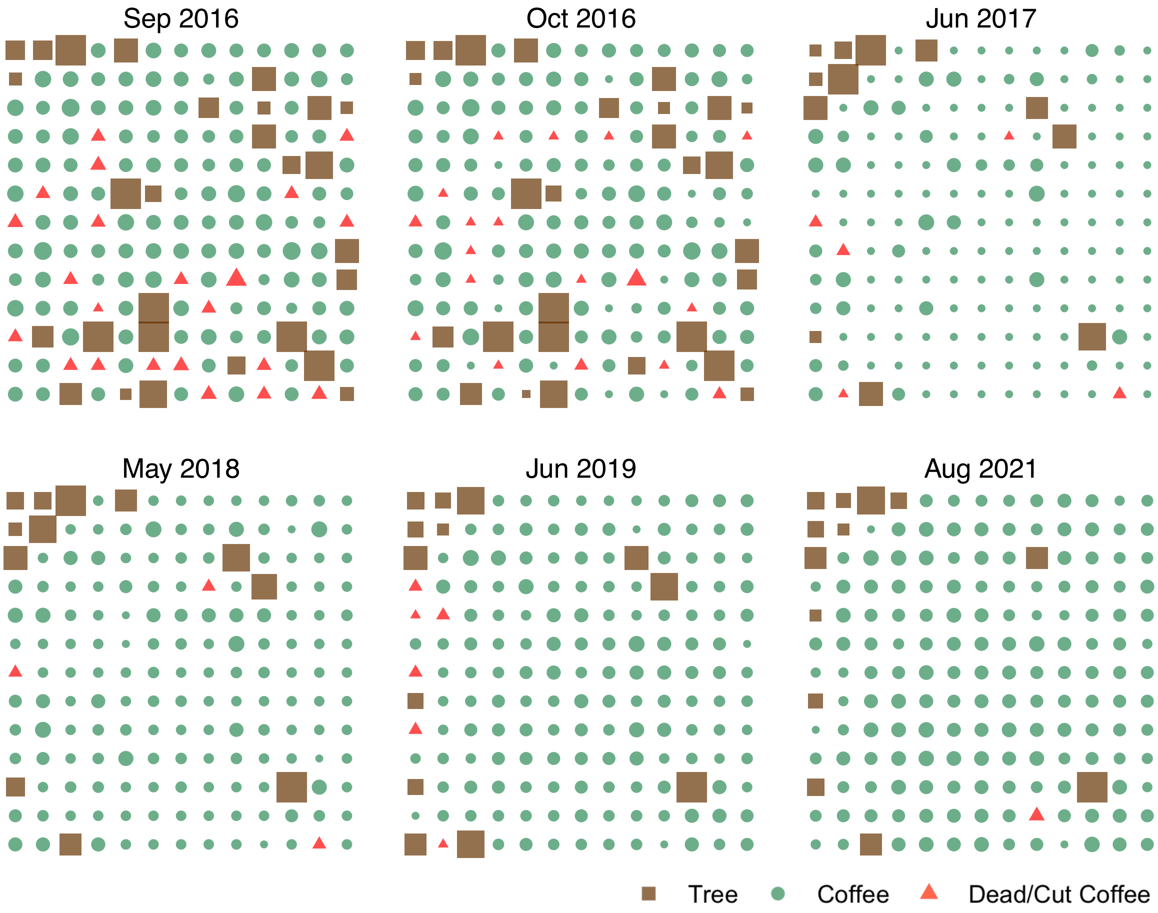


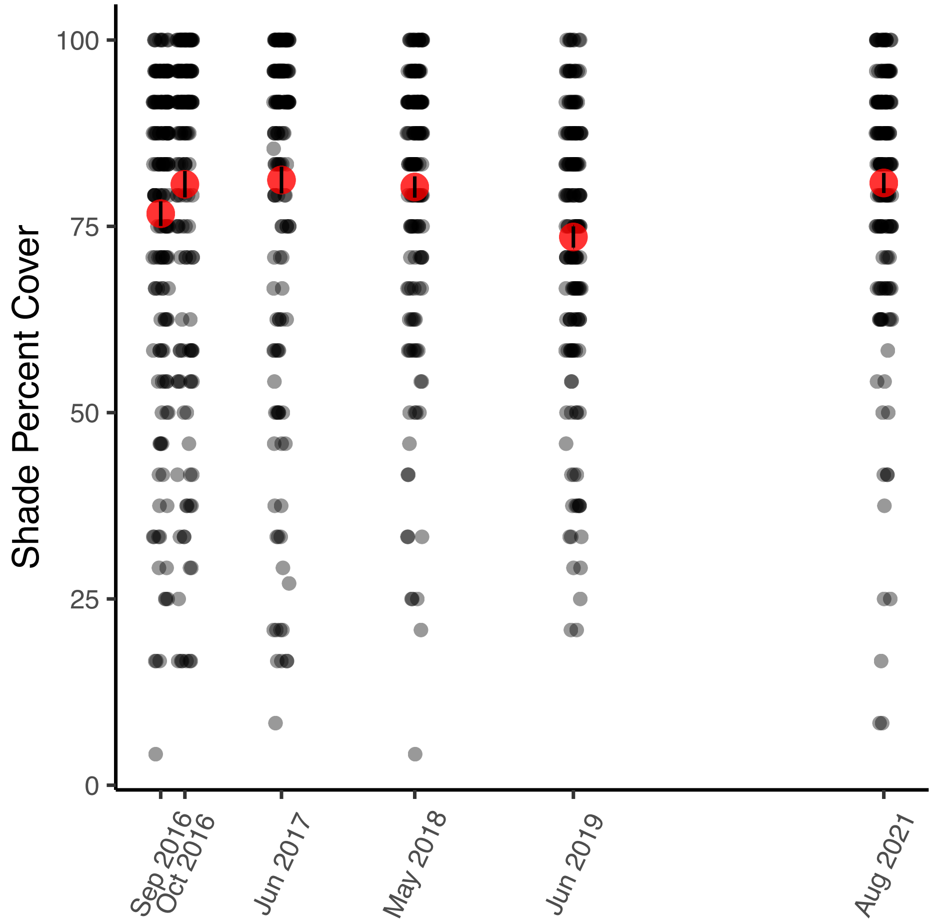
Figure 4.9 – Environment-Ant Relationships: Ground Cover to Ant Richness and Activity. a-c) Show the relationship between mean percent ground vegetation cover and **a)** mean ant species richness by strata, **b)** mean ant activity per bait by strata, and **c)** overall mean ant activity per bait. **d & e)** Show the relationship between mean percent leaf litter cover and **d)** mean ant activity per bait by strata and **e)** mean ant activity per bait overall. All points represent means of each variable across the plot for each sampling time point. In all figures, model lines were fitted with simple linear regressions, where the R^2 and *p-values* displayed in figures correspond to those models.



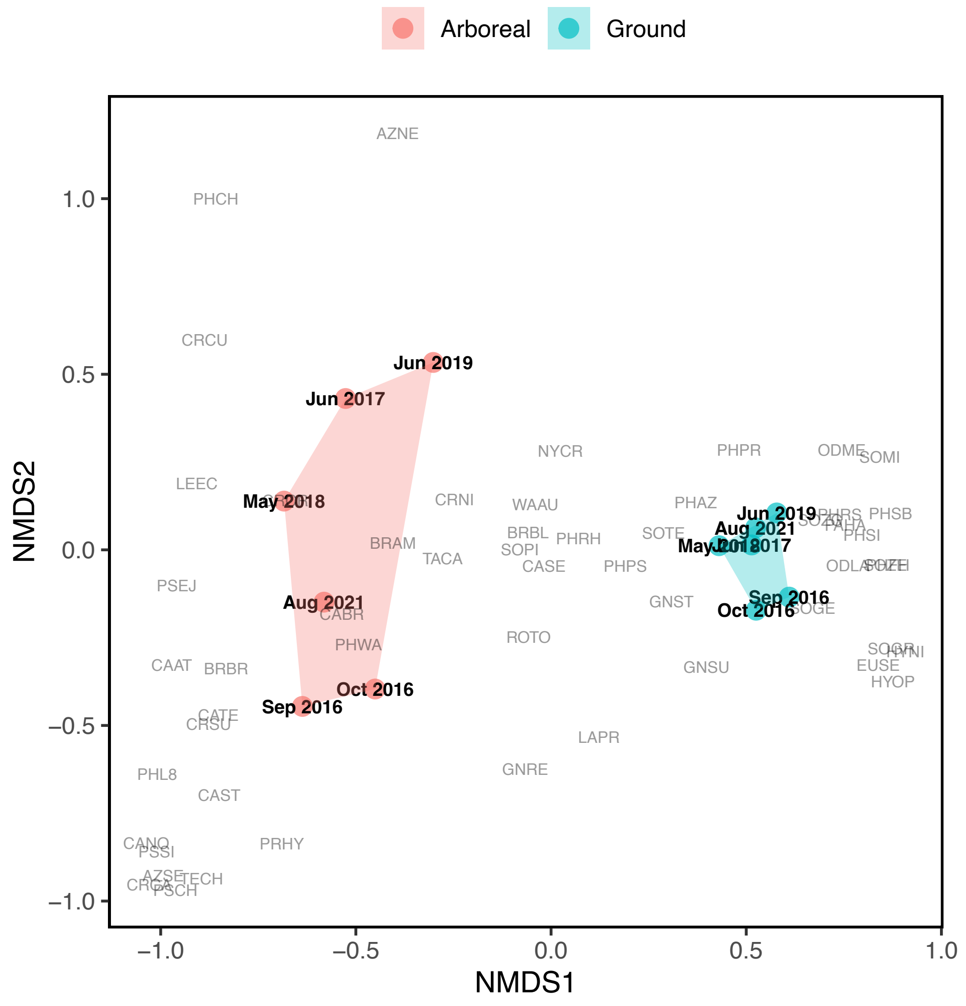
Supplementary Figure 4.1 – Spatial plots of ground cover through time. Green represents vegetation, brown represents exposed soil, and white spaces represent leaf litter. The size of each square depicts the relative proportion for each ground cover metric measured with a randomly placed quadrant at each bait sample location.



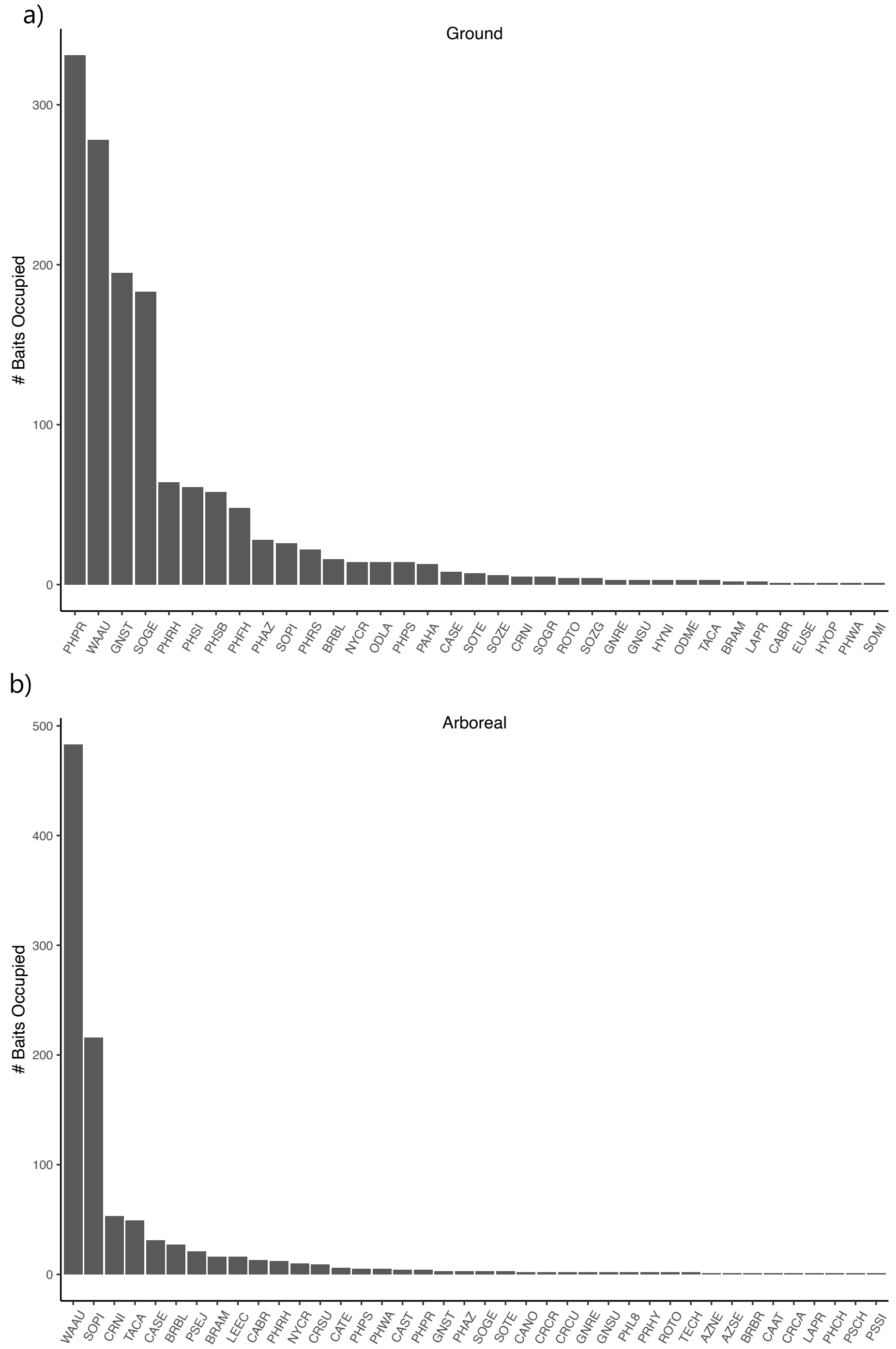
Supplementary Figure 4.2 – Spatial plots of baited plant identity through time. Green circles represent live coffee, red triangles represent heavily pruned or dead coffee, and brown squares represent other woody plants and shade trees. Point sizes depict relative plant height (m) at each point.



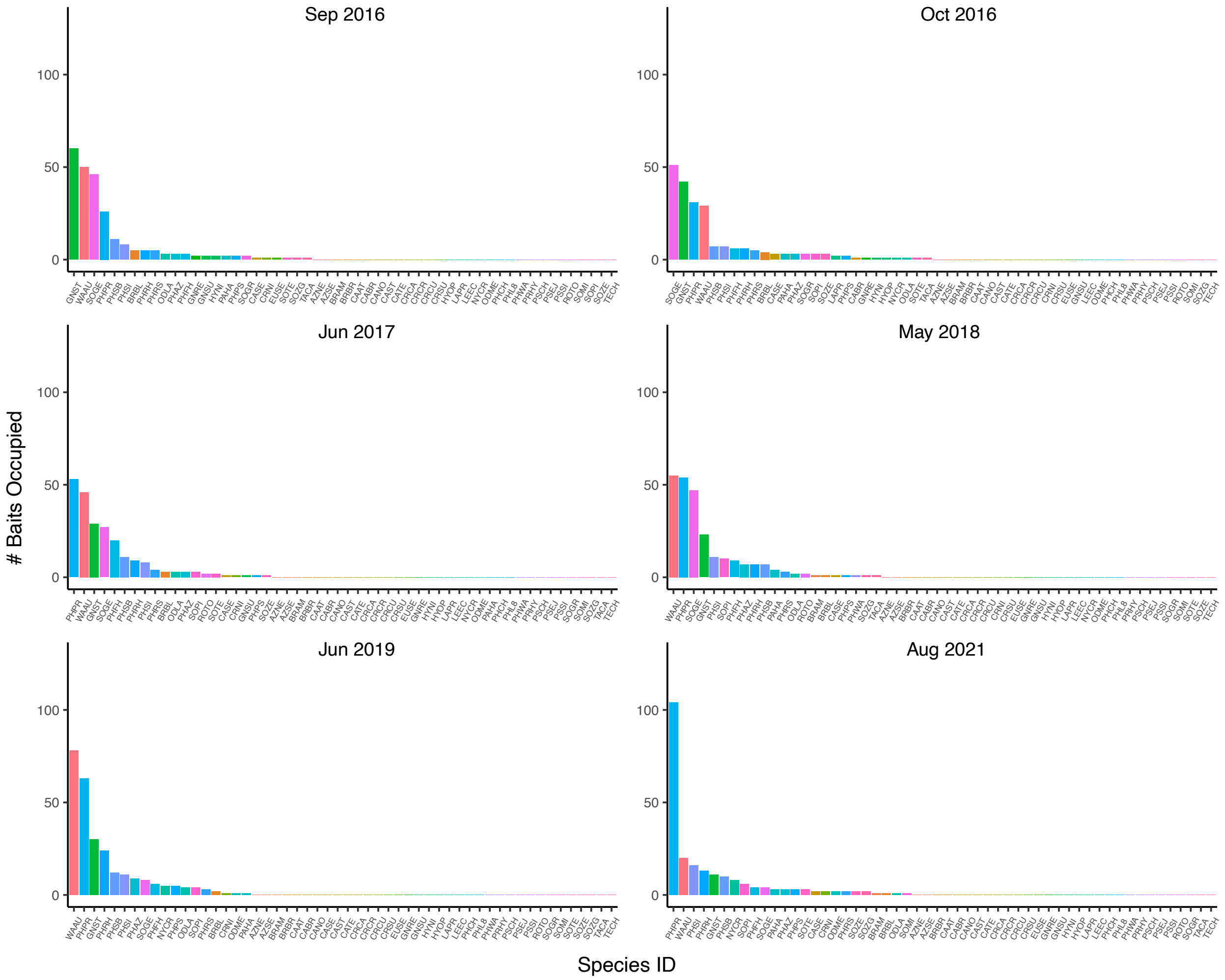
Supplementary Figure 4.3 – Percent shade (canopy cover) through time. Grey points represent raw data recorded at each bait location, while red points represent mean levels (±SE).



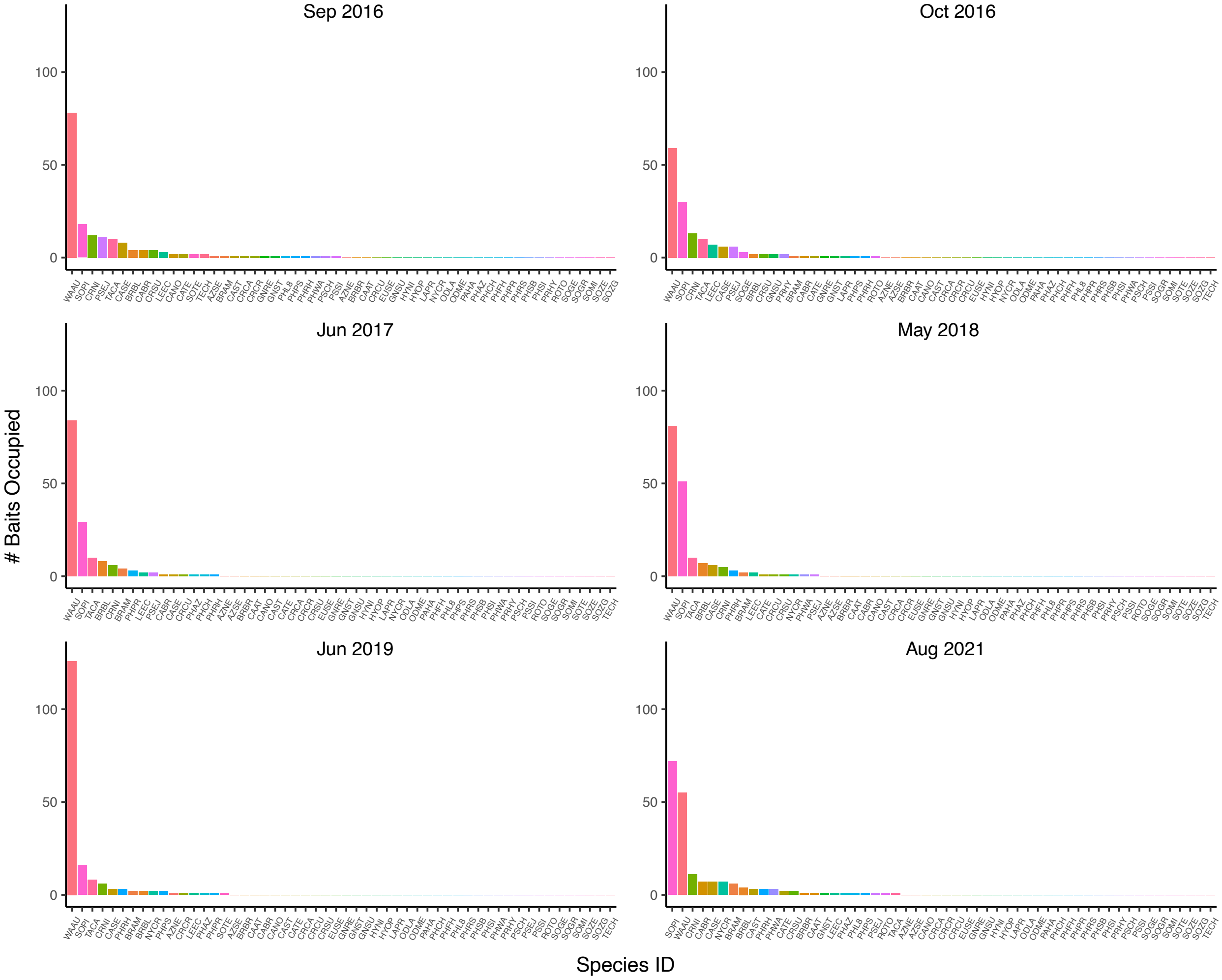
Supplementary Figure 4.4 – Shows a non-metric multidimensional analysis (NMDS) of arboreal and ground ant communities with survey time points as temporal "sites". The relative contribution of individual ant species to each stratum (ground or arboreal) in NMDS space is displayed in grey text (species abbreviation codes). NMDS coordinates and dissimilarity measures were calculated using Jaccard distance (species presence-absence only).



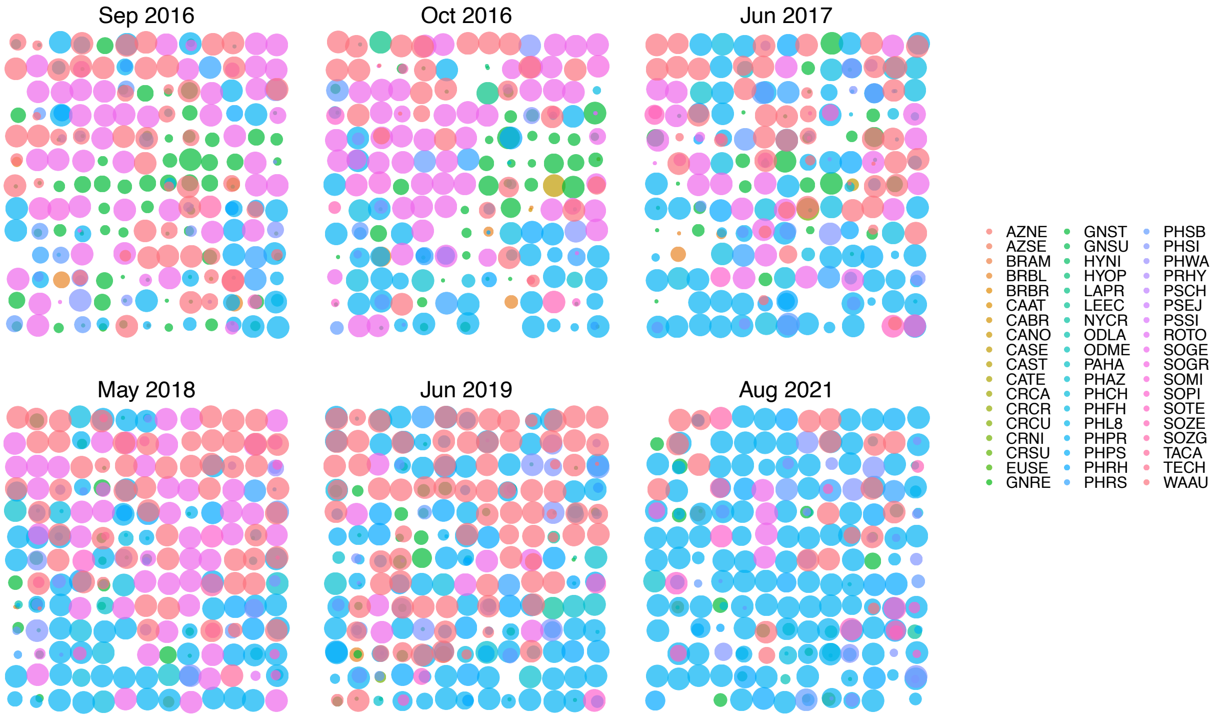
Supplementary Figure 4.5 – Species rank abundance curves of **a)** the ground ant community and **b)** the arboreal ant community through time for all sampling times combined. Species identity abbreviations are labeled on the x-axes of figures.



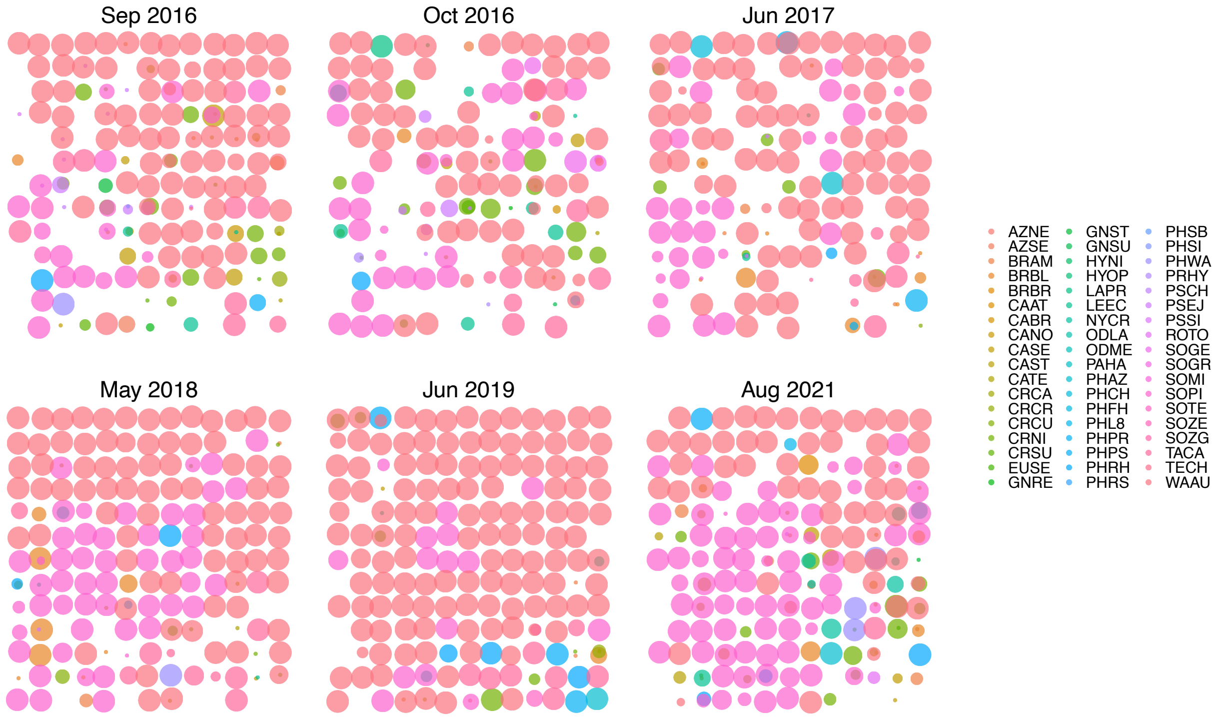
Supplementary Figure 4.6 – Species rank abundance curves of ground ants through time. Colors correspond to the same species across time and show shifts in relative abundance.



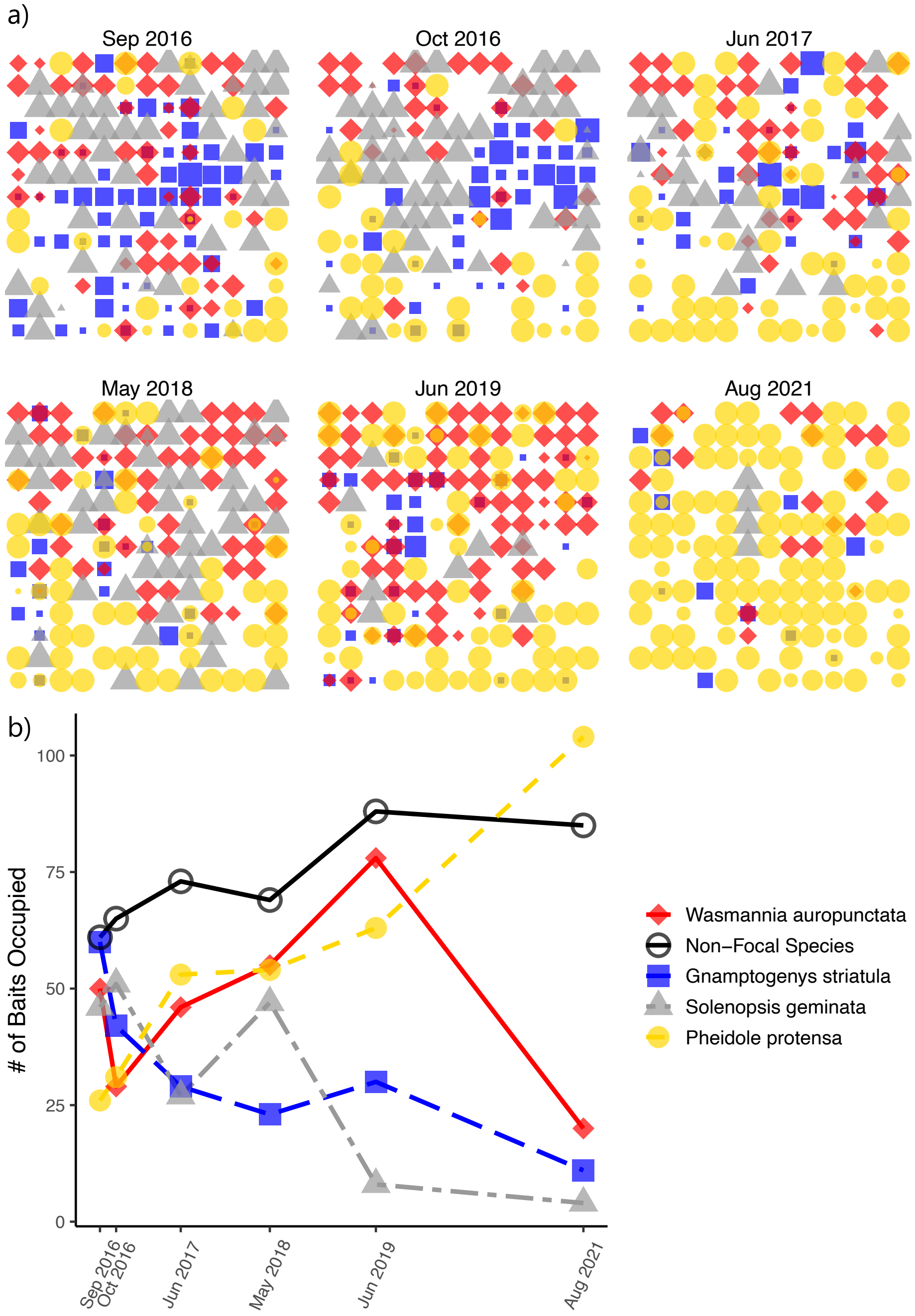
Supplementary Figure 4.7 – Species rank abundance curves of arboreal ants through time. Colors correspond to the same species across time and show shifts in relative abundance.



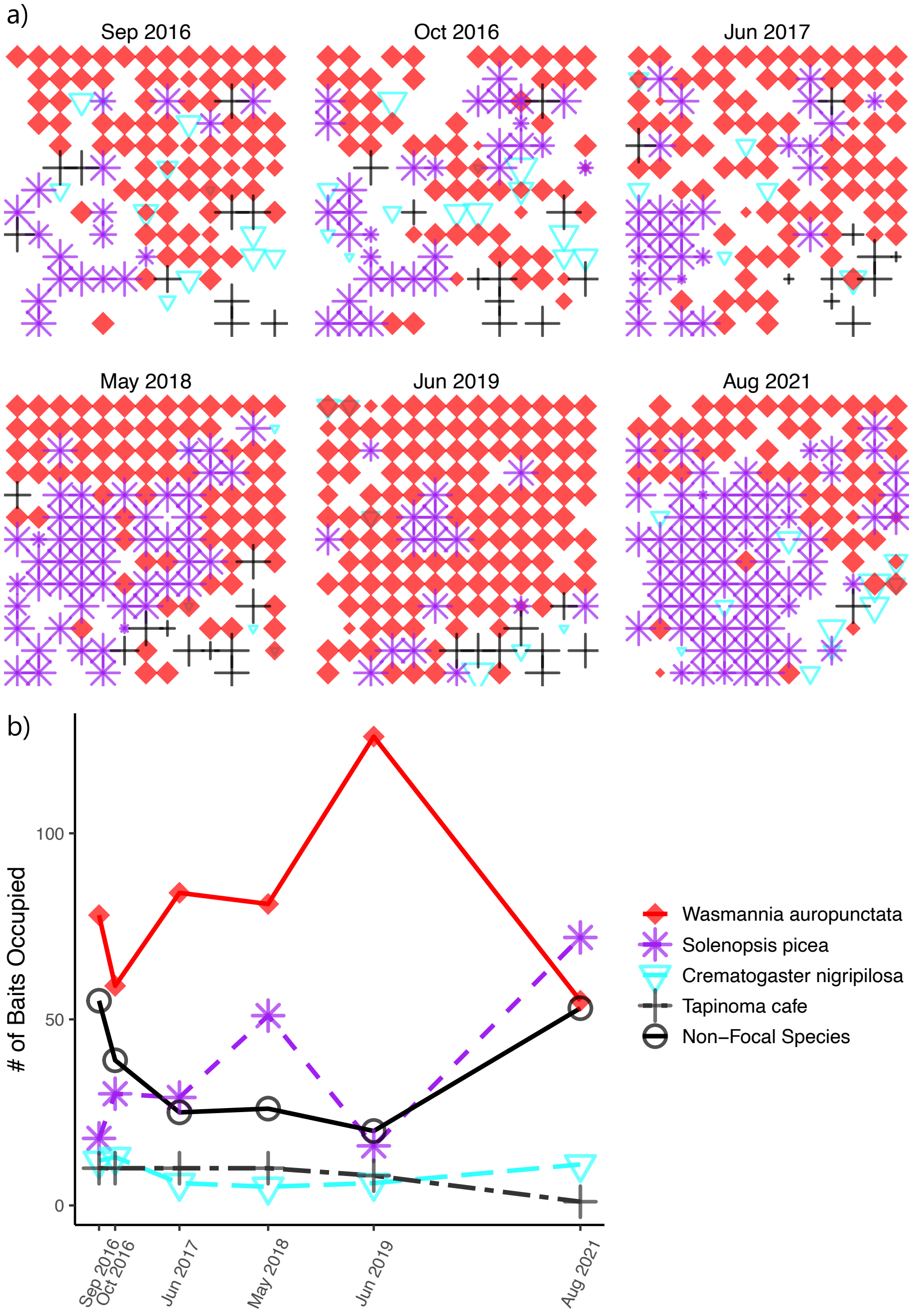
Supplementary Figure 4.8 – Spatial plots of all ground ant species through time. Point sizes depict the number of ant individuals (up to 20) for each ant species recorded at individual baits. The figure legend lists all species abbreviations and their corresponding color in plots.



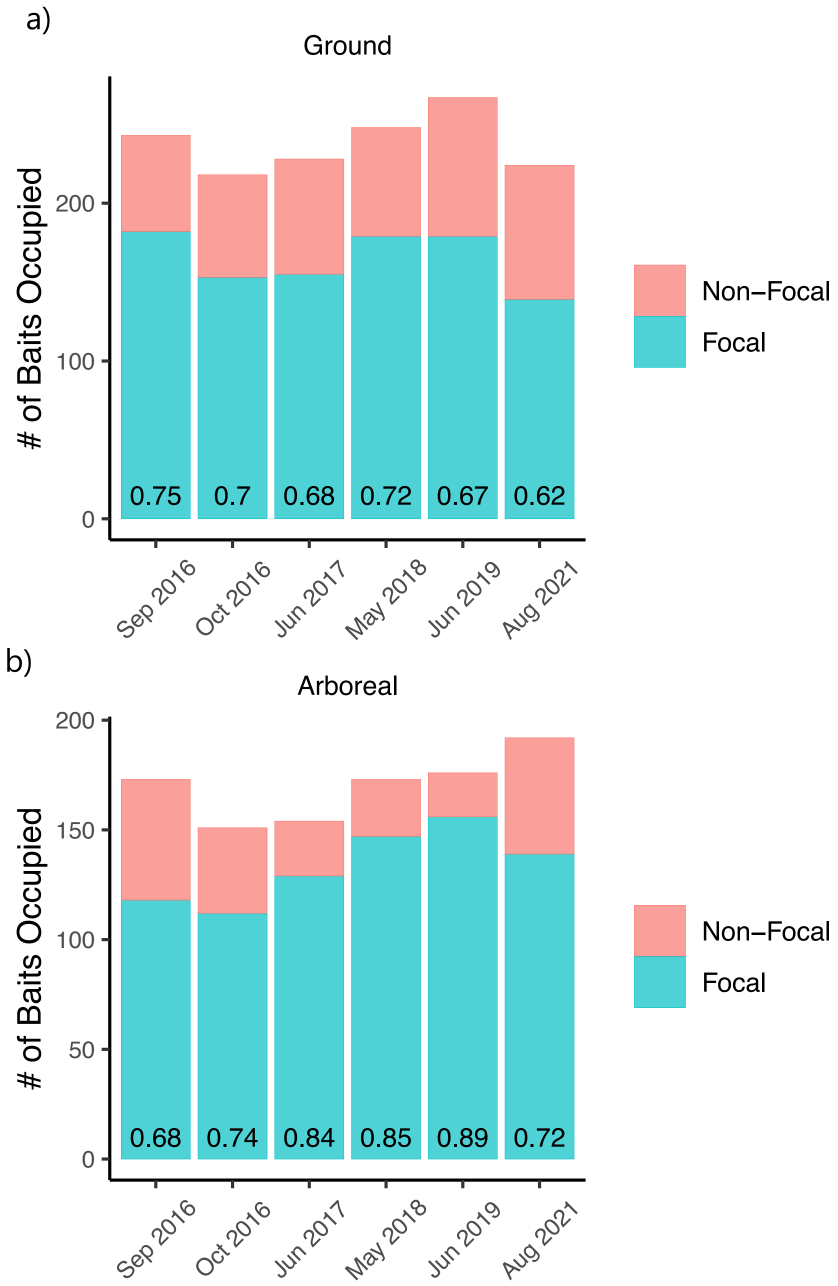
Supplementary Figure 4.9 – Spatial plots of all arboreal ant species through time. Point sizes depict the number of ant individuals (up to 20) for each ant species recorded at individual baits. The figure legend lists all species abbreviations and their corresponding color in plots.



Supplementary Figure 4.10 – a) Spatial plots of focal ground ant species through time. Point sizes depict the number of individuals (up to 20) for each ant species recorded at individual baits. **b)** Abundance (bait occupancy) of focal and non-focal ground ant species through time.



Supplementary Figure 4.11 – a) Spatial plots of focal arboreal ant species through time. Point sizes depict the number of individuals (up to 20) for each ant species recorded at individual baits. **b)** Abundance (bait occupancy) of focal and non-focal arboreal ant species through time.



Supplementary Figure 4.12 – Relative proportion of focal vs. non-focal ant species, based on bait occupancy, through time for sampled **a)** ground and **b)** arboreal ant communities. The proportion of focal species in communities is listed at the bottom of each bar per sampling time point.

Chapter 5 A Comparison of Coffee Epiphyte Communities Reveals Major Differences with Farm Management[4]

5.1 Abstract

Coffee farm management plays an important role in tropical conservation, where coffee agroforests can potentially provide habitat for a diverse array of unplanned biodiversity, including epiphytic plants. Epiphyte communities in coffee can be diverse and may include threatened species of orchids and bromeliads. Epiphytes in coffee may also provide important habitat for other ecological groups, like natural enemies that may be crucial for the regulation of coffee pests. The impact of coffee farm management has been explored for epiphyte communities on shade trees, and individual practices, such as desmusgue (epiphyte removal) have been investigated with regard to coffee-based epiphytes. Less research has explored how general farm management and coffee intensification impact the community of epiphytes on coffee. Here we report results from a survey study, comparing the epiphyte communities on coffee plants between two contrasting farms with different management, a shaded organic polyculture, and a high-input low-shade monoculture (where epiphyte removal is not regularly practiced in either farm). Overall, we found that the shaded polyculture has significantly more coffee epiphyte diversity, with higher total and mean species richness, and greater orchid and bromeliad abundance. Communities between the farms were also distinct, with higher evenness

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in the shaded polyculture. Our assessment of potential farm management drivers was less clear, where shade unexpectedly did not significantly explain coffee diversity patterns between farms. We did, however, find a significant impact of the distance to the nearest forest patch on epiphyte species richness in the shaded polyculture and an overall positive impact of individual coffee height on species richness and abundance. Our results demonstrate how the diversity of epiphytes on coffee plants may be significantly reduced by coffee intensification practices.

5.2 Introduction

While agricultural intensification poses a major threat to biodiversity around the world, agroecological approaches to crop production can make agriculture more favorable for biodiversity (Kremen 2020; Kremen & Merenlender 2018; Tscharntke *et al.* 2012). Coffee production typically occurs in the tropics, in biodiversity hotspot zones that are especially important for conservation (Moguel & Toledo 1999; Perfecto *et al.* 1996). The management of coffee agricultural systems spans a spectrum of intensification, from more traditional high-shade, low-input agroforests, to input-heavy monocultures (Jha *et al.* 2014; Moguel & Toledo 1999; Perfecto *et al.* 1996). It has also been demonstrated that coffee management and intensification can have major impacts on general biodiversity loss and conservation (Philpott *et al.* 2008).

Coffee systems may be especially important for the conservation of epiphytic plants since they are often located in the montane and cloud forest biogeographic zones that have a high diversity of these species (Hietz 2005). Because much of this habitat has been lost, coffee agroforest systems with shade trees may be essential for the conservation of rare species from these zones, especially certain orchids (García-González *et al.* 2017; Solis-Montero *et al.* 2005; Solís-Montero *et al.* 2019). Epiphytes in coffee can also have cascading consequences for other ecological groups by providing important habitat, especially for birds, ants, and other natural enemies (Cruz-Angón *et al.* 2009; Cruz-Angón & Greenberg 2005) that may play an important role in natural pest control.

Some important research has been conducted exploring the impact of coffee management practices on epiphytes. Most of this research has considered the arboreal communities of epiphytes on shade trees in coffee farms and nearby forests (Hietz 2005; Moorhead *et al.* 2010; Scheffknecht *et al.* 2012; Toledo-Aceves *et al.* 2012). One study demonstrated that farms with higher levels of shade can house more diverse arboreal epiphyte communities and buffer the drop-off in species richness observed when moving further away from nearby forest patches in farms with less shade (Moorhead *et al.* 2010). Other work has looked specifically at the epiphytes found on coffee plants and the impacts of epiphyte removal in farm management – a practice known as "desmusgue" (García-González *et al.* 2017; Mondragón *et al.* 2009; Solís-Montero *et al.* 2019). This research has been essential in demonstrating that coffee plants themselves, and not just shade trees, can house diverse communities of epiphytes and may be important reservoirs of rare epiphyte species (García-González *et al.* 2017; Solís-Montero *et al.* 2019). Despite this work, there has been less focus on the general effects of farm management and intensification (beyond desmusgue) on coffee-based epiphyte communities, even though coffee is the dominant plant on coffee farms and may provide the majority of vegetative niche space for epiphyte colonization.

With this study we aimed to fill this gap, combining the above approaches to investigate the broader impacts of coffee farm management on coffee epiphyte communities. We conducted a survey study to measure the diversity and community composition of coffee epiphytes in two farms of contrasting management and intensification, a high-shade, low-input polyculture, and a low-shade, high-input monoculture. Like Moorhead et al. 2010, we relied on a comparison of two neighboring farms, located in the same region and at the same site, to control for differences in the regional pool of epiphytes and to isolate the specific impacts of management on coffee epiphytes. We hypothesized that the high-shade farm would have greater coffee epiphyte diversity and abundance than the low-shade farm, since shade trees have been shown to be important reservoirs of epiphytes in coffee (Hietz 2005; Moorhead *et al.* 2010).

5.3 Methods

5.3.1 Study Site

Our study was based in the Soconusco of Chiapas, a major coffee-producing region in Mexico. We conducted surveys of epiphytes in two neighboring farms of contrasting management and agricultural intensification (Fig. 5.1). The first farm, Finca Irlanda, is a high-shade, low-input, organic polyculture. While the principal crop is coffee, the farm houses a diverse array of secondary crops, shade trees, and unplanned biodiversity. The second farm, Finca Hamburgo, is a low-shade, high-input monoculture. While there are some shade trees at this farm, they are typically much smaller, more dispersed, and individuals of only a few species, especially the genus *Inga*. For clarity, throughout the study, we refer to these two farms as “shaded polyculture” and “sun monoculture.” Both farms are located roughly at 1000-1100m elevation and 15°10'25"N and 92°19'57"W. The fact that these two farms are neighboring, but of contrasting management intensity made this an ideal site to investigate the relationship between management and coffee epiphyte communities. Because they are located in the same region, and nearly the same location, the regional pool of epiphyte species should be roughly the same for the two farms, helping to control for site-by-site community variation. Additionally, unlike farms from other nearby coffee-producing regions (Solís-Montero *et al.* 2019), it appears that neither of these farms practice intensive removal of epiphytes from coffee (known as “desmusgue”), making it possible to test for relationships between broader management practices, such as shade cover or coffee pruning, and epiphytes.

5.3.2 Epiphyte Survey

To systematically survey coffee epiphyte communities, we haphazardly selected ten sites on each farm. Sites were located a minimum of 53.7m apart, but on average were separated by 109.4m. At each site, we haphazardly selected a central coffee plant and laid two intersecting transect lines of 10m crossing at the central plant. We then selected coffee plants closest to 5m and 10m away from the central plant, in all four directions for a total of nine plants per site, including the central plant. We ensured that all selected plants were a minimum of 1.5m tall and 1in wide in base diameter. If the closest plant at each point did not meet these criteria, we selected the next nearest plant that did. At each central plant, for all sites, we recorded the latitude and longitude using a handheld GPS and estimated shade with a densiometer, taking the average of measurements in all four cardinal directions. We also visually estimated the height and width of all surveyed coffee plants. For our epiphyte surveys, we identified and counted the number of unique species of vascular epiphytes and the abundance of orchid and bromeliad species on all coffee plants. Abundance was only measured for orchids and bromeliads because most of the epiphytic fern species in the region have growth patterns that make it difficult to identify individuals. Additionally, we focused our surveys on true epiphytes and not hemi-epiphytes, which can exhibit vine-like growth after rooting in the soil and may be affected by other aspects of management.

5.3.3 Data Analysis

5.3.3.1 Farm Management & Environmental Metrics

To understand how the local environment and management at our sites influenced coffee epiphytes we analyzed a number of related metrics. We tested for differences between percent shade cover, coffee height (m), and coffee diameter (in) between the two surveyed farms. For shade cover, we compared levels across our sites between the two farms with a generalized linear model using a quasibinomial error distribution to account for proportional data. Coffee height and diameter were compared between farms directly using generalized linear mixed models (GLMMs) with gamma error distributions (log link) and site as a random effect to control for spatial non-independence between coffee plants. These variables were also included as environmental covariates in models testing differences in our epiphyte communities between farms, which we describe further below. For coffee diameter, we discovered a few missing entries in our data (four plants out of 180 total). We replaced these with the mean value from the rest of our data set in order to use this variable as a covariate in our statistical models. Additionally, we estimated the distance of each site from the nearest forest patch to understand how natural habitat interacts with farm management to influence coffee epiphyte communities. Distances were calculated as the most direct line from the recorded GPS coordinates to the nearest forest edge, based on manually digitized land cover polygons (De la Mora *et al.* 2013). We included distance to forest as a fixed effect in our models described below.

5.3.3.2 Epiphyte Community

To assess differences in coffee epiphyte communities between farms we conducted a number of analyses on species richness, abundance, and community composition. We first ran species rarefaction curves on the survey data to determine how well communities were sampled and to test for differences in total epiphyte species richness between farms. Because our sampling effort was fixed, with the same number of sites and coffee plants per site, we assumed that if species rarefaction curves were asymptotic, we should be able to make reliable comparisons of the data. Estimated means and standard deviations for rarefaction curves were generated using the Mau Tao method (Colwell *et al.* 2012) for site-level rarefaction. We then compared epiphyte species richness per coffee plant between farms. For this, we ran a generalized linear mixed model, using a Poisson error distribution to fit count data, and included species richness, distance to forest, and their interaction as fixed effects. Similarly, to assess differences in the summed abundance of orchids and bromeliads on coffee plants, we ran a GLMM using a negative binomial error distribution to fit count data (the original model with a Poisson distribution was overdispersed), with species richness, distance to forest, and their interaction as fixed effects. For both models, we included coffee height, coffee width, and shade cover as covariate fixed effects, which were centered and scaled, along with the distance to forest variable, to assist model convergence. Additionally, both models included site as a random effect to account for variation between sites and spatial non-independence between coffee plants at each site.

We assessed differences in epiphyte community composition between farms by calculating community dissimilarity distance matrix between sites. We did this using Bray-Curtis similarity to include both species identity and abundance and Jaccard similarity for species identity alone. We then ran PERMANOVs to test for statistical differences in the multivariate centroids of treatment groups. To visualize these differences, we conducted non-metric multidimensional scaling (NMDS) of community multivariate space to collapse the variation in community composition across all species into two dimensions. Finally, to complement our analysis, and further assess compositional differences between communities, we constructed species rank abundance curves and calculated the probability of interspecific encounter (PIE) for each community.

All data analysis was performed in R version 4.3.1 (R Core Team 2023). All GLMMs were run using either the “glmer” or “glmer.nb” functions from the “lme4” package. For GLMMs, fixed effect coefficients and random effect variance were estimated using maximum likelihood (with Laplace approximation). GLMs were run using the base “glm” function. Distance analysis was performed using the “sf” package for geospatial processing. Rarefactions, community dissimilarity matrices, and NMDS plots were all run using the vegan package, while PERMANOV tests were run with 999 permutations using the “adonis2” package.

5.4 Results

Our analysis of farm management and environmental variables revealed important differences between the two farms. As expected, shade levels were very different, with nearly 90% mean shade cover in the shaded polyculture vs. less than 40% shade cover in the sun monoculture (Table 5.1 & Fig. 5.2). Coffee basal diameter was also significantly different between farms and coffee height was marginally significantly different, with thicker plants on average in the sun monoculture, but taller plants in the shaded polyculture (Table 5.1).

Analysis of species rarefaction showed that both farm communities were thoroughly sampled, with both curves appearing asymptotic (Fig. 5.3). Given this, and our equal sampling effort between farms, the pronounced difference in total species richness observed between farms is both reliable and impressive. We observed nearly double the total species in the shaded polyculture than in the sun monoculture, with 16 and nine coffee epiphyte species, respectively. On a per plant basis, mean species richness on individual coffee plants was also significantly greater in the shaded polyculture, with an average of 2.62 species per plant compared to 1.49 in the sun monoculture (Table 5.2 & Fig. 5.4a). This trend was also reflected in the abundance data for orchid and bromeliad individuals, with over 5 times more individual orchids and bromeliads on coffee plants in the shaded polyculture (Table 5.2 & Fig. 5.4b). In our model of species richness, several environmental covariates were significant factors, including a significant positive overall impact of coffee plant height and diameter (Table 5.2 & Fig. S5.1). Interestingly, distance to the nearest forest patch was a significant driver of epiphyte richness only in the shaded polyculture farm, with less richness observed farther from forest (Table 5.2 & Fig. 5.5). Although richness levels were much lower in the sun monoculture overall, they were consistent across all levels of forest distance for the sites that we sampled (Fig. 5.5b). Contrastingly, in our model of epiphyte abundance (orchids and bromeliads) none of the covariates tested were significant, however there was a marginally significant positive effect of coffee height (Table 5.2 & Fig. S5.2). Surprisingly, in both models we did not find a significant effect of shade on epiphytes.

Community composition analysis of epiphytes also revealed significant differences between farms. The communities were different both using Bray-Curtis distance (Fig. 5.6a, R^2=0.233, F=5.483, p=0.001; NMDS stress=0.132) and with Jaccard distance (Fig. 5.6b,

R^2=0.229, *F*=5.340, *p*=0.003; NMDS stress=0.186). Species rank abundance curves also demonstrated higher evenness in the community of epiphytes on coffee in shaded polyculture (Fig. 5.7), which was confirmed by a comparison of the probability of interspecific encounter metrics (PIE: Sun Monoculture=0.614; Shaded Polyculture=0.824).

5.5 Discussion

Coffee farms exist along a spectrum of management intensification, are often located in biodiversity hotspots, and have the potential to be managed agroecologically to better promote conservation and ecosystem services, like natural pest control (Jha *et al.* 2014; Moguel & Toledo 1999; Perfecto *et al.* 1996). Epiphyte communities in coffee can be diverse (Hietz 2005), housing rare species of orchids and bromeliads (García-González *et al.* 2017; Solis-Montero *et al.* 2005), and can also provide resources for pollinators and habitat for important natural enemies, which may contribute to pest regulation (Cruz-Angón *et al.* 2009; Cruz-Angón & Greenberg 2005). Our survey study revealed significant differences in the epiphyte communities on coffee plants between two farms of contrasting management. Overall, the more intensively managed sun monoculture farm had significantly less epiphyte diversity, richness, and abundance than the shaded monoculture, with nearly double the total species observed. Furthermore, communities between the two farms were compositionally distinct, where the sun monoculture also had less even species distribution. These results, collectively, imply a significant impact of farm management on coffee epiphytes.

Between the two farms, clear management differences were observed, including more than twice as much shade cover in the shaded polyculture. Despite this stark difference and the difference in coffee epiphyte diversity, we did not find a significant direct impact of shade on epiphyte communities in our statistical models. This disagreed with our initial hypothesis and is surprising given the fact that many of the orchid, bromeliad, and fern species in these communities are wind-dispersed (Madison 1977) and large shade trees are probably an important source of individuals for many of these populations (Moorhead *et al.* 2010). While shade cover did not appear to be a significant factor, we did find that distance to the nearest forest patch was an important driver of epiphyte communities, but interestingly, only in the shaded polyculture, where species richness declined with distance.

This finding was also surprising and contrasts somewhat with previous work on arboreal epiphytes at this site (Moorhead *et al.* 2010). Moorhead et al. 2010 found a decline in arboreal epiphytes on shade trees with forest distance in the same two farms studied here, but a more pronounced decline in the sun monoculture, suggesting that the higher levels of shade cover and shade tree diversity in the shaded polyculture buffer the distance effect. Contrastingly, for coffee epiphytes, we found no effect of distance in the sun monoculture, but a decline with forest distance in the shaded polyculture, despite higher overall levels of species richness across all distances. This finding is perplexing but may suggest that other management factors are more relevant for coffee epiphyte communities in the sun monoculture farm. For both farms, we found a significant positive overall effect of coffee height on epiphyte species richness and a marginally significant effect of coffee height on orchid and bromeliad abundance. In the sun monoculture, we also found shorter coffee plants on average (marginally significant in our model). This is likely the result of aggressive coffee pruning management that we have observed at this farm, where coffee plants are pruned almost entirely to their base after a few years and left to resprout, presumably to stimulate productivity. This reduces vegetative niche space for epiphytes and would significantly limit coffee epiphyte colonization and accumulation compared to the coffee plants in the shaded polyculture, which are not typically pruned so aggressively. This factor may explain why forest distance had no effect in the sun monoculture, where pruning might essentially override that driver. However, this trend could also be explained by other management factors that we did not measure in this study, such as the use of herbicides or potential epiphyte removal. While we have not observed regular desmusgue at either of these sites, if it occurs it would add noise to the assessment of the other management signals (Mondragón *et al.* 2009).

To add complexity to the interpretation of our results, none of the management or environmental factors we measured, except for a marginally significant effect of plant height, explained the 5-fold difference in orchid and bromeliad abundance on coffee plants between farms, with greater abundance in the shaded polyculture. While coffee plant height may be an important driver of abundance in these two epiphyte groups, it is not clear why shade cover or distance from the forest were not, given their natural history as primarily wind-dispersed plants (Madison 1977). It is possible that while forest patches are important source pools that determine the regional epiphyte community, coffee plant height (and by proxy age) is more important for driving abundance on a site-by-site basis. In this case, the increased vegetative niche space provided by large coffee plants could promote more individual orchid and bromeliad colonization, and as individuals reproduce locally, their wind-dispersed seeds would be more likely to colonize the same host coffee plant, increasing their abundance.

Regardless of the precise mechanism, it is clear that overall farm management is driving major differences in coffee epiphyte communities at this site. Importantly, intensification in the form of a sun monoculture farm was related to a major reduction in the overall diversity of coffee epiphytes, when compared to a shaded polyculture. This is a significant finding, considering that coffee plants are likely the most abundant vegetative niche space available for epiphyte colonization in coffee plantations. While important work has focused on the conservation of epiphyte communities on shade trees in coffee systems (Hietz 2005; Moorhead *et al.* 2010; Solis-Montero *et al.* 2005; Toledo-Aceves *et al.* 2012), the epiphyte communities on coffee may be of equal importance for conservation and are likely to be more immediately impacted by farm management. This is known from studies on the direct removal of epiphytes from coffee, known as desmusgue (García-González *et al.* 2017; Mondragón *et al.* 2009; Solís-Montero *et al.* 2019), but few studies have explored the overall impact of farm management and intensification on coffee epiphytes, specifically. Our results here, along with the highlighted literature, suggest that the intensification of coffee farm management may significantly reduce the capacity of coffee farms to house diverse epiphyte communities. This is of obvious conservation concern since many of these epiphyte species have already lost significant native habitat in tropical montane and cloud forest regions (Hietz 2005; Solis-Montero *et al.* 2005), but also may have cascading negative consequences on ecosystem services, like natural pest control, since epiphytes provide important resources for many natural enemies in coffee (Cruz-Angón *et al.* 2009; Cruz-Angón & Greenberg 2005). Future work should explore the precise management mechanisms driving the patterns we observed, especially regarding coffee plant pruning frequency, to better understand how coffee farms can be more sustainably managed to promote diverse epiphyte communities and their associated ecosystem services.

5.6 Acknowledgements

We are indebted to Floridalma Marleni Velásquez, Miriam López Pérez, Gabriel Dominguez Martinez, and Gustavo López Bautista for their invaluable support and friendship in the field. We thank Ricardo Alberto Castro Chan at ECOSUR, and Gerardo Adolfo Salazar Chávez, Laura Calvillo, Ana Gabriela Martínez-Becerril, and Rafael Torres Colín at the MEXU Herbarium at the Universidad Nacional Autónoma de México for assistance with epiphyte specimens. We thank Blanca Hernández Hernández and Graciela García Guzmán at the Institute of Ecology at UNAM for logistical assistance. We thank Annie Damon for helpful discussion of the project. Finally, we thank both the Perfectomeer and Parcela lab groups for general feedback on the project. Funding for this project was provided by a Fulbright-Hays Doctoral Dissertation Research Abroad Fellowship, a Rackham Graduate Student Research Grant from the University of Michigan, and a Graduate Research Fellowship from the Institute for Global Change Biology at the University of Michigan, all awarded to Jonathan R. Morris. Additional funding was provided by National Science Foundation Grant (DEB 1853261).

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5.8 Tables & Figures

Table 5.1 – Model Outputs: Farm Management & Environmental Variables. Results from generalized linear models (GLM) and generalized linear mixed models (GLMMs) with model fixed effect estimates, standard errors (SE), test statistics, and p-values listed.

Parameter	**Estimate**	**SE**	**t/z value**	**Pr(>|t/z|)**
GLM: Shade Cover~				
Intercept	-0.436	0.242	-1.804	0.088
Farm	2.610	0.459	5.692	**<0.001**
GLMM: Coffee Height~				
Intercept	0.727	0.094	7.728	**<0.001**
Farm	0.259	0.133	1.950	0.051
GLMM: Coffee Diameter~				
Intercept	1.552	0.085	18.226	**<0.001**
Farm	-0.313	0.121	-2.593	**0.010**

Table 5.2 – Model Outputs: Epiphyte Species Richness and Abundance. Results from generalized linear mixed models (GLMMs) with model fixed effect estimates, standard errors (SE), z value test statistics, and p-values listed.

Parameter	**Estimate**	**SE**	**z value**	**Pr(>|z|)**
Species Richness (Farm Reference: Sun Monoculture)~				
Intercept	0.276	0.140	1.979	**0.048**
Farm	0.794	0.225	3.523	**<0.001**
Distance to Forest Patch	-0.071	0.099	-0.716	0.474
Coffee Height	0.129	0.052	2.485	**0.013**
Coffee Width	0.116	0.051	2.256	**0.024**
Shade Cover	-0.112	0.113	-0.991	0.322
Farm-Distance Interaction	-0.180	0.118	-1.523	0.128
Species Richness (Farm Reference: Shaded Polyculture)~				
Intercept	1.071	0.114	9.403	**<0.001**
Farm	-0.794	0.225	-3.523	**<0.001**
Distance to Forest Patch	-0.251	0.065	-3.838	**<0.001**
Coffee Height	0.129	0.052	2.485	**0.013**
Coffee Width	0.116	0.051	2.256	**0.024**
Shade Cover	-0.112	0.113	-0.991	0.322
Farm-Distance Interaction	0.180	0.118	1.523	0.128
Orchid & Bromeliad Abundance (Farm Reference: Sun Monoculture)~				
Intercept	-2.864	0.610	-4.698	**<0.001**
Farm	2.535	0.917	2.764	**0.006**
Distance to Forest Patch	-0.255	0.389	-0.656	0.512
Coffee Height	0.300	0.159	1.886	0.059
Coffee Width	-0.065	0.190	-0.342	0.732
Shade Cover	-0.589	0.440	-1.339	0.181
Farm-Distance Interaction	0.140	0.430	0.327	0.744
Orchid & Bromeliad Abundance (Farm Reference: Shaded Polyculture)~				
Intercept	-0.329	0.398	-0.827	0.408
Farm	-2.536	0.917	-2.764	**0.006**
Distance to Forest Patch	-0.114	0.185	-0.620	0.535
Coffee Height	0.300	0.159	1.886	0.059
Coffee Width	-0.065	0.190	-0.342	0.732
Shade Cover	-0.589	0.440	-1.339	0.181
Farm-Distance Interaction	-0.140	0.430	-0.327	0.744

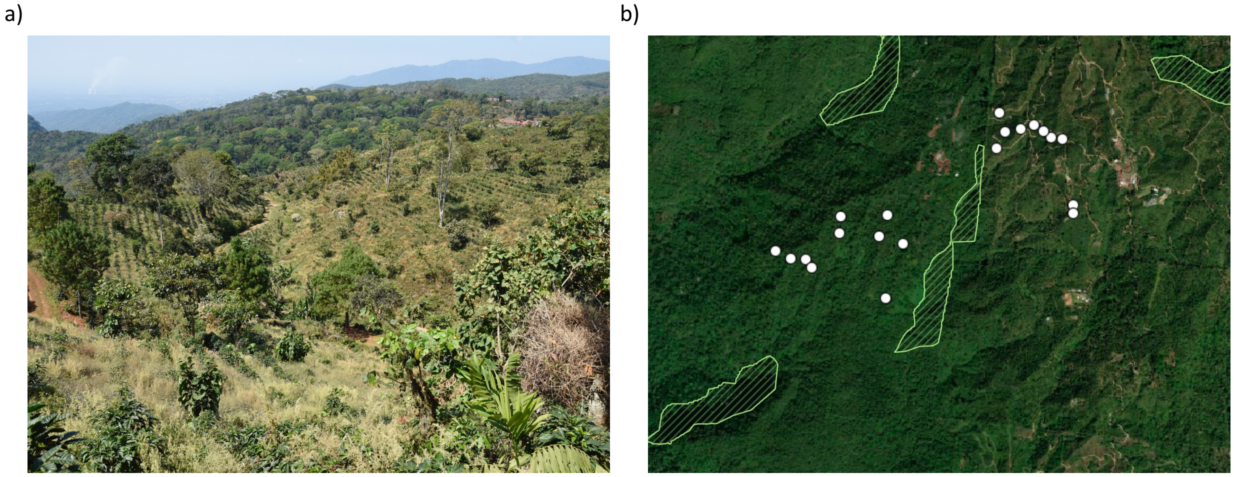


Figure 5.1 – Farm Comparison and Satellite Image of Site Locations. a) Displays a photo taken of the coffee landscape at our site, with the sun monoculture farm in the foreground, and the shaded polyculture farm in the background. **b)** Shows a satellite image of the site with the sun monoculture in the top right of the image and the shaded polyculture in the central part of the image. White points represent surveyed sites in each farm and light-green shaded polygons represent forest patches. Photo in **a)** by Jonathan R. Morris.

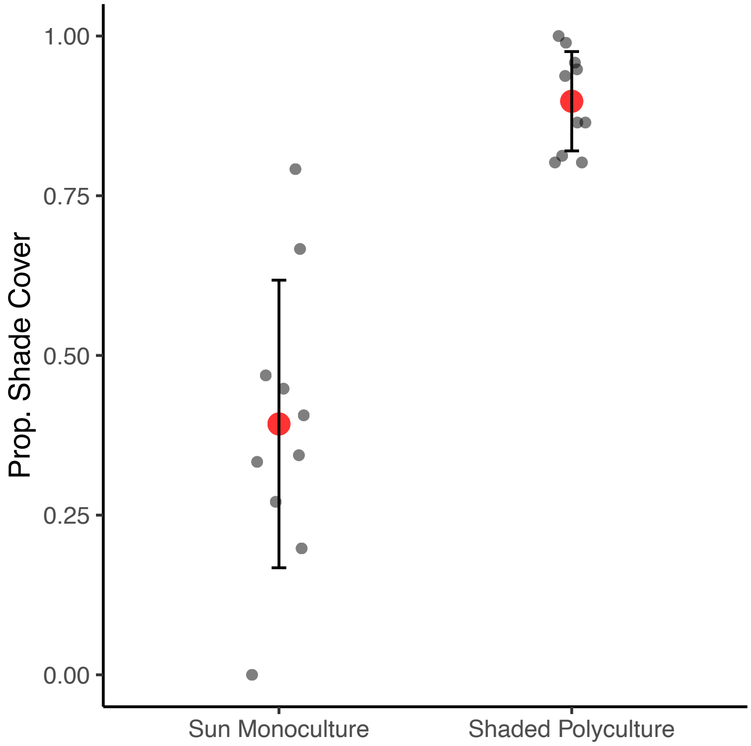


Figure 5.2 – Shade Cover. Shows proportional shade cover for each site (grey points) with mean points in red (±SE).

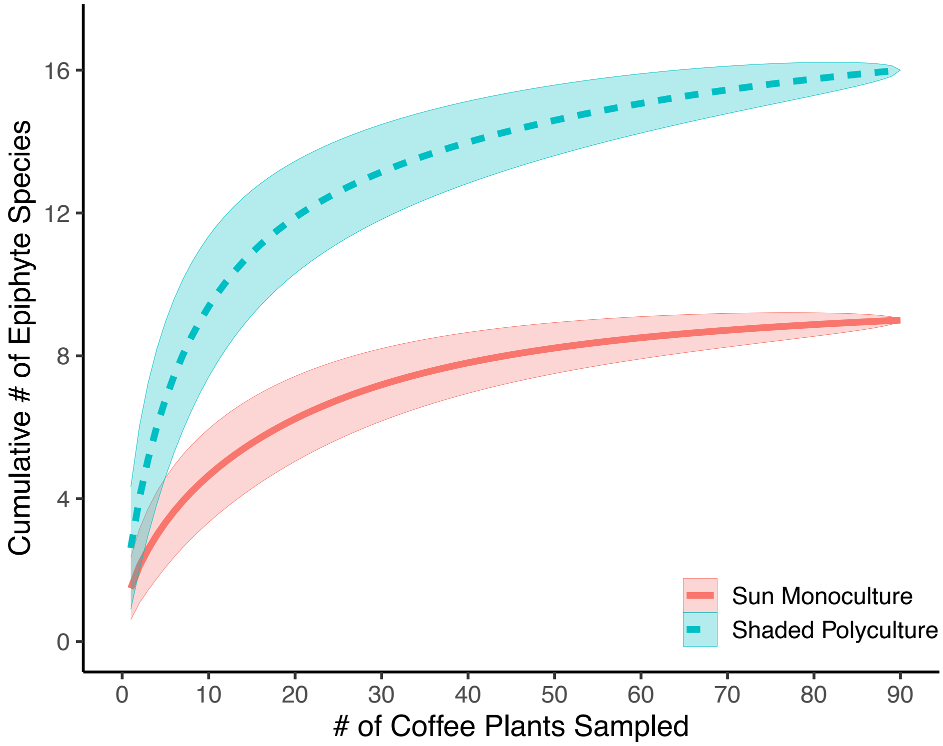


Figure 5.3 – Species Rarefaction. Displays species accumulation curves for each farm calculated using the Mao Tau sample-based estimator. Darker lines represent rarefied means and lighter areas show the standard deviation.

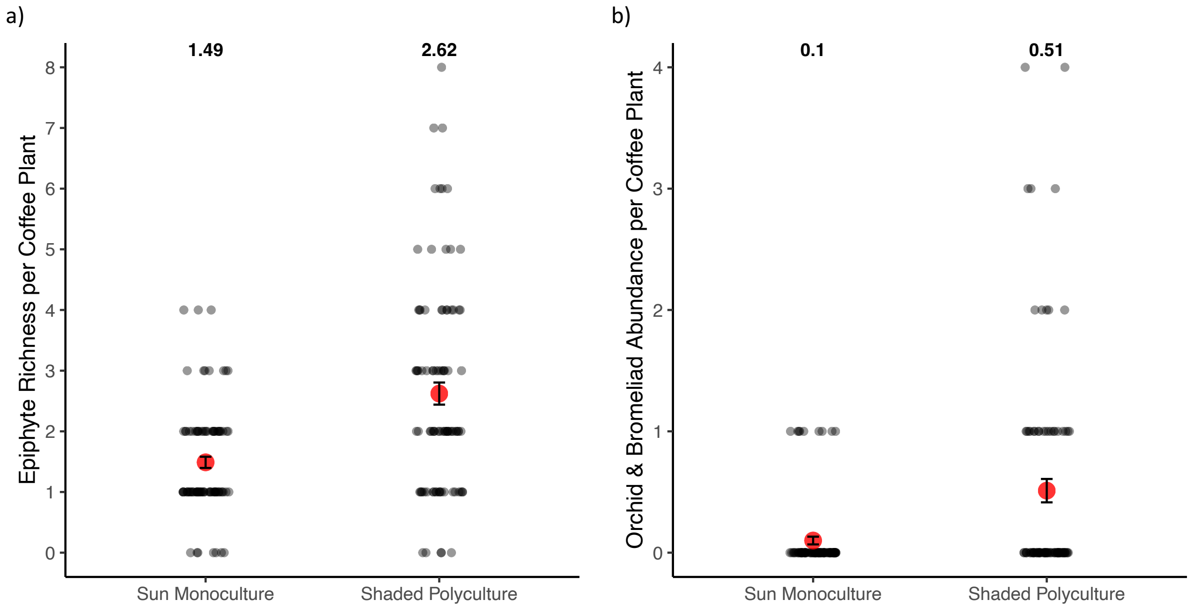


Figure 5.4 – Epiphyte Richness and Abundance. Shows per coffee plant **a)** epiphyte richness and **b)** abundance of orchids and bromeliads for both farms. In both figures, dark points are the raw data, and red points are the mean (±SE). Mean values for each farm are also displayed above the data in bold.

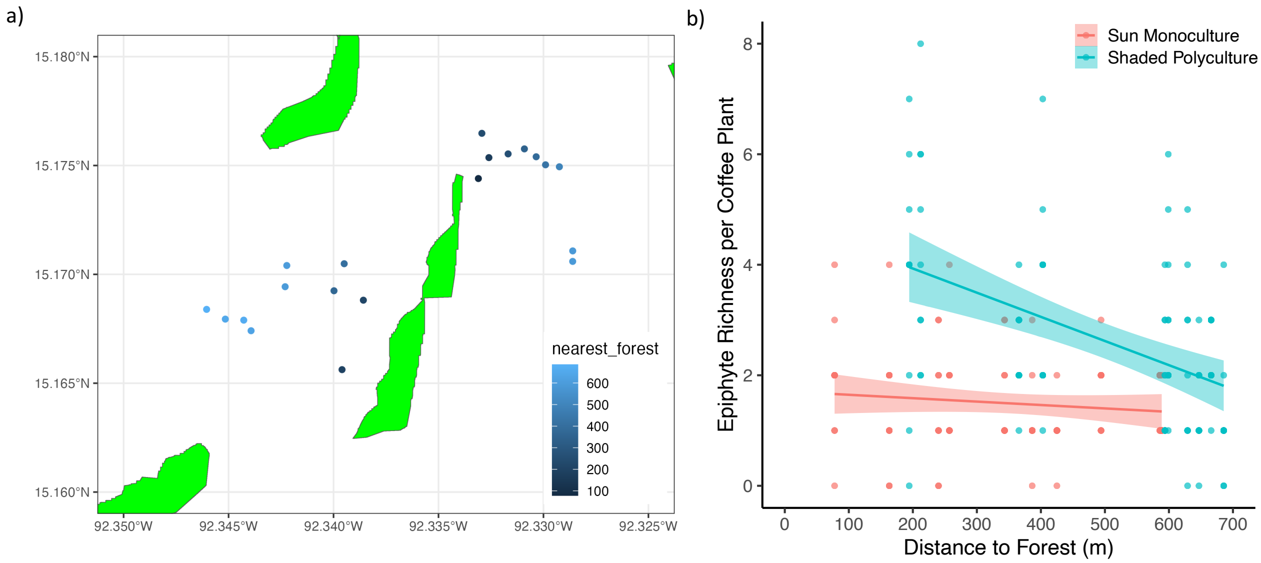


Figure 5.5 – Distance From Nearest Forest Patch. a) Shows a latitude-longitude coordinate map of the surveyed sites with green polygons roughly showing forest patches in the coffee landscape and the darkness of points representing the distance of sites to the nearest forest patch. **b)** Shows the relationship between distance to nearest forest patch and epiphyte species richness by farm. Lines are from linear regressions displayed here to show the qualitative relationships between variables.

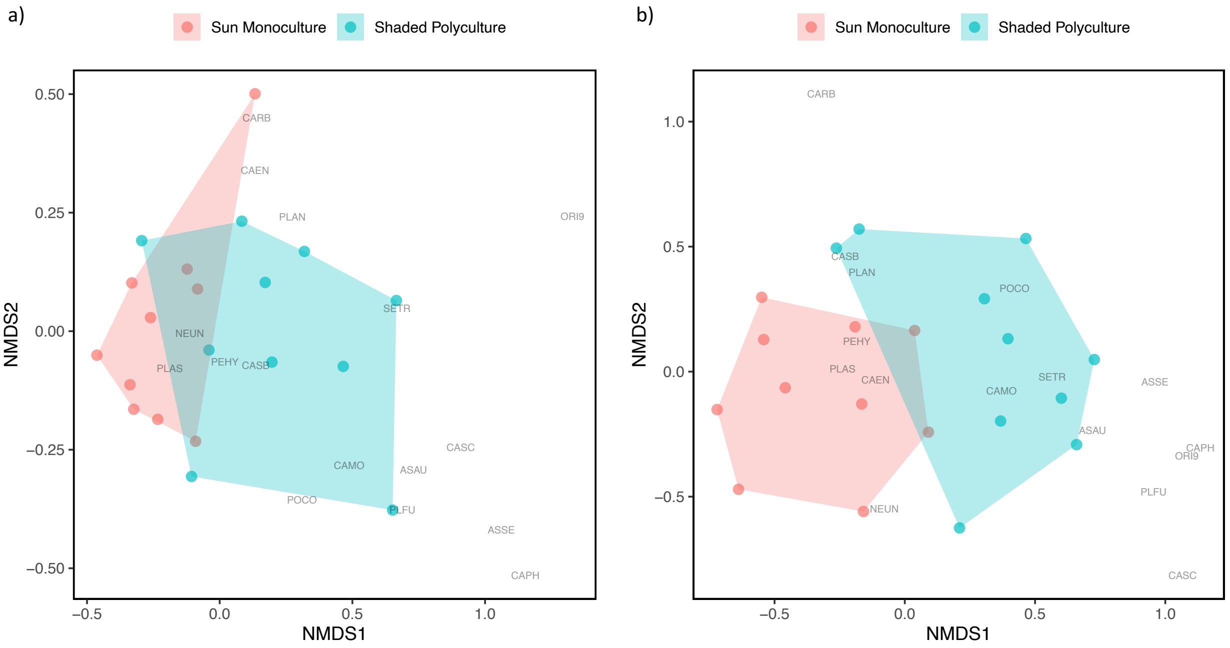


Figure 5.6 – Community Composition: Non-Metric Multidimensional Scaling (NMDS). Both plots show NDMS analyses for community composition data between the two farms, where **a)** is with Bray-Curtis dissimilarity and **b)** with Jaccard dissimilarity. Grey text in both images shows the relative position of individual species in the collapsed multivariate space.

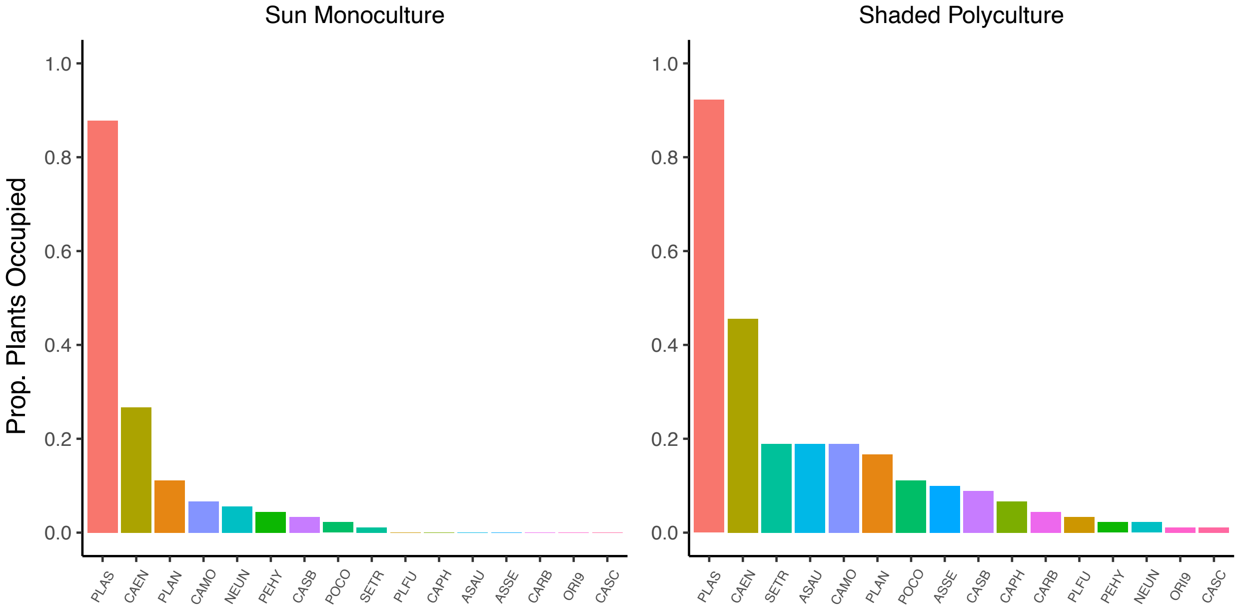
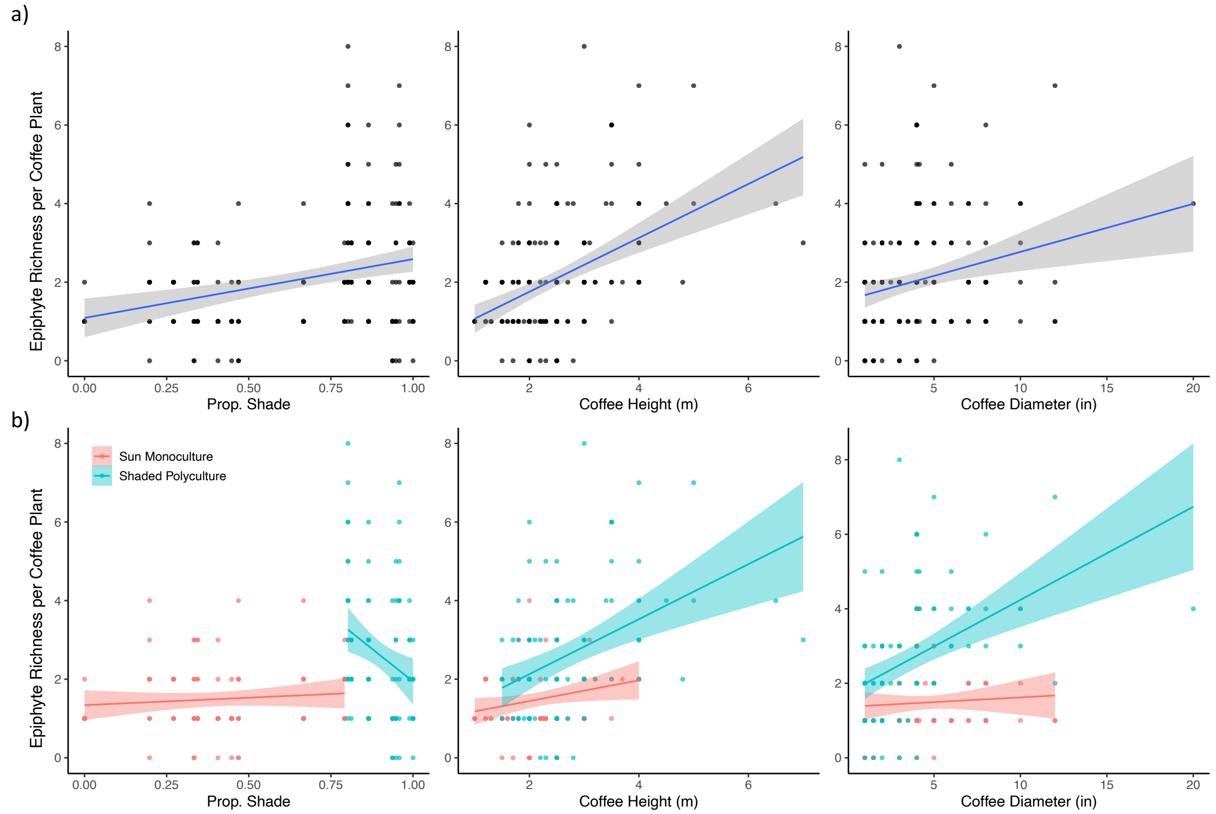
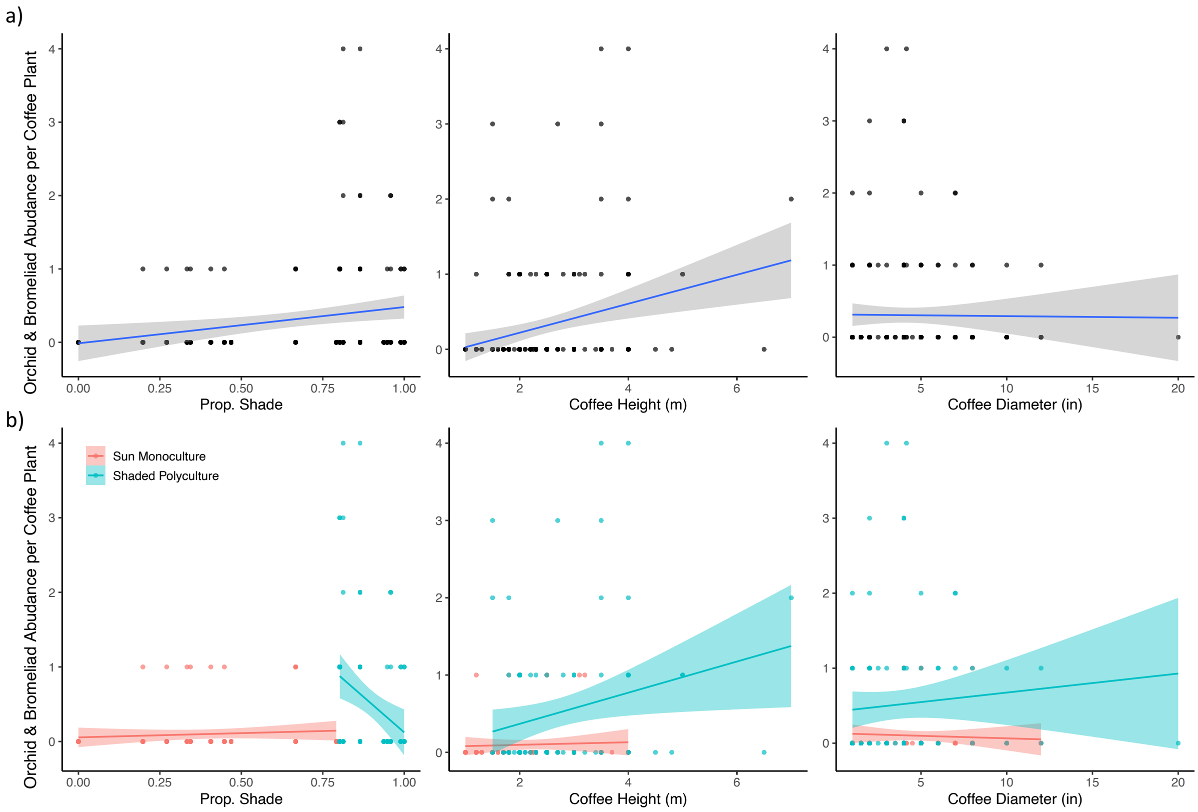


Figure 5.7 – Rank Abundance Plots. Both figures show the proportion of occupied coffee plants for each species in each farm, ranked. Colors are consistent between the plots for each species to show how the relative position of species changes in each farm.



Supplementary Figure 5.1 – Epiphyte Richness vs. Environmental Variables. Shows the **a)** overall relationship between variables and **b)** separated by farm. Lines are from linear regressions displayed here to gauge qualitative relationships.



Supplementary Figure 5.2 – Orchid & Bromeliad Abundance vs. Environmental Variables. Shows the **a)** overall relationship between variables and **b)** separated by farm. Lines are from linear regressions displayed here to gauge qualitative relationships.

Chapter 6 Agricultural Intensification, via Epiphyte Removal, Interacts with Drought Stress to Suppress Ant Communities and Associated Pest Control in Coffee[5]

6.1 Abstract

Agricultural intensification and the increased use of pesticides is a major driver of biodiversity declines and environmental degradation. Recent research has highlighted the impact of agricultural intensification on terrestrial insect declines, which may be magnified by interactions with climate change. This has resulted in repeated calls to reduce global pesticide use and shift to more sustainable pest regulation strategies, such as natural pest control (NPC), by conserving natural enemy habitat in agricultural landscapes. Coffee agricultural systems are well positioned to benefit from this approach, since they can be managed as agroforests with shade trees and high vegetation complexity, and are typically located in tropical biodiversity hotspots, potentially providing habitat for diverse communities of natural enemies. While recent NPC research has focused on the role of landscape composition, local agricultural management that impacts vegetation complexity on farms is also crucial, especially for less mobile natural enemies, like ants, which play an important role in natural pest control across many agricultural systems. An important feature of local coffee management and farm intensification is the removal of epiphytes on coffee plants, also known as “desmusgue”, which may have detrimental consequences for ant communities and ant-mediated pest control. We conducted a manipulative

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epiphyte removal experiment to test the impact of this practice on ant communities and natural pest control over the course of a growing season and understand how this practice interacts with seasonal changes in precipitation. We found that epiphyte removal significantly disturbed ant communities on coffee plants, resulting in reduced species richness, ant activity, and abundance. This trend was apparent immediately after epiphyte removal, but also persisted for 15 months over the course of the experiment. Additionally, ant community composition between our treatment groups was significantly different, and compositional turnover rates were consistently higher on desmusgue plants. We also found indirect negative impacts of epiphyte removal on ant-mediated pest control, as measured by the removal rate of the coffee berry borer on plants and by the abundance and activity of a focal pest control agent. Lastly, our analysis of effect size difference in our ant metrics suggested that the impact of epiphyte removal occurred immediately after disturbance and then was amplified following an extended period of extremely low precipitation, suggesting the interaction of this agricultural perturbation with seasonal drought stress. Collectively, our results demonstrate the negative impact of this practice on both ant diversity and associated natural pest control, and suggest how these effects may be amplified with climate change. This interaction, and similar practices in agroecosystems, should be explored more broadly with regards to natural pest control.

6.2 Introduction

Globally, the intensification of agriculture is a leading driver of biodiversity declines and environmental degradation (Kremen & Merenlender 2018; Vandermeer *et al.* 2018). In particular, the increased use of pesticides in intensive agriculture has been linked to recently documented declines in terrestrial insects (Raven & Wagner 2021; Wagner 2020; Wagner *et al.*

2021) and detrimental health consequences from chemical pollution (Larsen *et al.* 2017). To compound this problem, climate change interacts with agricultural intensification to amplify its negative impacts on insect populations (Outhwaite *et al.* 2022; Raven & Wagner 2021). This grim environmental picture has led to repeated calls to reduce the use of pesticides in agriculture and shift to more sustainable approaches such as natural pest control (Forister *et al.* 2019; Harvey *et al.* 2020; Kremen & Merenlender 2018). Natural pest control (NPC) relies on the conservation of natural enemy habitat in and around agricultural systems to promote naturally occurring predator populations and the sustainable reduction of pest damage (Bianchi et al. 2006; Tscharntke et al. 2016).

Coffee agricultural systems have the potential to benefit greatly from natural pest control (Karp *et al.* 2013), especially mediated by ants (Morris *et al.* 2018; Philpott & Armbrecht 2006). Coffee systems can house diverse communities of natural enemies and exist along a spectrum of agricultural intensification, where farms managed as polyculture agroforests have the potential to significantly increase the natural habitat available for natural enemies (Jha *et al.* 2014; Moguel & Toledo 1999; Perfecto *et al.* 1996). While natural pest control management typically focuses on the quantity of nearby forest in agricultural landscapes (Dainese *et al.* 2019; Karp *et al.* 2018; Tscharntke *et al.* 2016), natural enemy communities in coffee agroforests may also benefit from local shade trees and epiphytic vegetation that grows both on trees (Hietz 2005; Moorhead *et al.* 2010) and coffee plants (Solís-Montero *et al.* 2019).

In natural systems, epiphytes provide important habitat for ants through both obligate (Huxley 1980; Janzen 1974) and facultative (Blüthgen *et al.* 2000; Ellwood *et al.* 2002) relationships, which can result in indirect protective relationships, where epiphyte dwelling ants reduce herbivory on the plants that host their epiphytes (Dejean *et al.* 1995). Some research has explored this relationship on trees in agriculture (DaRocha *et al.* 2015; Fayle *et al.* 2013), and one more general study, investigating arthropod communities, found positive impacts of shade-tree epiphytes in coffee on ants (Cruz-Angón *et al.* 2009). While these interactions are likely important for supporting general communities of natural enemies in agriculture, in agroforests, especially, epiphytes that grow directly on crops may have significant potential to promote natural pest control (Rogy *et al.* 2019; Rost-Komiya *et al.* 2022). This is especially the case with ants, since they may be impacted more significantly by local farm management factors than larger, more mobile natural enemies like birds (Gonthier *et al.* 2014).

Some important work has been conducted to better understand the relationship between coffee farm management, intensification, and epiphyte communities. It is known that higher densities of shade trees and the presence of nearby forest cause can increase epiphyte diversity and abundance (Hietz 2005; Moorhead *et al.* 2010). More recent work has explored the management of epiphytes on coffee plants, investigating the role of epiphyte removal practices on epiphyte communities (Mondragón *et al.* 2009; Solís-Montero *et al.* 2019). This process, known in some regions in Latin America as "desmusgue," involves the direct manual removal of epiphytic vegetation (mainly moss, ferns, orchids, and bromeliads) on coffee plants (Solís-Montero *et al.* 2019). From a conservation standpoint, this practice is unfortunate; since coffee plants are by nature so abundant on coffee farms, they provide ample vegetative niche space to house diverse and abundant epiphyte communities, including some species of endangered orchids (García-González *et al.* 2017; Mondragón *et al.* 2009; Solis-Montero *et al.* 2005; Solís-Montero *et al.* 2019). Furthermore, this practice, which is promoted by regional government outreach programs in some areas, seems to have negligible impacts on coffee yields (Solís-

Montero *et al.* 2019). Importantly, coffee epiphytes may provide crucial habitat for ant natural enemy communities directly on coffee, promoting ant-mediated natural pest control.

While a few recent studies have explored the impact of epiphyte removal on arthropods and ants on citrus farms (Rogy *et al.* 2019; Rost-Komiya *et al.* 2022), there is much less work exploring this relationship in other agricultural systems, and almost no research test this on coffee plants. This is despite the fact that coffee has high potential for natural pest control and conservation when managed as agroforestry systems, since they are commonly located in tropical regions with high biodiversity (Moguel & Toledo 1999; Perfecto *et al.* 1996). Here we report the results of a manipulative experiment to test the impact of coffee epiphyte removal (desmusgue) on ant communities and associated natural pest control. We also ran the experiment over the course of an entire coffee growing season to understand how seasonal drought stress interacts with this agricultural perturbation. We aimed to answer four principal questions: 1) Do coffee epiphytes promote ant diversity, abundance, or activity? 2) Is there a relationship between coffee epiphytes and ant community composition? 3) Do coffee epiphytes have cascading impacts on ant-mediated natural pest control? And lastly, 4) does seasonal variation and potential climatic stress interact with epiphyte removal to disturb ant communities?

6.3 Methods

6.3.1 Study Site & Coffee Plant Replicates

To understand the impact of coffee epiphytes on ant communities and associated natural pest control, we conducted an epiphyte removal experiment. This study was conducted at Finca Irlanda in the Soconusco region of Chiapas, Mexico. Finca Irlanda is a shaded, organic, commercial polyculture coffee farm located at roughly 15°11´N, 92°20´W, and approximately

1050m elevation. While epiphyte removal, or “desmusgue”, is practiced in other nearby regions (Solís-Montero *et al.* 2019), it is not a part of the regular management of coffee on this farm, making it possible to study its effects. Throughout this study, we use epiphyte removal and “desmusgue” interchangeably to refer to this practice.

We conducted our experiment on 100 haphazardly selected coffee plants distributed across the farm. For the purposes of this study, we selected plants with heavy epiphyte loads to ensure that any differences observed between treatment groups were due to epiphyte removal. We also did our best to ensure coffee plants were not directly touching shade trees to limit the community of ants to coffee foragers. On all plants, epiphyte communities consisted mainly of a mixture of moss and fern species, often forming a layer covering the main trunks of coffee plants (Fig. 6.1a), but also included several species of bromeliads and orchids, and in a few cases mistletoe. Coffee plant replicates were paired at each site, located no more than five meters apart, and randomly assigned to control and desmusgue treatment groups. While there was a fair amount of variation across plants, we attempted to select pairs that were of a similar size and structure (similar number of trunks), with comparable epiphyte loads. To ensure that coffee replicates were equivalent between the treatment groups, we made visual estimates of plant height (m) and plant diameter at base (in), and visually estimated epiphyte loads. We also recorded shade cover with a densiometer for each replicate. While most plant replicates were used repeatedly over the 15-month period of the study for ant surveys, a few plants were altered due to inevitable farm management. This occurred closer to the end of the study, where a few plants were pruned, had branches broken or bent during the harvest, or had partial epiphyte removal. Plants that were completely lost toward the end of the project due to pruning were removed from analysis.

6.3.2 Desmusgue

To perform epiphyte removal, plant pairs were randomly split into desmusgue and control treatment groups. Desmusgue was accomplished by manually removing all moss and vascular epiphytic growth on coffee plants within arm's reach (Fig. 6.1). On a few plants, some epiphytic material that grew higher up on branches (mostly moss) could not be reached, but this was a very small fraction of the overall epiphyte vegetative growth on coffee plants. During epiphyte removal, we also made observations of ant species that were seen nesting on, in, or underneath epiphytes. While we aimed to minimize damage to coffee plants during the process, on a few occasions minor branches were broken off of coffee plants. When this occurred, we placed the broken branches in the crotch of other branches on the plant to minimize any potential non-desmusgue disturbance to ant communities (in case they were being used by twig-nesting ants). We also did our best to avoid damaging vines and herbaceous growth on and around coffee plants to control for any non-epiphyte-related effects this might have had on ants, however, vine growth was minimal on our replicates.

6.3.3 Ant Surveys

We assessed coffee ant communities repeatedly throughout our study, before and after desmusgue, in April 2022, June 2022, October 2022, February 2023, and again in June 2023. While most of these dates fell within the rainy season at our site (April-November) we included one survey during the dry season to understand how the perturbation to ants from epiphyte removal interacted with seasonality (Rogy *et al.* 2019). To thoroughly assess ant communities on coffee we conducted both visual and bait surveys (Gotelli *et al.* 2011), since it is known that some species of ants in these communities do not recruit to baits (Perfecto & Vandermeer 2013). For visual surveys, all ants observed during a three-minute window on coffee plants were recorded. We scanned plants systematically, typically starting at the base and moving our way up the trunk(s) and ending with a scan of branches and leaves. While it is likely that this approach did not account for all ant individuals on coffee, the fixed time window for visual surveys should have standardized our effort across plant replicates. We also assessed ants with tuna bait surveys, where five individual baits of tuna (thumbnail size, in oil) were distributed in the vertical space of coffee plants to increase sampling coverage of different species which may forage in different strata within coffee. On each coffee plant, two to three of these baits were placed closer to the base of plants (0.5-1m up) where the highest densities of epiphytes were typically observed. After placement, we waited a minimum of 30 minutes to begin checking baits, where most baits were assessed roughly 30-45 minutes after placement. For both surveys, ants were identified in the field to morphospecies, and ant activity was recorded per species on each bait, up to 20 individuals. When ants could not be reliably identified in the field, individuals were collected and stored in alcohol for later identification in the lab. While ant communities are highly diverse in this tropical montane region, our research group has accumulated many years of experience and resources to assist with ant identification. We also used resources from AntWeb to assist with identification in the laboratory (AntWeb 2023).

6.3.4 Pest Control Assessment

To assess the indirect impact of epiphyte removal on ant-mediated natural pest control in coffee, we collected data on coffee fruits and fruit damage at the end of our experiment, in June

2023. This assessment focused on the coffee berry borer (CBB), the most damaging insect pest of coffee around the world (Damon 2000; Vega *et al.* 2009). On each remaining coffee replicate in June, we haphazardly selected four coffee branches near chest height at the center of the coffee plant, if possible. We selected only branches with a minimum of five coffee fruits per branch. When sufficient fruits or branches were not available, we moved on to the next coffee replicate. On each surveyed branch, we recorded the total number of fruits, the number of bored fruits, and the number of incomplete bored fruits, both as a measure of coffee pest control. Bored and incomplete bored fruits are assessed by observing the ostiole on the end of fruits, where the CBB typically bores (Barrera 2008). If borers are removed or consumed by natural enemies during the process, which can take over an hour, they leave an incomplete gallery entry, which we counted as incomplete bored fruits to indirect gauge the removal rate of borers on coffee plants.

6.3.5 Data Analysis

6.3.5.1 Ant Community Impacts

We explored differences in our ant community data using generalized linear mixed models (GLMMs) where we included coffee plant treatment group (desmusgue or control), time, and their interaction as fixed effects in all models. This allowed us to differentiate between overall differences in treatment groups and the impact of time on those differences, to test for potential impacts of seasonality or possible recovery of the ant community after initial desmusgue perturbations. We first tested for differences in ants by assessing ant species richness at coffee baits with a GLMM using a Poisson error distribution to fit count data. We then tested for differences in the total number of ant individuals on coffee plants (pooled across species) as a general metric of ant activity, using a GLMM with a negative binomial error distribution to fit overdispersed count data. While we acknowledge that pooled ant activity masks important differences in natural history across ant species, we used this as a rough assessment of the overall activity of the ant community, which may reveal more immediate changes that are not apparent when only considering the presence or absence of species. We then assessed differences in the abundance of ants on plants, both at the bait level and plant level. We tested for differences in the proportion of baits occupied per coffee plant using a GLMM with a binomial error distribution to fit proportional data and ran a similar model on the proportion of total baits occupied, with a binomial error distribution to fit binary presence-absence data. For our models of species richness per bait and total baits occupied, we included plant identity and bait identity as a nested random effect to control for variation and temporal non-independence between repeatedly sampled coffee plants, and spatial non-independence between baits on individual plants. For our models of ant activity per plant and the proportion of baits occupied per plant, we included plant identity as a random effect to control for variation and temporal non-independence between repeatedly baited coffee plants. Since we did not observe any differences in shade, coffee height, coffee diameter, or epiphyte load estimates between our treatment groups, we chose to leave these variables out of our models to facilitate model fitting and simplify the interpretation of model outputs.

To assess differences in overall ant species richness in these communities, we conducted species rarefactions of sampled ants for each treatment group separately across all time points. Rarefactions were run on both tuna bait survey data and visual survey data, using the Mau Tao estimator for sample-based rarefaction (Colwell *et al.* 2012). Because our sampling effort was standardized across the study, with the same number of baits per plant, and mostly the same number of plants across time, we assumed comparison of asymptotic rarefaction curves would allow for fair comparisons of total species richness. However, a few plants that were heavily pruned or cut during regular farm management were not included in the February 2023 and June 2023 surveys.

Differences in the composition of ant communities on control and desmusgue plants were assessed by calculating community dissimilarity metrics across all time points for our bait level data from tuna bait surveys. This was done using Bray-Curtis dissimilarity to account both for species identity and relative abundance within communities and Jaccard dissimilarity, which assesses species identity alone. Using these metrics, we created distance matrices of all communities (each treatment group across all sampling times) and ran PERMANOVAs to test for differences in the centroid of treatment groups in multivariate space. To support this analysis, we created non-metric multidimensional scaling (NMDS) plots of our community dissimilarity data to collapse multivariate space into two dimensions so that community differences could be more easily visualized. This was done both with Bray-Curtis and Jaccard dissimilarity. Finally, we created species rank abundance plots of communities to assess differences in the relative abundance of species and evenness of communities across time between treatment groups and quantified community evenness using the probability of interspecific encounter (PIE) metric.

6.3.5.2 Pest Control Impacts

To assess the indirect impacts of epiphyte removal on ant-mediated natural pest control, we conducted statistical tests of coffee berry borer damage and coffee fruit loads. We ran generalized linear mixed models on the proportion of bored fruits, the proportion of incomplete bored fruits, and total fruit load per branch. GLMMs for the proportion of bored and incomplete bored fruits per branch were run with a binomial error distribution to fit proportional data. The GLMM for total fruit load per branch was run with a Poisson error distribution to fit count data. For all models of pest control, coffee plant and branch identity were included as a nested random effect to control for variation between coffee plants and spatial non-independence between branches.

6.3.5.3 Interacting Stressors – Epiphyte Removal & Precipitation

To assess how the perturbation from epiphyte removal may have interacted with seasonal effects (mainly precipitation) across time we measured the effect size difference in various ant metrics from our tuna bait survey data. For this, we calculated Glass' Δ $((\overline{x}_{desmusgue} - \overline{x}_{control})/s_{control})$ to assess the scaled impact of epiphyte removal on ants at different time points. We then visually assessed changes in the overall impact of epiphyte removal across time to explore any additional changes that may have occurred with seasonality at our site. This was done using mean species richness per bait, mean ant activity per plant (total pooled individuals), and the mean proportion of baits occupied per plant.

All data analysis was conducted in R version 4.3.1 (R Core Team 2023). GLMMs were run using the "glmer" or "glmer.nb" functions from the "lme4" package. For all GLMMs, fixed effect coefficients and random effect variance were estimated with maximum likelihood using Laplace approximation. Species rarefactions, community dissimilarity matrices, and NMDS plots were run using the "vegan" package. PERMANOVA tests were run with 999 permutations using the "adonis2" package.

6.4 Results

6.4.1 Ant Community Impacts

Overall, we documented 55 species of ants on coffee plants, with 47 species on control plants and 45 species on desmusgue plants, and 37 species found on plants in both treatment groups (Table S6.1). Across all plant and bait level metrics, there was a significant negative impact of epiphyte removal on ant communities (Fig. 6.2, Table 6.1). In all cases, these measures started out essentially the same between treatment groups before epiphyte manipulation, followed by a pronounced drop off in the desmusgue group only. After epiphyte removal, the difference between the treatment groups persisted for the entire course of the study with the greatest difference seen during our final sampling effort in June 2023, nearly 15 months after the initial perturbation. Immediately after epiphyte removal, mean ant species richness per bait declined by 28.3% (Fig. 6.2a), mean ant activity declined by 41.6% (Fig. 6.2b), and the mean proportion of baits occupied per plant decreased by 21.8% (Fig. 6.2c), all in the desmusgue plant treatment group. For all ant metric GLMMs, there was a significant overall effect of treatment group and a significant interaction between treatment and time, reflecting the trends we saw in reduced ant metrics in the desmusgue group after epiphyte removal and across all time points (Table 6.1). While time had a significant negative effect on all metrics for the desmusgue plants, it was not a significant factor driving the trends in our control group except for total bait occupancy, suggesting that this group was essentially unchanged after epiphyte removal, given that no manipulation took place on these plants (Table 6.1). While ants on control plants stayed mostly unchanged during the first three survey timepoints, in February, during the dry season, we saw a substantial drop off in both treatment groups, with subsequent recovery on control plants and partial recovery on desmusgue plants. This trend is addressed further in our discussion section.

Interestingly, despite the clear impacts we observed on ants at the plant (and bait) level, we did not find a clear effect of epiphyte removal on total species richness across the ant community (Fig. S6.1). While rarefaction analysis revealed some slight differences in our tuna bait surveys, with more species in the control plant group, these differences were not consistent and not significant enough to suggest any meaningful reduction in total richness after desmusgue (Fig. S6.1a). This was also true for the rarefaction analysis of our visual survey ant data, while although the differences were slightly more pronounced, the communities appeared to start at different places in terms of overall richness before the manipulation, making comparison challenging (Fig. S6.1b).

Despite this, analysis of ant community composition across all baits suggested that the compositional make-up of ant communities was significantly altered by epiphyte removal. PERMANOVA tests revealed that ant communities were significantly different between our control and desmusgue treatment groups (Fig. 6.3). This was true using both Bray-Curtis dissimilarity (Fig. 6.3a, R^2=0.236, F=2.472, p=0.028; NMDS stress=0.157) and Jaccard dissimilarity (R^2=0.206, F=2.079, p=0.01; NMDS stress=0.331). Ant communities also appeared to diverge through time, with higher turnover in the desmusgue community, where the greatest change in dissimilarity, compared with the original community sampled in April 2022, was observed immediately after epiphyte removal on desmusgue plants (Fig. 6.3b and 6.3c). This trend of higher turnover in the desmusgue ant community was true both for Bray-Curtis (Fig. 6.3b) and Jaccard dissimilarity (Fig. 6.3c), although with the Jaccard metric we saw similar values of dissimilarity between both treatment groups in February 2023, during the dry season.

Visual analysis of species rank abundance curves (Figs. S6.2 and S6.3) also suggested that control plant communities had more even species distributions across the experiment, which was confirmed by our calculation of the probability of interspecific encounter (PIE). However, this metric also suggested higher evenness in control plant communities before desmusgue occurred, making it difficult to assess the overall impact of epiphyte removal on community evenness.

6.4.2 Pest Control Impacts

While we did not find an impact of epiphyte removal on the overall amount of coffee fruits infested with borers per branch at the end of our experiment (Table 6.2), we did see a significantly higher proportion of incomplete bored fruits per branch on control plants (Fig. 6.4, Table 6.2), suggesting that undisturbed ant communities have greater pest removal rates. We also did not observe a difference in the overall quantity of fruits on branches (Table 6.2), suggesting that while ants on control plants may reduce pest damage at higher rates, they are not significantly increasing coffee fruit load. However, this finding also suggests that the removal of epiphytes also did not have a significant impact on fruit load.

We also observed significant impacts of desmusgue on one of the focal pest control agents at this site, *Solenopsis picea*. There was a significant decrease in *S. picea* abundance on desmusgue plants after epiphyte removal compared to control plants (Fig. 6.5a & 6.5b), measured by both the proportion of baits and plants occupied. After this initial drop-off, abundance levels of this species stayed consistently lower than on control plants across the duration of the experiment. While there was also variability in *S. picea* abundance on control plants, it mostly varied around the original values observed at the beginning of the experiment. Additionally, we also observed significant reductions in *S. picea* activity on desmusgue plants with a flattening and contraction of ant activity across the vertical space of plants, compared to control plants, which typically had higher levels of ant activity further away from the ground (Fig. 6.5c).

6.4.3 Interacting Stressors

We also documented a significant reduction in ant communities across all metrics in both treatment groups during the dry season (Feb. 2023) (Fig. 6.2). Although this occurred during the normal dry season at our field site, this was reportedly an unusual dry spell for the region, which has been trending toward reduced rainfall in recent years (Li *et al.* 2022). Interestingly, ants fully recovered on control plants after the dry season, while they did not on desmusgue plants, suggesting a potential interaction of epiphyte removal and climatic stressors (Fig. 6.2 & Fig. 6.6a). This was supported by our analysis of effect size differences in our ant metrics across time, as measured by Glass Δ (Fig. 6.6a), where we saw an initial reduction across all metrics immediately after desmusgue in June 2022, followed by a compounding reduction after the dry season in June 2023. Importantly, the initial decline in effect size difference between the control and desmusgue groups stayed mostly unchanged over multiple sampling points across the year until the secondary reduction observed immediately following the drought period (Fig. 6.6a).

6.5 Discussion

In this study, we investigated the impact of coffee epiphyte removal as a management perturbation on ant communities and ant-mediated pest control. We also explored how this agricultural perturbation interacts with seasonal stress and potential drought to impact ants.

Overall, we demonstrated that coffee epiphyte removal perturbs ants by reducing their diversity, activity, and abundance, shifts ant community composition, and reduces elements of ant-mediated pest regulation. We also found evidence that these effects may be compounded by climatic stress at the study site where we conducted this research.

Across nearly all metrics of ant diversity and community composition, we saw a significant negative impact of coffee epiphyte removal. On individual coffee plants, we observed a reduction in the species richness of ant communities, the overall pooled activity of ants, and the abundance of ants, measured by their presence at baits and on plants.

While the overall richness of ant communities was not consistently impacted, which may be explained by potential extinction lags which take more time to present at the community level after disturbance, all plant-level metrics of ants made the negative impact of desmusgue clear. Importantly, the reduction we observed in all ant metrics after epiphyte removal, measured by the difference between control and desmusgue treatment groups, occurred immediately after the perturbation and persisted throughout the full length of the study over nearly 15 months. While there was also substantial variability with seasonally on control plants, overall, there was not a significant impact of time on ant communities on undisturbed plants for nearly all metrics. The negative impacts from epiphyte removal were also apparent at the community level, where we observed a shift in the composition of ant communities between treatment groups, and increased rates of turnover on desmusgue plants, demonstrating the effect of the perturbation on whole coffee ant communities (in contrast with total species richness).

Beyond direct impacts on ants, we also found indirect impacts on some measures of ant-mediated pest control. While we did not find a difference in the proportion of fruits bored by the coffee berry borer, we found a significant difference in the amount of incomplete bored fruits, with more on control plants, suggesting higher rates of pest removal. Since both measures are indirect, the overall proportion of bored fruits may not be the most reliable indicator of ant-mediated pest control, since borer levels can also be the result of other factors like incomplete harvest in coffee (Damon 2000). In fact, on some coffee plants and branches, we found nearly 100% infestation levels, and also observed old heavily bored fruits that were probably missed during the previous year's harvest and likely explain the high levels of borer damage, since these fruits are a known pool for borers (Damon 2000). The level of incomplete bored fruits on coffee, however, while still indirect, may be more relevant for gauging the regulatory impact of ant natural enemies.

Our broader analysis of focal species in coffee ant communities also revealed detrimental impacts of epiphyte removal on an important natural enemy and known pest control agent of the coffee berry borer (Constantino-Chuaire *et al.* 2022; Morris & Perfecto 2016), the ant *Solenopsis picea*. While this was one of the most abundant ants documented during our experiment, we observed a significant reduction in its abundance on baits and plants in the desmusgue treatment group after epiphyte removal. This quantitative finding corresponded with observations of this species commonly nesting underneath epiphytes on coffee, especially between the mat of moss and fern rhizomes when covering crevasses and hollowed-out pits on plants. These findings were accompanied by a reduction and flattening of ant activity in the vertical space of coffee plants, suggesting that fewer individuals were available higher up in plants after desmusgue, closer to the areas where coffee berry borers infest fruits.

Beyond these specific impacts on potential ant-mediated natural pest control and focal pest control agents, it should be noted that the general reductions in ant communities that we documented also probably have an overall impact on pest regulation. Previous research has demonstrated the importance of ant diversity in pest regulation in coffee systems, particularly with functional diversity (Gonthier *et al.* 2015; Philpott *et al.* 2012), in the complex interaction webs found in coffee agroecosystems (Perfecto *et al.* 2014; Vandermeer *et al.* 2010, 2019). Having higher levels of species richness on individual coffee plants, for example, may increase the probability that certain species will help to regulate specific pests, when other ants do not under changing environmental conditions (Philpott *et al.* 2012) – the noted biodiversity insurance hypothesis (Yachi & Loreau 1999). While overall richness in the community was not impacted consistently over the time in this study, plant level richness, which did significantly decline with epiphyte removal, is probably more important for potential insurance pest control effects. Furthermore, while we did not find a significant impact of epiphyte removal on coffee fruit loads, which was measured as a proxy for yields, the fact that there was no difference in fruits load between our control and desmusgue treatment groups further illustrates that desmusgue probably serves little practical benefit, as demonstrated more thoroughly in previous research (Solís-Montero *et al.* 2019).

Perhaps most significant of our findings, was the apparent interaction of this agricultural perturbation with seasonal drought stress on ants. While the disruptive impact of epiphyte removal on ant communities was apparent immediately after the disturbance, we also observed an increase in the effect size of this perturbation on ants after the dry season at our site. While a significant reduction in precipitation is typical during the dry season in this region (Li *et al.* 2022), our study took place during an extended drought, with almost no rainfall over a period of nearly two months leading up to our sampling during February 2023. This resulted in a significant reduction in ants across all coffee plant-level metrics, but eventually, the largest differences in effect size between our control and desmusgue treatments several months later in

June 2023. This suggested that drought stress during the dry season may exacerbate the impacts of epiphyte removals on ants. Interpreting this result ecologically, epiphytes that provide important habitat for ants, in the form of microclimate refugia, may become even more important during the dry season, when precipitation and moisture levels are very low, and ants reduce their foraging activity to survive these stressful conditions. On desmusgue plants without epiphytes, the negative impacts on ants may not become fully apparent until compounded by this additional environmental stress. A related result was found in citrus farms, where positive relationships between bromeliads, invertebrate communities, and ants were only detected during the dry season (Rogy *et al.* 2019; Rost-Komiya *et al.* 2022), which the authors suggested reflects the importance of bromeliads as refugia from climate extremes. This result also aligns with recent broader research demonstrating the interactive negative effects of agricultural intensification and climate change on global insect declines (Outhwaite *et al.* 2022).

Collectively, our results, along with other related research (Solís-Montero *et al.* 2019), provide important perspective on the management of epiphytes and natural pest control in coffee agroecosystems. The removal of epiphytes from coffee plants has clear negative impacts on the epiphyte communities themselves (Mondragón *et al.* 2009; Solís-Montero *et al.* 2019), but also negatively impacts ants by reducing their richness, activity, and abundance on coffee plants. This had indirect negative impacts on pest removal rates and focal pest control agents at our site. Furthermore, we found evidence that the impact of epiphyte removal may be amplified by seasonal-related drought stress, which is likely to become more frequent with climate change. Additionally, we did not find a positive impact of epiphyte removal on coffee fruit loads, confirming other research that has explored this question more thoroughly (Solís-Montero *et al.* 2019). Given that this practice is also costly from a farm labor standpoint, we believe these results, collectively, suggest that coffee epiphyte removal, or “desmusgue”, should be avoided. This management shift would represent a potential multi-win scenario, where shifting farm management away from desmusgue can increase the capacity of farms to conserve epiphytes and ants, reduce labor expenses, promote natural pest control (and correspondingly, reduce pesticide use), and potentially allow farmers to market coffee as “epiphyte friendly,” similar to other campaigns that have focused on birds, shade, and other elements of biodiversity in coffee (Jha *et al.* 2014). Beyond these specifics, and perhaps most notably, these results highlight the potential for the interaction of agricultural intensification and climate stressors on biodiversity loss (Outhwaite *et al.* 2022) in agricultural landscapes, which should be considered more broadly in the management of natural pest control in other agroecosystems.

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6.8 Tables & Figures

Table 6.1– Model Outputs: Ant Richness, Activity, & Abundance. All model outputs are from generalized linear mixed models (GLMMs) evaluating the relationship between various ant community metrics, our treatment groups (desmusgue and control), and time. For all models, the estimated coefficients are listed with estimate standard error (SE), z value test statistics, and p-values.

Parameter	Estimate	SE	z value	Pr(>|z|)
Ant Species Richness per Bait (Treatment Reference: Control)~				
Intercept	-0.254	0.048	-5.237	**<0.001**
Treatment	-0.194	0.070	-2.785	**0.005**
Time	-0.028	0.032	-0.892	0.373
Treatment-Time Interaction	-0.127	0.048	-2.644	**0.008**
Ant Species Richness per Bait (Treatment Reference: Desmusgue)~				
Intercept	-0.448	0.051	-8.751	**<0.001**
Treatment	0.194	0.070	2.785	**0.005**
Time	-0.155	0.036	-4.320	**<0.001**
Treatment-Time Interaction	0.127	0.048	2.644	**0.008**
Ant Activity per Plant (Treatment Reference: Control)~				
Intercept	3.559	0.090	39.347	**<0.001**
Treatment	-0.268	0.121	-2.211	**0.027**
Time	0.025	0.065	0.382	0.703
Treatment-Time Interaction	-0.184	0.091	-2.030	**0.042**
Ant Activity per Plant (Treatment Reference: Desmusgue)~				
Intercept	-0.448	0.051	-8.738	**<0.001**
Treatment	0.194	0.070	2.780	**0.005**
Time	-0.155	0.036	-4.304	**<0.001**
Treatment-Time Interaction	0.127	0.048	2.634	**0.008**
Prop. Bait Occupancy per Plant (Treatment Reference: Control)~				
Intercept	1.023	0.177	5.777	**<0.001**
Treatment	-0.543	0.248	-2.193	**0.028**
Time	-0.189	0.114	-1.661	0.097
Treatment-Time Interaction	-0.361	0.161	-2.244	**0.025**
Prop. Bait Occupancy per Plant (Treatment Reference: Desmusgue)~				
Intercept	0.480	0.175	2.744	**0.006**
Treatment	0.543	0.248	2.193	**0.028**
Time	-0.550	0.114	-4.814	**<0.001**
Treatment-Time Interaction	0.361	0.161	2.244	**0.025**
Total Prop. Bait Occupancy (Treatment Reference: Control)~				
Intercept	0.816	0.144	5.675	**<0.001**
Treatment	-0.447	0.202	-2.206	**0.027**
Time	-0.153	0.065	-2.375	**0.018**
Treatment-Time Interaction	-0.291	0.091	-3.192	**0.001**
Total Prop. Bait Occupancy (Treatment Reference: Desmusgue)~				
Intercept	0.369	0.143	2.588	**0.010**
Treatment	0.447	0.202	2.206	**0.027**

Time	-0.445	0.065	-6.879	**<0.001**
Treatment-Time Interaction	0.291	0.091	3.192	**0.001**

Table 6.2 – Model Outputs: Pest Control & Coffee Yield. Model outputs are from GLMMs evaluating the difference in pest control and coffee yields between our treatment groups (desmusgue and control). For all models, the estimated coefficients are listed with estimate standard error (SE), z value test statistics, and p-values.

Parameter	Estimate	SE	z value	Pr(>|z|)
Prop. Incomplete Bored Fruits~				
Intercept	-4.073	0.309	-13.162	**<0.001**
Treatment	-1.068	0.447	-2.388	**0.017**
Prop. Bored Fruits~				
Intercept	-2.534	0.786	-3.223	**0.001**
Treatment	-0.356	1.151	-0.309	0.757
Total Fruits~				
Intercept	2.978	0.144	20.646	**<0.001**
Treatment	0.068	0.214	0.318	0.750

Supplementary Table 6.1 – Ant Species List and Overall Abundance. Numbers represent the total count of coffee plants that each species was observed on for the duration of the experiment. Counts include ants observed both before and after epiphyte removal.

Species	**Control**	**Desmusgue**
Azteca sericeasur	8	12
Azteca sp.	2	2
Brachymyrmex sp. 1	70	85
Brachymyrmex sp. 2	46	30
Brachymyrmex sp. 3	1	3
Camponotus albicoxis	1	1
Camponotus bretesi	5	7
Camponotus sp.	0	1
Camponotus striatus	1	0
Camponotus textor	6	3
Cardiocondyla sp.	0	1
Cephalotes basilius	0	2
Crematogaster crinosa	1	2
Crematogaster curvispinosa	9	1
Crematogaster nigripilosa	7	1
Crematogaster sumichrasti	2	8
Gnamptogenys regularis	1	0
Gnamptogenys striatula	5	2
Gnamptogenys sulcata	2	3
Hypoponera nitidula	2	0
Myrmelachista skwarrae	0	1
Neoponera carinulata	8	1
Neoponera curvinodis	1	0
Nesomyrmex echanatinodis	8	9
Nesomyrmex pittieri	2	1
Nylanderia sp.	5	1
Pheidole exigua	61	26
Pheidole indistincta	9	7
Pheidole protensa	15	8
Pheidole psilogaster	3	1
Pheidole punctatissima	10	5
Pheidole purpurea	0	1
Pheidole simonsi	0	1
Pheidole sp. 1	3	0
Pheidole sp. 2	1	0
Pheidole sp. 3	1	0
Pheidole sp. 4	3	2
Platythyrea punctata	1	0
Procryptocerus hylaeus	0	4

Pseudomyrmex ejectus	12	2
Pseudomyrmex elongatus	3	3
Pseudomyrmex filiformis	2	0
Pseudomyrmex simplex	9	6
Pseudoponera stigma	1	0
Rogeria tonduzi	21	16
Solenopsis geminata	1	2
Solenopsis geminata RED morph	0	3
Solenopsis picea	94	84
Solenopsis sp.	3	8
Solenopsis tericola	10	12
Solenopsis zeteki	28	14
Tapinoma sp.	2	1
Technomyrmex sp.	4	4
Wasmannia auropunctata	20	30
Wasmannia rochai	3	0

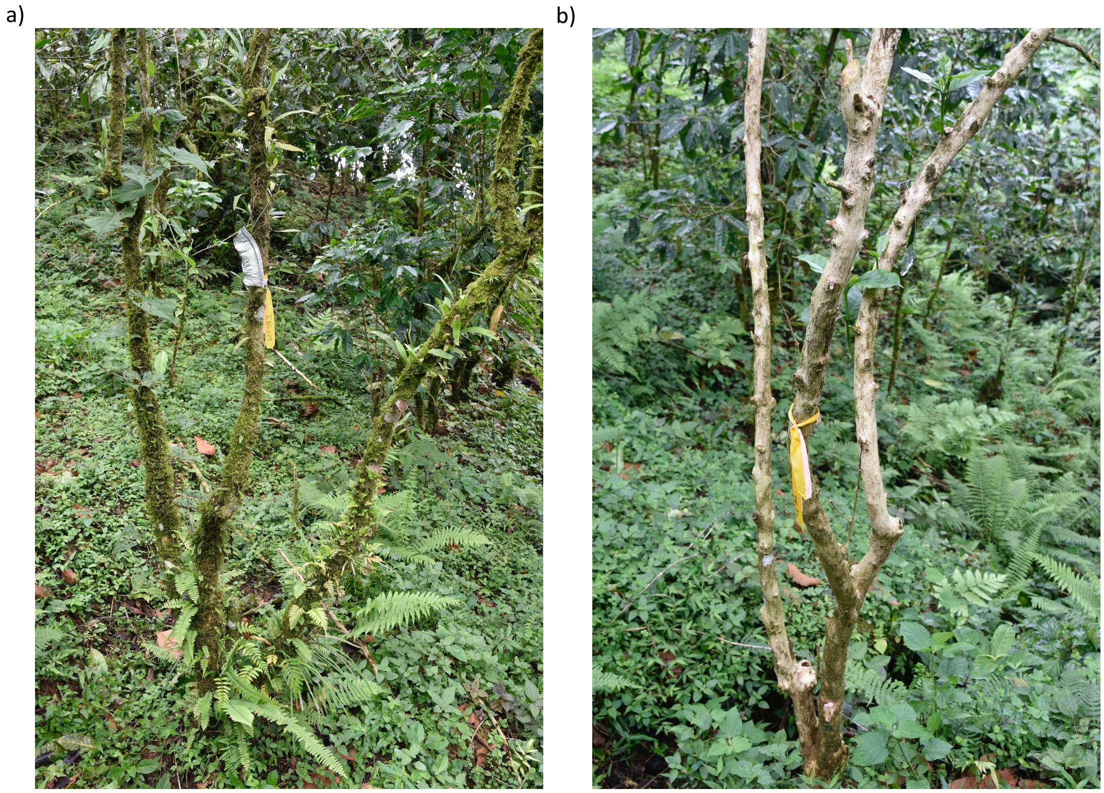


Figure 6.1 – Epiphyte Removal Example Photos. Photos show the contrast between our treatment groups for **a)** control and **b)** desmusgue plants after experimental manipulation. Photos are taken at the base of coffee plants. Both photos by Jonathan R. Morris.

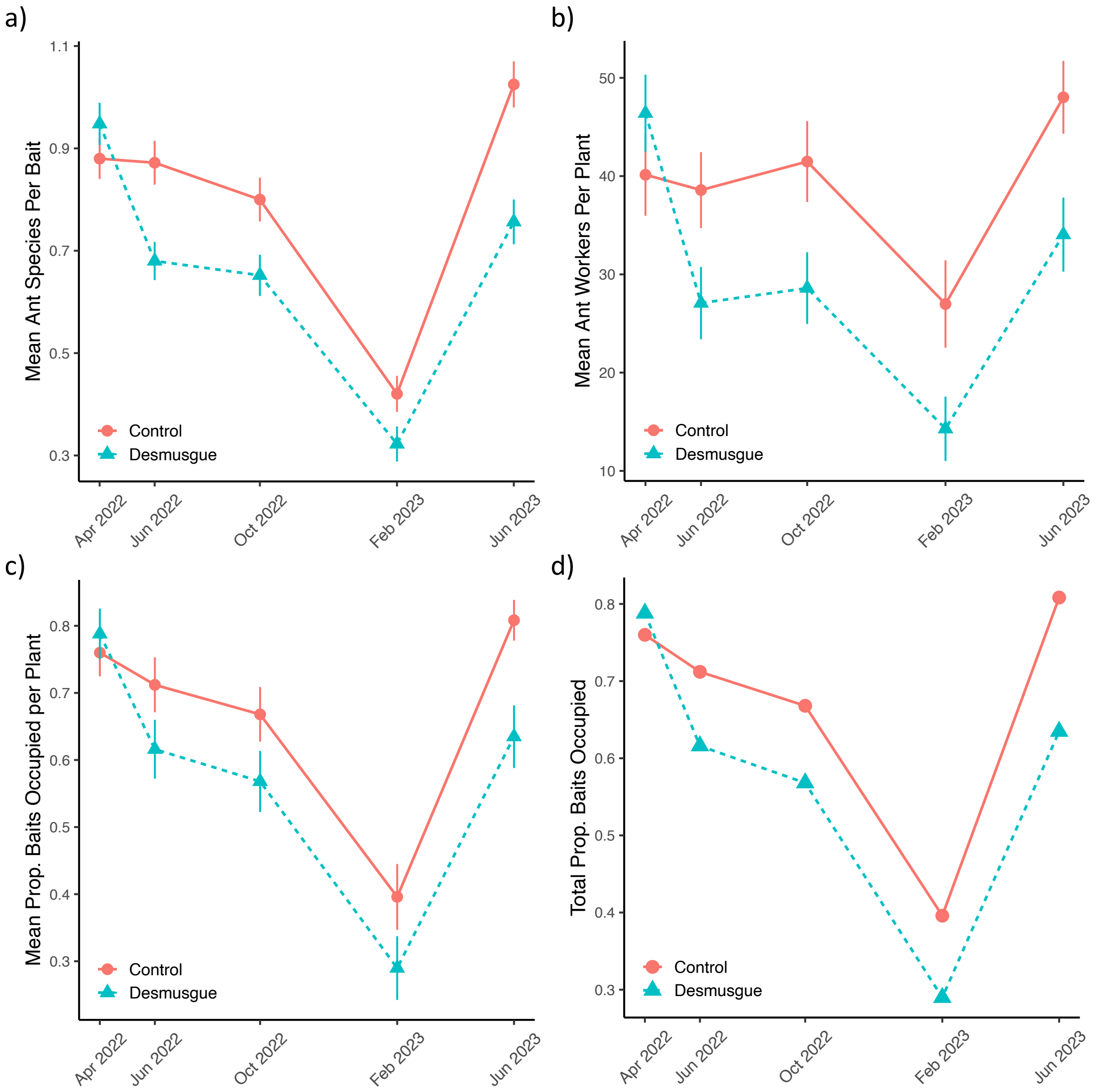


Figure 6.2 – Ant Richness, Activity, & Abundance. All plots depict changes in various ant community metrics over time by treatment group (control and desmusgue). **a)** Shows mean ant species richness per bait on coffee plants, **b)** shows the mean number of pooled ant individuals per coffee plant, **c)** shows the mean proportion of baits occupied per coffee plant, and **d)** shows the total proportion of baits occupied across all plants. For **a-c)** error bars depict the standard error (SE) of the mean.

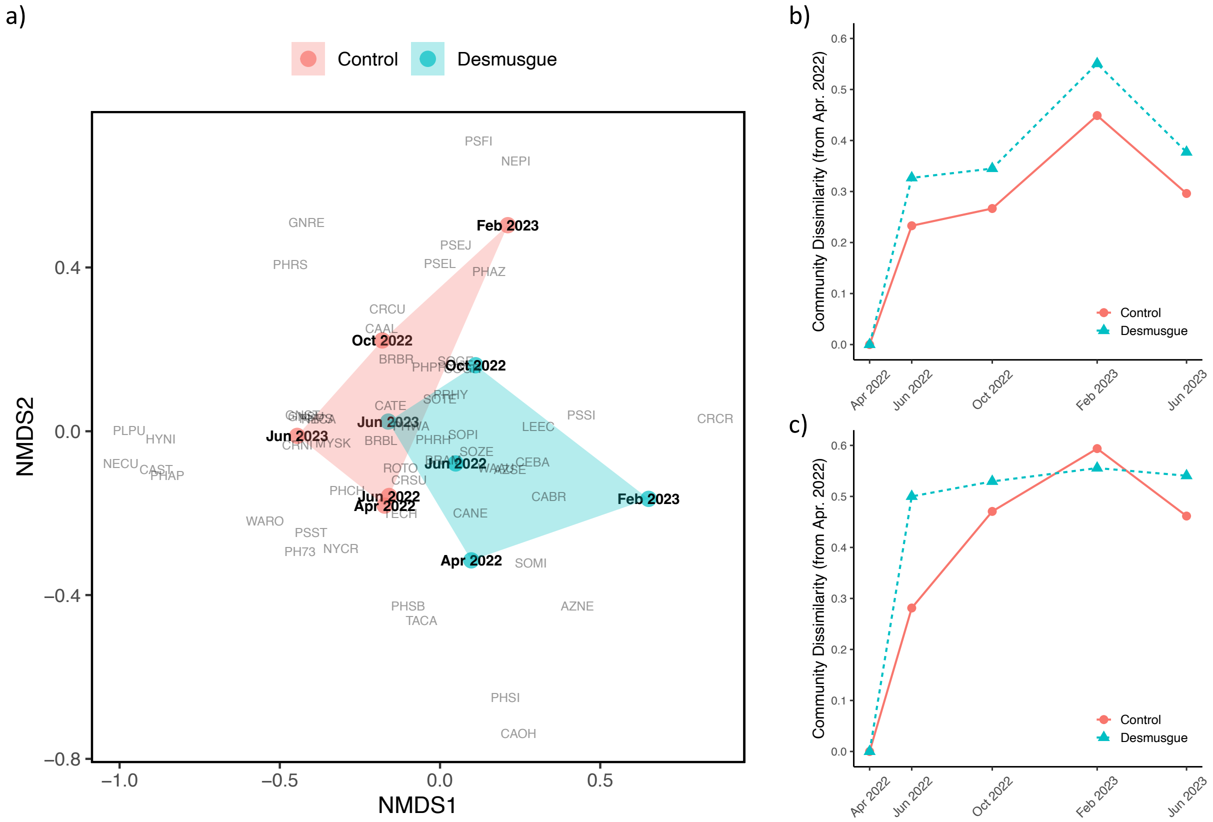


Figure 6.3 – Ant Community Composition & Turnover. **a)** Shows a non-metric multidimensional analysis plot (NMDS) for the ant communities between treatment groups. Sampling time points are shown (labeled in bold), along with estimated polygons for each community in the collapsed multivariate space. Grey labels show the relative position of species in both communities in NMDS space. In **b & c)** community dissimilarity metrics are depicted across time between the treatment groups to assess compositional turnover, where **b)** shows values using Bray-Curtis distance and **c)** using Jaccard distance.

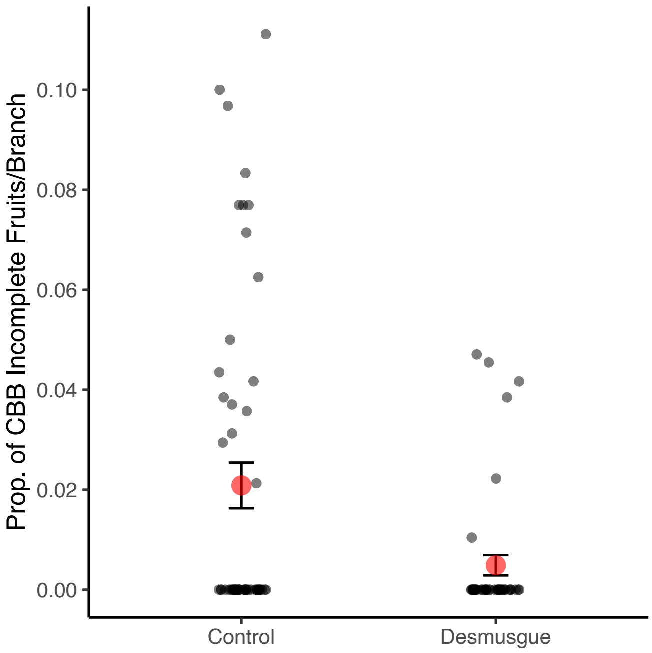


Figure 6.4 – Coffee Pest Control. Shows the proportion of coffee berry borer incomplete bored fruits per branch between treatment groups. Grey points represent the raw data, while red points show the mean (±SE).

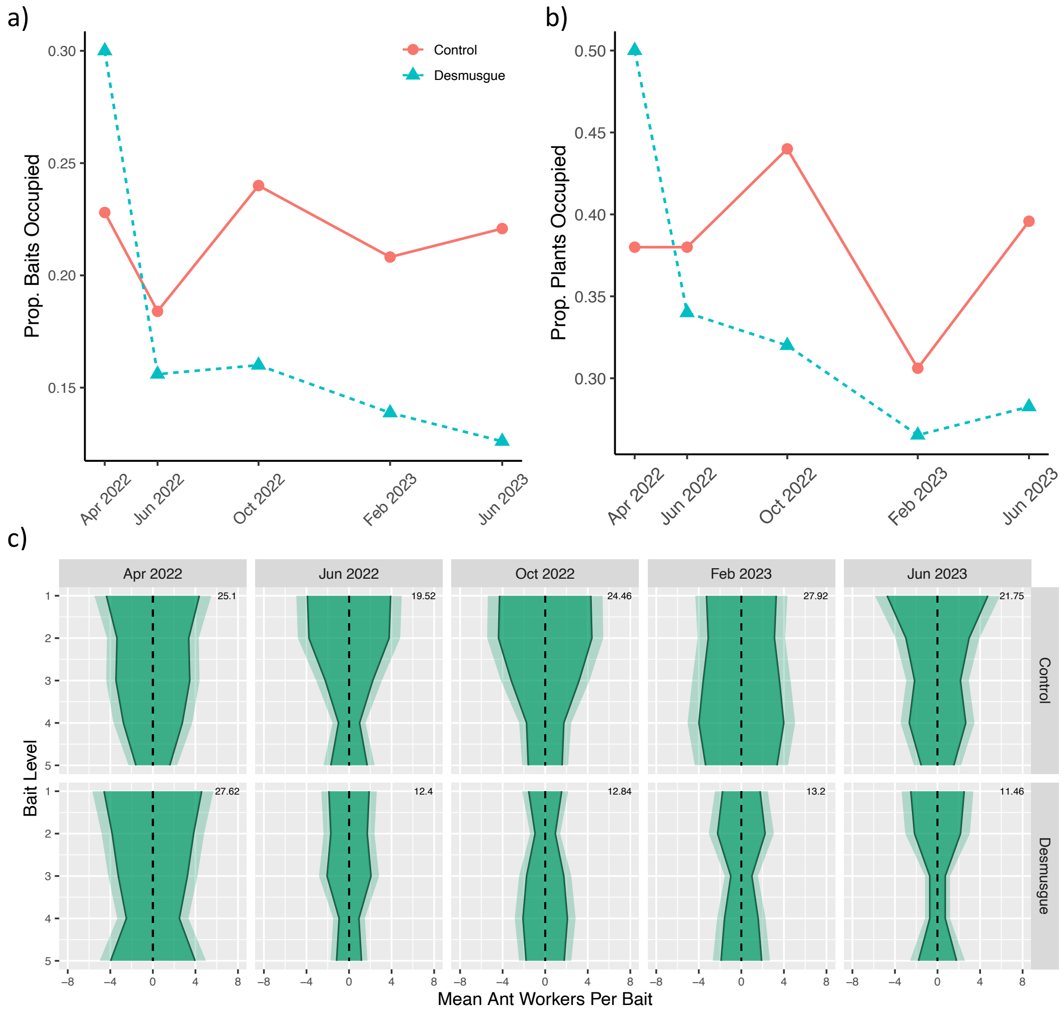


Figure 6.5 – Focal Pest Control Agent Abundance & Activity. All plots depict data for *Solenopsis picea,* one of the most abundant ant species observed in our study and an important natural enemy of the coffee berry borer. In **a & b)** the total proportion of baits and plants occupied across time is shown by treatment group. In **c)** ant activity is depicted, where each figure shows the spatial distribution of mean activity across baits on coffee plants arranged vertically in plant space, to demonstrate where ants are most actively foraging on plants. The width of the dark green area corresponds to mean activity and the lighter green edges show the standard error of means. As an estimate of total mean ant activity across plant space, the area between the curves is included in the top right corner of plots. Plots are shown for both treatment groups across all sampling times.

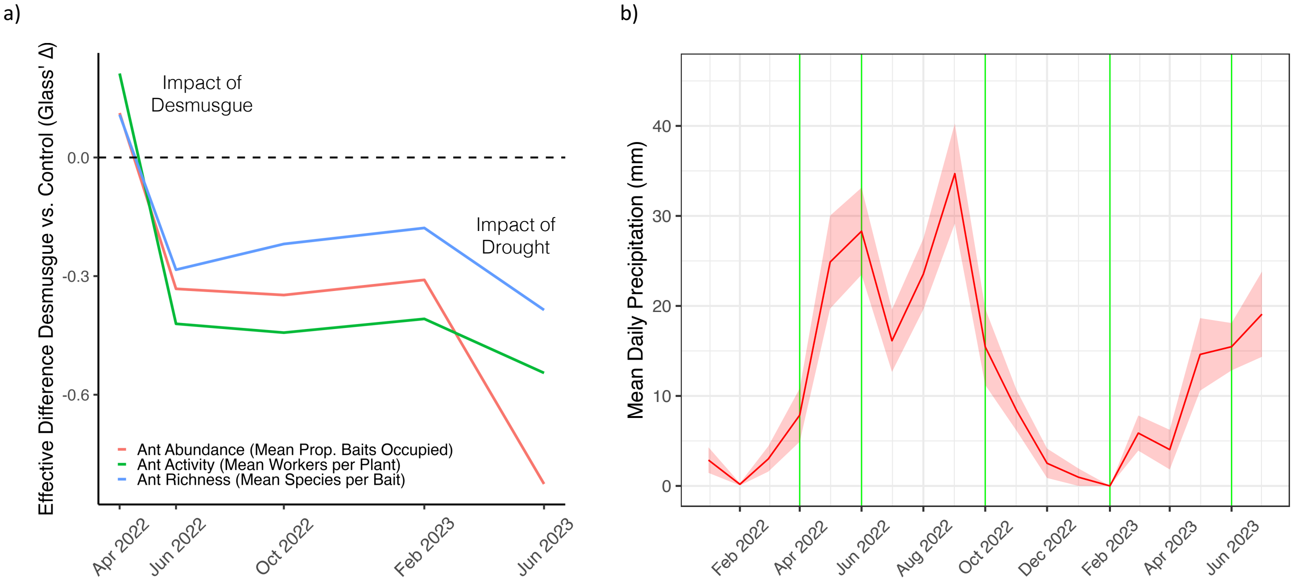
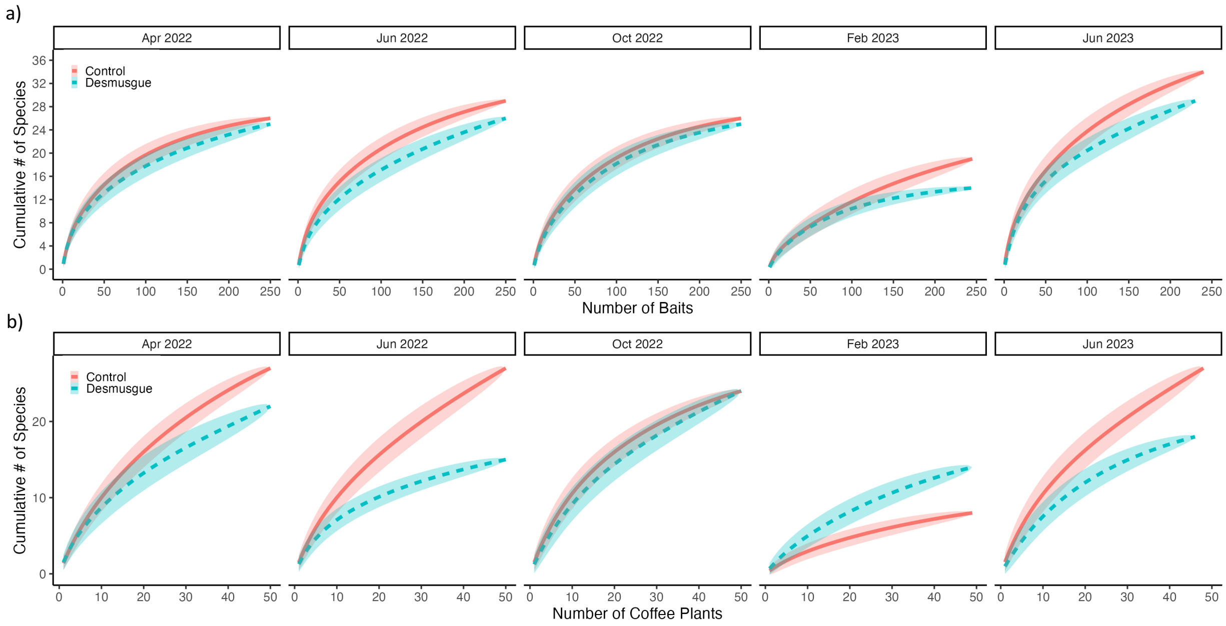
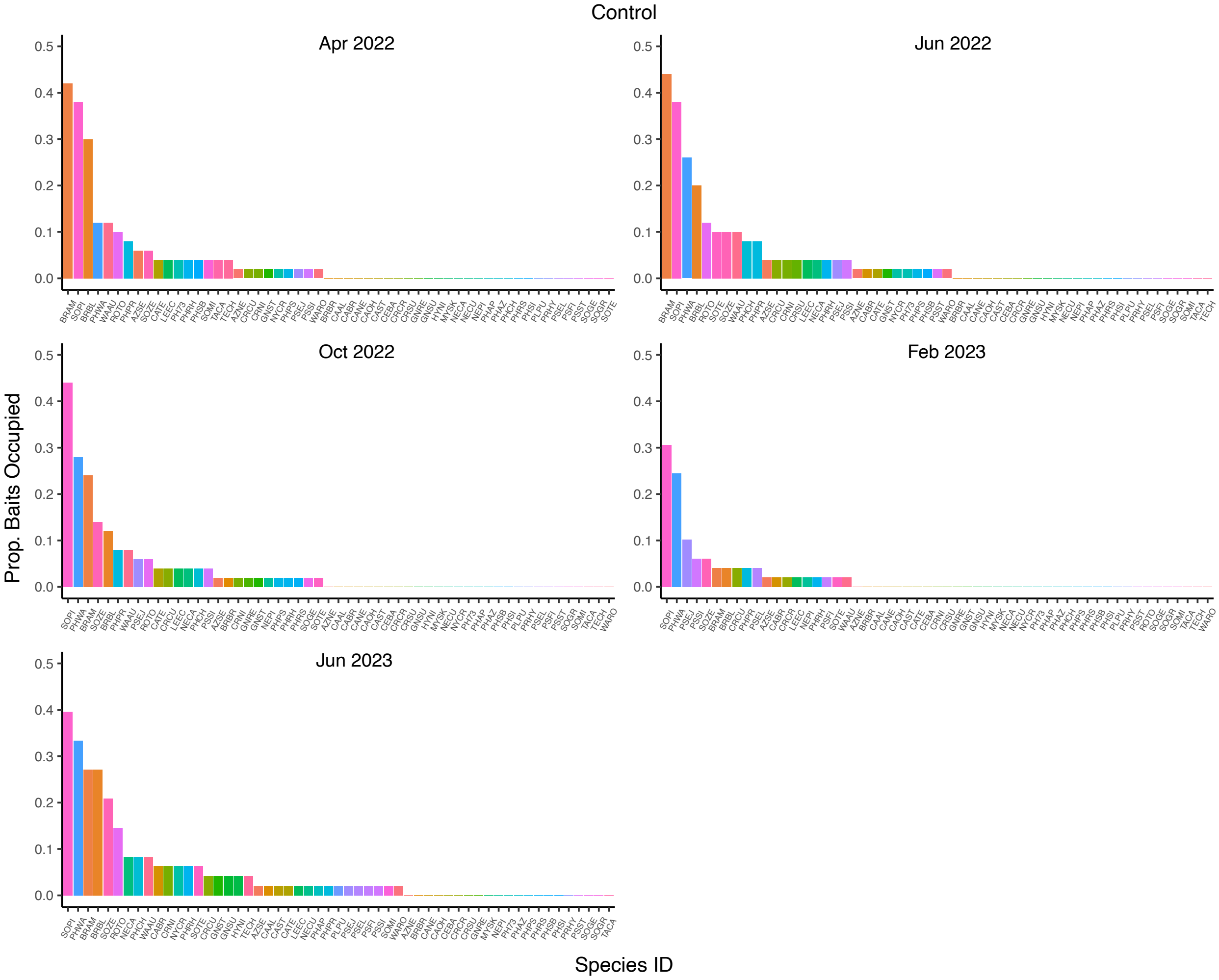


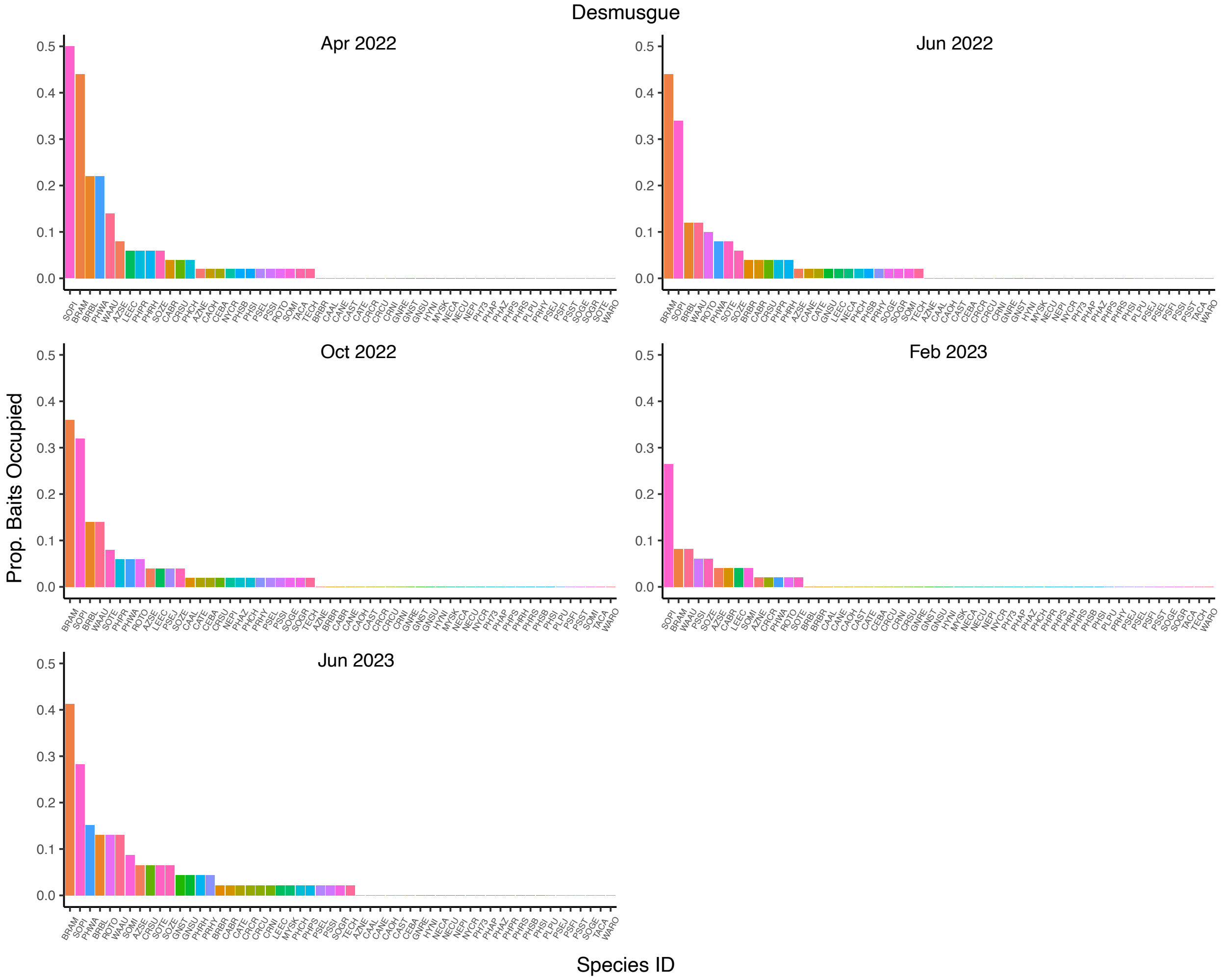
Figure 6.6 – Interacting Stressors & Precipitation. a) Shows the effect size of epiphyte removal through time, where lines depict the estimated difference between treatment groups using Glass' Δ. Effect size by time is shown for three different metrics of ant communities, where values below the dashed horizontal line indicated a negative overall impact of desmusgue on the given metric. We label the initial reduction in all metrics as the initial impact of desmusgue and the secondary reduction after February 2023 as the potential interacting impact of drought or dry season stress. In **b)** we show the recorded mean daily precipitation per month (mm) at our site across time, with green vertical lines marking the approximate dates of our ant surveys and the dark red line depicting mean precipitation (standard error is shown by the lighter shaded area).



Supplementary Figure 6.1 – Species Rarefaction. Figures show species rarefaction curves for ant communities between treatment groups for each sampling time point in our **a)** tuna bait and **b)** visual ant surveys. Rarefactions were conducted using the Mao Tau estimator for sample-based rarefaction, where solid lines represent estimates for rarefied means and shaded areas depict the standard deviation.



Supplementary Figure 6.2 – Species Rank Abundance for Control Plants. All plots depict the proportion of coffee plants occupied for all observed species at each time point on control plants. For each time point, species are ranked according to their relative abundance, where colors are consistent across plots to gauge changes in the relative positions of species in communities through time.



Supplementary Figure 6.3 – Species Rank Abundance for Desmusgue Plants. All plots depict the proportion of coffee plants occupied for all observed species at each time point on desmusgue plants. For each time point, species are ranked according to their relative abundance, where colors are consistent across plots to gauge changes in the relative positions of species in communities through time.

Chapter 7 Conclusion

To curb the impacts of agricultural intensification on biodiversity loss, environmental pollution, and climate change, a holistic, agroecological transition to crop production is paramount (Kremen & Merenlender 2018; Vandermeer *et al.* 2018). A key pillar of this shift will require drastic reductions in chemical pesticide application and the implementation of natural pest control (NPC) as a sustainable alternative (Forister *et al.* 2019; Harvey *et al.* 2020; Lewis *et al.* 1997). However, important gaps in our understanding must be addressed to facilitate this shift. To encourage the widespread adoption of natural pest control, we need to improve our scientific understanding of the community ecology (Vandermeer & Perfecto 2018) and management (Tscharntke *et al.* 2016) of these systems. In particular, there is urgent need to better understand the role of interaction complexity and dynamics in these systems (Eubanks & Finke 2014; Settele & Settle 2018; Vandermeer *et al.* 2010, 2019), especially regarding non-consumptive effects, since pest control has historically placed greater focus on consumptive interactions (Culshaw-Maurer *et al.* 2020). While landscape scale management factors have received increased attention more recently (Dainese *et al.* 2019; Karp *et al.* 2018; Rosenheim *et al.* 2022), the impacts of local farm management may be equally or even more important for natural enemies that are less mobile (Gonthier *et al.* 2014), such as ants. Furthermore, despite its challenges, we need more research which experimentally tests the impact of specific management factors to better understand the mechanistic impacts of intensification. To address these research needs, I grounded my dissertation research in coffee agroecosystems to explore the dynamics, interactions, and management of ant-mediated natural pest control.

In my first two research chapters, I explore interaction complexity in natural pest control, focusing on the role of non-consumptive effects. In Chapter 2, I report the results of a mesocosm experiment testing the impact of a non-consumptive effect (NCE) on multi-predator interactions between ants and pest control function. This interaction drives spatial structure in the population of the pest (the coffee berry borer), where the plant-based ant removes borers from the plant and throws them to the ground. This reduces borer damage on the plant, but results in increased damage to fruits on the ground, as a proxy for damage that could occur on other plants if borers survive attacks. It also increased resources for the ground-foraging ant, which reduced the elevated damage on the ground when present in mesocosms with the arboreal ant. Overall, the two-ant system resulted in the lowest level of damage on the plant, with the subsequent combined reduction in damage on the ground – suggesting spatial complementarity between the two ant species. In this study, I also presented field data showing that the density of hemipteran insects (scale), which the arboreal ant tends for honeydew, and season (rainy vs. dry) can regulate the frequency of the non-consumptive throwing. Additionally, with my field data, I found that the actual consumption of the borer by this arboreal ant species is quite low. Overall, this study contributes novel findings to the literature by demonstrating positive multi-predator effects through a non-consumptive effect between ant species in pest control, and that alternative resources for the non-consumptive predator can regulate NCE strength. Additionally, it revealed that this interaction occurs primarily through natural enemy aggression and not because of prey refuge-seeking behavior, which is far less common in the NCE literature (Bolker *et al.* 2003; Culshaw-Maurer *et al.* 2020).

In Chapter 3, I explore the dynamics of this interaction with a consumer-resource model. I model this interaction in a more general framework, inspired by the natural history of this study system, where two spatially separated predators are connected by a pest that occurs in two spatial pools, on plants and the ground. The prey move in both directions between these pools and their downward migration is amplified by the non-consumptive aggressive behavior of the plant predator. Simulations of this model across the three-dimensional parameter space of downward and upward migration, and the non-consumptive effect revealed important dynamical impacts of the NCE. First, the base system with one-way downward migration exhibited complex dynamics with distinct chaotic and quasiperiodic zones, and multipoint cycles. Fully coupling the system with two-way migration and the NCE stabilized dynamics by reducing dynamical complexity back to simple limit-cycle behavior. Increasing the strength of the NCE increased resources for the ground predator, which increased its overall density, but reduced the density of the plant predator. Interestingly, however, this resulted in reduced borer populations on the plant and the ground, across most parameter conditions, appearing to enhance overall pest regulation across the studied parameter range. Additionally, increasing NCE strength mostly reduced the variability of both predator populations. This study is novel in exploring the dynamical consequences of non-consumptive effects in pest control and, more broadly, linking NCE dynamics to an empirical system, which there are fewer efforts to do in the literature (Bolker *et al.* 2003; Culshaw-Maurer *et al.* 2020). Additionally, our non-consumptive modeling framework is also novel in modeling the effect explicitly through a spatial interaction and not through a consumer functional response, which most NCE theoretical studies have done (Larsen 2012; Terry *et al.* 2017). While our literature search has not been exhaustive, we know of no other studies to model non-consumptive effect dynamics in pest control systems.

In my next three chapters, I study the impacts of farm management and local intensification perturbations on ant communities, their resources, and ant-mediated pest control. In Chapter 4, I explore the impacts of a broad farm disturbance that occurred through coffee cutting, clearing, and replanting on ant communities in coffee, as a proxy for agricultural intensification. I found that this process of plot renovation significantly disturbed ground vegetation cover and the proportion of exposed soil, and significantly reduced coffee plant vegetative complexity. The disturbance had an overall negative effect on ant communities, both on the ground and arboreally (mainly coffee plants), where overall species richness in the study plot was significantly reduced and stayed below pre-disturbance levels for several years before partially recovering five years after the disturbance. Mean species richness in both communities was also significantly reduced after the disturbance, as well as ant abundance, measured by presence-absence at baits, in the arboreal community. Furthermore, ant community composition changed dramatically immediately after the disturbance, but then was mostly unchanged for the rest of the study, both for arboreal and ground ants, suggesting that the perturbation temporarily increased species turnover in both communities. This study is unique in the literature on coffee management impacts on ants, by using a farm management disturbance as a “natural” experiment to test whole community impacts over a multi-year time frame (see references in Morris et al. 2018). While agricultural systems are typically difficult spaces for researchers to implement these kinds of manipulative experiments, closely following the impacts of farm management perturbations, when they do happen, can provide essential insight into the broader implications of agricultural intensification. Additionally, the multi-year time frame of the study allowed for understanding of ant community recovery, which may be facilitated by the patchy nature of this disturbance (only occurring in certain places) and the overall management of the high-shade commercial polyculture at the study site.

In Chapter 5, we assess the impacts of management more broadly by comparing neighboring farms of contrasting degrees of intensification and their impact on coffee epiphytes, which may be important resources for ants. We compared a high-input, low-shade monoculture to a low-input, high-shade polyculture and found that the shaded polyculture had nearly double the total species richness of vascular epiphytes and five times higher orchid and bromeliad abundance on coffee. We also found that the communities of epiphytes were distinct between farms, with increased species evenness in the shaded polyculture. Surprisingly, shade levels at our sites did not explain the differences we found, but coffee plant height was a significant overall driver of these effects, suggesting that heavier, more frequent coffee pruning in the low-shade monoculture may drive some differences. This may also explain why the distance to nearest forest was only a factor in the shaded polyculture, where epiphytes richness declined with distance – in the low-shade monoculture, heavy coffee pruning may negate this effect. While other important work has demonstrated similar patterns with epiphytes on shade trees in farms (Moorhead *et al.* 2010), and has explored the impact of coffee epiphyte removal (Mondragón *et al.* 2009; Solís-Montero *et al.* 2019), we believe this is the first comparative study to explore how overall farm management and intensification drives the coffee epiphyte community.

In Chapter 6, we follow up on these results by exploring the relationship between coffee epiphytes, and their removal, on ant communities on coffee. We designed an experiment to simulate the practice of coffee epiphyte cleaning and removal, known as “desmusgue,” where we removed epiphytes from coffee plants in a desmusgue treatment group, compared to an undisturbed control coffee plant group, and tracked ant communities before and after over 15 months. Overall, we found that this disturbance had significant impacts on most aspects of the ant community. While the total species richness of communities was not consistently impacted (perhaps due to extinction lags), mean species richness, ant activity, and abundance were reduced on coffee plants immediately after the perturbation, and remained significantly below control group levels over the course of the full experiment. We also found elevated levels of species turnover between our treatment groups and distinct community composition, suggesting that epiphyte removal amplifies community shifts through time. Additionally, we found that epiphyte removal had overall negative impacts on pest damage regulation, by reducing coffee berry borer removal rates by ants, and by negatively impacting focal pest control agent populations and activity. Interestingly, while the total fruit load between treatment groups was not different, this also suggests that desmusgue has little practical benefit, confirming previous research testing this question (Solís-Montero *et al.* 2019). Finally, we found an unexpected significant interaction with seasonality, where drought stress during the dry season exacerbated the impacts of epiphyte removal on ants, resulting in the largest differences between treatment groups across several ant metrics nearly 15 months after the initial disturbance. This project is novel in testing the impacts of coffee intensification (through reduction in farm vegetation complexity) on the community of ant natural enemies directly on coffee. While other important work has explored how management impacts epiphytes on shade trees (Moorhead *et al.* 2010), and how coffee epiphyte removal impacts epiphyte communities (Mondragón *et al.* 2009), we link this to the natural enemies that are important for pest control on the crop. Furthermore, our manipulative study demonstrates the clear cascading impacts of epiphyte removal on ant-mediated pest regulation by reducing removal rates and pest control agent abundance and activity. Finally, this study is significant in demonstrating the potential for interacting stressors with agricultural intensification and climate change on pest control, which has been shown more broadly to be a major driver of global insect declines (Outhwaite *et al.* 2022).

Review & Future Directions

Collectively, through my dissertation research, I shed light on two important areas of investigation that may improve our overall understanding of how complex natural pest control systems operate – interaction complexity and the impact of farm management perturbations. My work on non-consumptive effects in Chapters 2 and 3 highlights the importance of interaction complexity on pest regulation through multi-predator interactions and reveals some less explored elements, including how alternative resources impact NCE strength, and how NCEs may have unexpected impacts on pest dynamics. These interactions may be equally important compared to consumptive interactions in pest control, however, there is much less research on them, since pest control is typically thought of as a consumptive process (Culshaw-Maurer *et al.* 2020). Given this gap, and the results I present here, non-consumptive effects, and especially their dynamics, should be further explored in natural pest control systems, where they are likely ubiquitous (Culshaw-Maurer *et al.* 2020; Sheriff *et al.* 2018). Additionally, future work should aim to develop theory on NCEs for other empirical pest control examples (Bolker *et al.* 2003) to understand how often and under what general conditions the dynamics we observed occur. Ideally, attempts should be made to expand this component of natural pest control ecology to community-level dynamics, perhaps borrowing from some of the more general ecological network theory on multiplex systems (Kéfi *et al.* 2012, 2016).

The findings of the epiphyte farm comparison study in Chapter 5 are important in clearly demonstrating the impacts of farm management and intensification on a component of biodiversity and resources that are likely important for the maintenance of natural enemy communities in coffee systems directly on the crop. It also revealed how different scales of management intensification, through both local and landscape factors (Gonthier *et al.* 2014), may simultaneously impact components of natural pest control ecology.

The results of my farm management and perturbation experiments in Chapters 4 and 6 are important contributions, since the bulk of work exploring how agricultural management impacts natural enemy communities is typically correlative. These studies demonstrate not only clear effects of farm perturbations on ant natural enemy communities, but also help to pinpoint the mechanistic impacts of these perturbations. Additionally, the repeated survey of ant communities in both of these projects allows for some understanding of the temporal dynamics of ants in these systems and the potential, or lack thereof, for community recovery after disturbance. In Chapter 6, our findings on the cascading impacts of epiphyte removal on pest control and the interaction of this perturbation with climatic drought stress paint a thorough picture of the overall potential impacts of farm perturbations and management intensification, even though this disturbance was limited to a select group of coffee plants. While whole farm manipulations are rare and challenging for ecologists to conduct, the kind of manipulative disturbance experiments that are presented here can provide inference into what those broader impacts may be. However, this work should be scaled up to the whole farm and landscape level, which, with a little foresight can be facilitated by the general monitoring of natural enemies and pest control services in less intensively managed agricultural landscapes, anticipating future farm intensification events (Wagner *et al.* 2021). By scaling up the kind of study we conducted on coffee plot renovation to the whole farm level and expanding our baseline data collection, ecologists can better understand and predict the broader and long-term consequences of management disturbance on natural pest control communities and their ability to regulate pests.

Importantly, this research on natural pest control should be pursued in other study systems to explore the generality or context-dependence of the patterns we observed. Because the entirety of this dissertation research was conducted in or motivated by the coffee agroecosystem, it will be important to explore these questions in other agroforestry and non-agroforestry systems. For example, the dynamics we explored through non-consumptive interactions are mediated by spatial structure, where predators and prey occur in distinct pools on coffee plants and the ground. This spatial complexity may be reduced in non-agroforests, perhaps reducing the strength of these interactions or changing their dynamics altogether. Regarding management, perennial agroforestry systems with shrubby or woody crops, such as coffee and cacao, allow for the management of vegetation complexity such as epiphytes that grow directly on crops. This may enhance the ability of ant communities to recover from disturbance after local farm perturbations, whereas other systems with less vegetation complexity may exhibit more long-term negative effects of disturbance on ants. Furthermore, a recent meta-analysis on ant-mediated pest control in agriculture found that the benefits of ants are most significant in agroforests (Anjos *et al.* 2022), suggesting that other natural enemies may play a more important role in other crop systems.

Only through these efforts, both to expand our general understanding of ecological complexity and broader farm management impacts on natural pest control, can we better promote this sustainable approach to pest regulation and contribute to the *agroecological* intensification of global agriculture (Carlisle 2022; Kremen 2020; Mier y Terán Giménez Cacho *et al.* 2018), advancing toward a more sustainable future.

7.1 References

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