Biological Control, Biodiversity, and Multifunctionality in Coffee Agroecosystems

by

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DEDICATION

To Emily, who has seen me through it all and has made the journey possible and so much more enjoyable. And to Andre, who never ceases to make me smile. Welcome to this world, amiguito.

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PREFACE

Modern agriculture has succeeded enormously in producing quantities of food at a level that is unparalleled in human history. It is now clearly evident, however, that this level of productivity cannot be sustained indefinitely, and the negative impacts on humanity, biodiversity, and various ecosystem services lead us to question the very framework of modern intensified agriculture. Indeed, the expansion of agriculture is one of the primary causes of biodiversity loss. At the same time, biodiversity is critical for maintaining several ecosystem services on which agriculture depends—services such as biological control of insect pests and pollination. Therefore, agricultural systems that incorporate biodiversity *and* ecosystem services offer an alternative framework—one that focuses on services provided directly from the ecosystem rather than from external inputs. In the following chapters of my dissertation, I address this alternative framework—a multifunctional framework—to consider how ecological complexity in and around farms can benefit both ecosystem services and biodiversity, and whether or not this framework is financially viable for farmers.

I begin by addressing the ecosystem service of biological control. Mounting concern over environmental toxins, as well as an interest in applying ecological principles to agriculture, has led to considerable attention on biological control. In many cases, this has followed the path of classical biological control, where one or a suite of biocontrol agents are intentionally released into an agroecosystem to control specific pests. However, increasing attention is turning to autonomous biological control, where the agroecosystem, with its complex network of interactions, achieves biological control not through one or a couple predator-prey interactions, but through this inherent ecological complexity, full of nonlinearities, multi-trophic interactions, and indirect effects, to name a few. Over the past two decades, researchers at Finca Irlanda, a large coffee farm in Chiapas, Mexico, have taken this whole-system approach, which has allowed for several intriguing, and sometimes counter-intuitive, ecological discoveries about biological control (Vandermeer et al 2010).

As an example, farmers and agronomists in the region have long noted that one arboreal ant, *Azteca sericeasur*, increases the population of the green coffee scale, *Coccus viridis* (Hemiptera: Coccidae), a mutualist with *Azteca* and a minor coffee pest. A logical approach to pest control, as some agronomists have recommended, would therefore be to eliminate the *Azteca* ants, which would then eliminate *C. viridis*. However, researchers have discovered, paradoxically, that the *Azteca* ants create a situation that actually controls *C. viridis* populations. The ants, due to their tending of *C. viridis*, provide clusters of high-density resources for a primary *C. viridis* predator, the ladybeetle *Azya orbigera* (Coleoptera: Coccinellidae). These clusters of food allow the voraciously feeding and relatively immobile *Azya* larvae to find sufficient prey. Without the *C. viridis* clusters, it is unlikely that *Azya* beetles could persist in the system at low densities of *C. viridis*, and a classical boom-and-bust predator-prey cycle could develop.

In Chapter 1, I expand our knowledge of the complex ecological interactions underlying effective autonomous biological control of *C. viridis*. Particularly, I studied how a second ladybeetle, *Diomus* sp., which also preys upon *C. viridis*, contributes to biological control in addition to that provided by *Azya* beetles. I found that the two beetles niche-partition, where *Azya* prefers the high-density areas of *C. viridis* close to the *Azteca* ants, and *Diomus* primarily feeds in areas outside of *Azya*'s primary range, likely due to competitive exclusion. Therefore, I suggest that the presence of both beetles provides enhanced, complementary control of *C. viridis*—with *Azya* as primarily control agent in the areas immediately around *Azteca* and *Diomus* as a primary control agent beyond those areas. Further, I show that *Diomus* larvae, which are physically unprotected from aggressive ants, engage in chemical mimicry of *C. viridis* or chemical camouflage, allowing them to consume *C. viridis* under the watchful eye of the *Azteca* ants.

I then broaden my research focus to the question of how farms and agricultural landscapes can be managed to maximize the effectiveness of biological control. A large body of literature supports the importance of environmental heterogeneity for the abundance and diversity of natural enemies of crop pests. However, although most enemies respond positively to heterogeneity, a large amount of variation in their response is typically found. This is not surprising, given that these studies cover a diverse range of organisms across varied habitats. Furthermore, organisms respond to their local habitat as well as the larger landscape, and the relative importance of these scales of heterogeneity undoubtedly differs between organism groups. In Chapter 2, with a synthetic review, I attempt to dissect why we see these differing responses of arthropod natural enemies, such as parasitoids, beetles, and spiders, to environmental heterogeneity. I found that an organism's guild is important, where parasitoids and plant-foraging insects were particularly sensitive to changes in environmental heterogeneity. Ground-foraging predators, such as ground-foraging ants, spiders, and carabid beetles, were less affected, possibly due to their high sensitivity to soil conditions and tillage regimes, which may trump vegetation-based heterogeneity. Furthermore, I found that arthropod natural enemies responded more strongly to landscape heterogeneity than farm-level heterogeneity (e.g.

polycultures). This work thus highlights the importance of broad-scale efforts to increase environmental heterogeneity in the landscape for the ecosystem service of biological control.

Much of the research on natural enemies and environmental heterogeneity, including that reviewed in Chapter 2, is based on temperate studies, whereas much less is known about these dynamics in the tropics. Therefore, in Chapter 3, I examine a specific example of how natural enemies, in this case parasitoid wasps in coffee farms in Mexico, respond to local management and landscape context. I found that parasitoids were influenced by both the local habitat, primarily the size of shade trees, and landscape complexity, where they benefitted from forests and low-intensity farmland. Vegetation structural components of the environment were more important than plant species diversity, presumably due to the high diversity context of that region of Chiapas—where even the most intensified farms still maintained high levels of plant diversity on and around the farms.

The first three chapters thus deal specifically with biological control and natural enemies, and how ecological complexity influences them. This information can be directly useful to farmers or agroecologists in terms of farm management. However, farms are inherently multifunctional—they provide multiple ecosystem services, support farmer livelihoods, and contribute towards biodiversity conservation. It is important, therefore, to consider these various components and how they interact to gain a more complete understanding of the system.

In Chapter 4, I address multifunctionality in agroecosystems by considering the important relationship between biocontrol and yield. Through meta-analysis, I ask whether it is possible, and under what conditions, to achieve farms that are both productive and that retain high biocontrol functioning, thus maximizing the win-win potential and minimizing tradeoffs. I found that farms with higher plant diversity (e.g. polycultures compared to monocultures) consistently provided higher biocontrol services. The effects on yield from plant diversity were more complex, and depended on planting densities and combinations. However, polycultural cropping systems did show promising potential for win-win scenarios between biocontrol and yield, especially if appropriate secondary crops (e.g. legumes that are saleable) are utilized.

Finally, in Chapter 5, I take a multifunctional approach to measure the response of biodiversity and ecosystem services to ecological complexity. I perform this research in the diverse ecological setting of Chiapas, and in the more ecologically simple setting of Puerto Rico. I assess the ecosystem service of coffee yield, as well as several services, primarily related to biological control, which affect yield or income. In terms of biodiversity, I surveyed taxa that were both functionally, phylogenetically, and morphologically diverse (vertebrates, invertebrates, and plants) and that were generally considered to be beneficial to coffee, such as biocontrol agents or pollinators. I found that the most ecologically complex farms do retain the most biodiversity, but are not always the most high-functioning with income-related ecosystem services. Therefore, achieving multifunctional farms that provide for both farmer livelihoods and for biodiversity conservation will be more likely under scenarios where there are incentives for ecologically complex farms, or where externalities for environmental degradation are properly incorporated.

This work was carried out in collaboration with multiple colleagues and mentors, and although I use a personal pronoun throughout this preface, it should rather be a hearty ‘we’. I am indebted to their hard work and insight, which made this research possible.

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ABSTRACT

In recent decades, ecologists have come to appreciate what many farmers have long known—that biodiversity and ecological complexity play essential roles in many of the processes occurring in agroecosystems. These include many ecosystem services and functions, such as biological control, pollination, and soil fertility and preservation. At the same time, ecologically complex agroecosystems are extremely important for the maintenance of biodiversity at local and at regional scales. In short, diverse agroecosystems help to maintain biodiversity, and biodiversity helps to maintain critical functions and services in these agroecosystems. As such, it is critical to understand the role of ecological complexity in and around agroecosystems so as to maximize the benefits to the conservation of biodiversity and of ecosystem services. I focused my questions on the service of biological control and the biodiversity of natural enemies, and I performed my research on coffee farms in Mexico and Puerto Rico. First, I addressed the importance of natural enemy diversity on biological control, where I studied two ladybeetle predators of a coffee pest. I concluded that through niche partitioning, these beetles coexist and enhance overall biocontrol through species complementarity. Then, using a quantitative review, I addressed how natural enemies are influenced by ecological complexity at various scales. I found that landscape complexity may play a more important role than local heterogeneity in determining enemy abundance and diversity. Next, I focused on one natural enemy guild, parasitoid wasps, and assessed how they respond to ecological complexity at local and landscape scales. Then, through meta-analysis, I addressed the challenge of attaining farms that are both productive and that retain high levels of biocontrol. I found that win-win relationships between biocontrol and yield are probable under certain planting conditions. Finally, I take a multifunctional approach and assess how multiple taxa and multiple ecosystem services respond to ecological complexity. I found that farms can be most profitable and conserve high biodiversity if incentive structures exist to support vegetationally complex farms. In summary, my dissertation research demonstrates the importance of ecological complexity in coffee farms for biodiversity conservation and ecosystem services, such as biocontrol.

CHAPTER I

Species complementarity and myrmecophily in two lady beetle species in a coffee agroecosystem: Implications for biological control[1]

Abstract

The family Coccinellidae contains many common aphidophagous and coccidophagous natural enemies, providing benefits to agroecosystems through their pest control services. Infrequently, but with great adaptive benefits, predatory coccinellid species form close relationships with ants and are facultatively or obligately myrmecophilous. We studied the behavior and distribution of two myrmecophilous coccinellids, *Azya orbigera* (Mulsant) and an undescribed species of *Diomus* to determine potential niche partitioning by the beetles. Both species feed on the green coffee scale pest, *Coccus viridis* (Green), in a coffee agroecosystem in Chiapas, Mexico. We also asked how the *Diomus* sp. is capable of avoiding ant aggression, given that its larvae appear to be physically rather vulnerable, unlike *A. orbigera* larvae, which are covered with long waxy filaments. Through field surveys and lab experiments we detected some degree of resource partitioning and significant spatial segregation among *A. orbigera* and the *Diomus* sp. We posit that the presence of both coccinellid species can lead to improved biocontrol of *C. viridis* populations through species complementarity. Furthermore, we deduce that these *Diomus* sp. larvae employ chemical mimicry or chemical camouflage, allowing them to coexist with aggressive ants. Our work supports the growing evidence that multiple natural

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[1] Co-authors are Burnham, R., Jackson, D., Perfecto,P., Vandenberg, N., Vandermeer, J.

enemies, under certain conditions, can be an important means to achieve more effective and stable autonomous biological control.

Keywords: ants, *Azya orbigera*, chemical mimicry, Coccinellidae, *Diomus*

Introduction

Biological control has long been appreciated for its role in regulating pests in agroecosystems (Root 1973; Risch et al 1983; Russell 1989). However, the role of natural enemy diversity in controlling pests is less certain (Bianchi et al 2006; Cardinale et al 2012). In some cases, one or just a few natural enemies may provide biocontrol that is as effective or better than diverse assemblages (Finke & Denno 2004, Rodríguez & Hawkins 2000). However, a body of evidence also supports the tenet that having multiple natural enemies present in an ecosystem improves the effectiveness of biocontrol (Letourneau et al 2009; Crowder and Jabbour 2014). One mechanism of how diversity may enhance biocontrol is species complementarity, where increased diversity leads to synergistic interactions (i.e. facilitation) between enemies or to a more complete service due to the occupation of more niche space (e.g. prey type, microhabitat, temporal period) (Tscharntke et al 2005; Bianchi et al 2006). In the current study, we investigate the distribution of two myrmecophilous lady beetles (Coleoptera: Coccinellidae) in order to understand how they coexist on the same resource and if their coexistence provides complementary benefits to biocontrol. Furthermore, we seek to understand how one of these beetles, a previously unstudied and undescribed species, is able to avoid ant aggression in order to feed on the *C. viridis* pest.

Two possible reasons for the contradictory findings regarding the effect of multiple natural enemies on biocontrol are the negative impacts of interference competition between enemies and intraguild predation, where enemies prey on each other rather than the pest. The influence of these effects on biological control is highly contingent on the strength and direction of the antagonism (Vance-chalcraft et al 2007). In fact, in some cases, strong negative interactions between natural enemies may actually stabilize biocontrol services, thus enhancing the overall effect (Ong and Vandermeer 2015). The influence of multiple natural enemies on biocontrol may also depend on the hunting mode of the predators and the habitat range of the predator and pest (Schmitz 2007). Here, predators that are able to at least partially segregate into microhabitats that are not completely overlapping can seek refuge from intraguild predation and strong competition.

Previous research has demonstrated the importance of expanding beyond solely considering the role of natural enemy diversity on biocontrol to include a larger ecological framework. For example, over the past 20 years, a dynamic example of autonomous biological control has been uncovered in the coffee agroecosystems of the Soconusco region of Chiapas, Mexico (Perfecto and Vandermeer 2008; Vandermeer et al 2010; Perfecto and Vandermeer 2015). In these agroecosystems, the green coffee scale, *Coccus viridis* (Green) (Hemiptera: Coccidae), believed to be native to East Africa, is a minor pest, despite its severity in other parts of the world (Young 1982; Murphy 1997). The primary natural enemies of *C. viridis*, identified as an entomopathogenic fungus and coccinellid beetles (Vandermeer et al 2010; Jackson et al 2012), manage to keep *C. viridis* in check despite the protection of an aggressive ant mutualist. Researchers have determined that *Azteca sericeasur* Longino (Hymenoptera: Formicidae) is a keystone ant species whose presence leads to spatial clustering of *C. viridis* around the ant nests

(Perfecto & Vandermeer 2008). In this mutualism, the *C. viridis* provide honeydew primarily in return for higher growth rates, likely through improved hygiene (Jha et al 2012). Although the ant species may thus be considered an accomplice of the pest, it is actually through the spatial clustering of the *C. viridis* that one of its primary predators, the myrmecophilous lady beetle *Azya orbigera* (Mulsant) (Coleoptera: Coccinellidae) (Fig. 1.1a,c), is able to persist in the coffee plantation (Vandermeer et al. 2010, Liere et al. 2012). This lady beetle benefits from the *A. sericeasur* ants in two primary ways: 1) high density food resources and 2) protection of larvae from their own parasitoids ('enemy-free space') (Liere and Perfecto 2008). The adults of *A. orbigera* benefit not only from the presence of high resource areas for feeding and ovipositing, but also from areas (outside of *A. sericeasur* influence) where they can escape the aggression of the ants (Liere et al 2012).

Recently, another *C. viridis* predator, representing an undescribed species of *Diomus* (Coleoptera: Coccinellidae) (N. Vandenberg, Pers. Comm.) (Fig. 1.1b,d), was discovered in *Finca* Irlanda, one of the coffee farms where the studies of the *A. sericeasur* system were conducted. Although ~one-half the length of *A. orbigera*, these *Diomus* sp. appear to also feed primarily on *C. viridis* and readily coexist with *A. sericeasur*. In order to coexist with ants, which are highly predaceous, multiple tactics of defense or deceit are employed by myrmecophilous coccinellids. In addition to physical protection, as is the case of *A. orbigera*, some coccinellids produce toxins or bioaccumulate toxic compounds produced by their prey (Sloggett and Majerus 2003; Pasteels 2007), whereas others use chemical mimicry or camouflage to escape ant attack (Dettner and Liepert 1994; Vantaux et al 2010). In the case of mimicry, an organism produces or sequesters compounds that resemble a model, whereas with camouflage an organism simply simulates scents of the background environment (Dettner and Liepert 1994). Although it is known that *A. orbigera* larvae utilize a physical mechanism of protection against *A. sericeasur* ant aggression through the production of a thick waxy covering over their dorsal surface (Fig. 1.1a) (Liere and Perfecto 2008), the *Diomus* sp. larvae in question lack such a covering (Fig.1b), and closely resemble their *C. viridis* prey. Although this observation suggests morphological mimicry, ants are highly dependent on olfactory cues, so it is unlikely that morphological mimicry would be their only mechanism of defense (Holldobler and Wilson 1990; Wolf and Wehner 2000).

In the current study, we seek to understand 1) if *Diomus* sp. and *A. orbigera* niche-partition to decrease competition for a shared resource, 2) whether the coexistence of these two predators enhances the biocontrol of *C. viridis* through species complementarity, and 3) how the relatively immobile *Diomus* sp. larvae withstand the aggressive *A. sericeasur* ants to form a myrmecophilous relationship?

Methods

Study site

We conducted field surveys and lab experiments in *Finca* Irlanda, a large, shaded, organic coffee plantation in the Soconusco region of Chiapas, Mexico ($15^0$20’N, $90^0$20’W), near the border with Guatemala. Elevations on the farm range from 950 to 1150 m asl and the area receives ca. 4500 mm of annual rainfall (Philpott and Bichier 2012). The farm’s shade tree canopy is diverse, with ~100 different species in the 300 ha area (Philpott and Bichier 2012). However, shade trees of the leguminous genus *Inga* are most common, comprising ca. 60% percent of all trees.

Resource partitioning and functional response

To determine if *A. orbigera* and *Diomus* sp. partition their resources, we tested the preferred size of *C. viridis* by *A. orbigera* and the *Diomus* sp. adults. We placed one beetle in a 10 cm petri dish with a determined number of *C. viridis* of sizes ranging from small nymphs to fully mature larvae. Each replication (N=21 *Diomus* sp. adults; N=25 *A. orbigera* adults) had approximately the same number of *C. viridis* from each size class, categorized as small (<1mm), medium (1-2.5 mm), and large (>2.5 mm). After 24 h, we assessed how many *C. viridis* of each size class each beetle consumed and compared differences in size preference within a species using a one-way ANOVA. We then tested the preference for small *C. viridis* compared to combined medium and large *C. viridis* with a Welch Two-Sample t-test. We also tested the functional response of *Diomus* sp. adults to small *C. viridis* by placing one adult with varying (5 to 129) numbers of *C. viridis* crawlers and monitoring the number consumed after 24 h. We only tested the functional response with small *C. viridis*, as this was their preferred size class (see 'Results').

Intraguild predation

As the *Diomus* sp. larvae physically resemble the main food resource of *A. orbigera*, and as the latter is much larger, intraguild predation of *A. orbigera* on the *Diomus* sp. larvae seemed possible. We tested for intraguild predation by placing one of the adult *A. orbigera* in a petri dish with an overabundant supply of *C. viridis*, plus 2 or 3 *Diomus* sp. larvae. We replicated this experiment 15 times, using a total of 38 *Diomus* sp. larvae, and monitored *Diomus* sp. mortality in each replicate over the course of 5 d. Although intraguild predation of *A. orbigera* larvae on the *Diomus* sp. larvae might also occur, we did not have enough *Diomus* sp. larvae available to adequately perform this experiment.

Field surveys

To determine the spatial distribution of *A. orbigera* and *Diomus* sp. in the field, we first selected 14 large *A. sericeasur* ant nests (located in canopy trees) within an established 45-hectare plot in *Finca* Irlanda. At each nest site, we selected the eight coffee bushes closest to each *A. sericeasur* nest and on each bush surveyed the number of *A. orbigera* and *Diomus* sp. larvae and adults, the approximate number of *C. viridis*, and any observed arboreal ant species. These ant species included *A. sericeasur*, but also several other species that are also known to tend scales, such as *Pheidole synanthropica* and *Crematogaster* spp. Upon first arriving at the site and before disturbing any of the vegetation, we performed visual surveys for *Diomus* sp. and *A. orbigera* adults, which scare easily. In all surveys, the same two people simultaneously searched the eight bushes for a 10-min period. As both beetles take refuge on the underside of leaves, these surveys were primarily focused on looking up from below without disturbing the branches. If a beetle was spotted, it was carefully collected to avoid double-counting, and released after the survey was completed. Following the visual adult counts, we surveyed each bush, one branch at a time, by turning over the branch and carefully screening each one visually for beetle larvae, *C. viridis*, and arboreal ants. Additionally, at three nest sites we surveyed the nearest 40 to 45 coffee bushes in order to observe the distribution at an extended scale (up to a 9.5 m radius). Here, we used the same survey techniques as in the other sites, but extended the visual survey for adult beetles to 30-min, again with two people observing. The locations of each of the bushes were measured, allowing for distance calculations of each bush from the *A. sericeasur* nest. We performed this survey twice, approximately 1 month apart, during the months of June and July of 2010.

Myrmecophily in Diomus sp.

We tested how the unprotected *Diomus* sp. larvae can avoid predation by aggressive ants through a four-step methodology. First, we observed whether *A. sericeasur* ants showed aggression towards *Diomus* sp. larvae compared to four other insect species: *C. viridis*, small flies (Diptera), small crickets (Orthoptera), and leafhopper nymphs (Hemiptera). These insects were selected for their similar size and their diversity of body type and phylogenetic relatedness. Five of each of these insects, including the *Diomus* sp. larvae, were first killed in a freezer and then placed on filter paper in five separate 10 cm petri dishes. All five dishes were then placed in a large bin that contained an *A. sericeasur* ant colony with thousands of individuals. Aggression of *A. sericeasur* ants towards each insect was scored according to the following four categories, in increasing order of aggression: 1) tapping of antennae on insect, 2) biting insect, 3) grabbing and carrying insect for short distance (within petri dish), and 4) removing insect completely from petri dish.

A second test allowed us to ascertain whether the avoidance of *Diomus* sp. larvae by *A. sericeasur* is largely due to morphological (resembling the shape and size of *C. viridis*) or chemical (mimicking the cuticular hydrocarbons of *C. viridis* or another organism) traits of the larvae. To do so, we made a paste of smashed *Diomus* sp. larvae and coated small crickets (ca. the same size as *Diomus* sp. larvae) that had been recently killed in a freezer with the paste. We placed one paste-covered cricket in a petri dish and a control (no paste) cricket in another petri dish. We then simultaneously placed these two dishes in a bin containing a large *A. sericeasur* ant colony and monitored if the crickets were removed by the ants (N=4).

A third test allowed us to determine if *A. sericeasur* avoids *Diomus* sp. due to a chemical defense (repellent). For this test, we replicated (N=20) a set of three petri dishes: one with a *C. viridis* individual, one with a *Diomus* sp. larva, and one with a black dot drawn on the underside of the petri dish. Like the preceding tests, we placed the three petri dishes in a bin containing an *A. sericeasur* colony and monitored the number of times an *A. sericeasur* ant crossed directly over the *C. viridis*, *Diomus* sp., or dot over a period of 5 minutes. We tested the difference between treatments for significance with a one-way ANOVA.

Lastly, to test whether a toxic compound was involved in *A. sericeasur*'s avoidance of *Diomus* sp., we fed 23 *A. sericeasur* ants a sugar solution containing smashed *Diomus* sp. larvae and monitored how long they survived compared to a control group of 23 ants that was fed the same sugar solution without the *Diomus* sp. paste.

Data analysis

Beetle spatial distributions

We calculated the spatial distribution of *Diomus* sp. and *A. orbigera* in relation to *A. sericeasur* ant nests through two methods. First, we tested for spatial niche partitioning by comparing the locations, in terms of the radial distance to the nearest *A. sericeasur* nest, of the *Diomus* sp. and *A. orbigera* populations. At each site, we calculated the difference between the median distance from the nest to the *Diomus* sp. observations and the median distance from the nest to the *A. orbigera* observations. Using a resampling approach, we compared this difference to what would be expected for two randomly distributed populations. For each site, we generated 10,000 synthetic populations by randomly allocating the *Diomus* sp. and *A. orbigera* observations to coffee bushes, i.e., by resampling with replacement from the observation locations. The difference between an observed value and the random expectation was considered significant if less than 5% of the 10,000 synthetic populations were as or more distant from each other than the observed populations. Specifically, we tested the hypothesis that the *Diomus* sp. populations were further from the nests than the *A. orbigera* populations. The results for individual sites were combined to generate a single P-value using Fisher's method (Fisher 1970). Secondly, we calculated the average number of *A. orbigera* and *Diomus* sp. larvae and adults per bush as a function of increasing distance from the *A. sericeasur* nest and tested for significant trends with linear regression. We separated the analyses by life stage, as the larvae and adults are likely influenced by different factors due to the larvae's ability to withstand ant aggression.

Predictors of beetle abundance

We used a hierarchical Bayesian framework with a Poisson regression to model the influence of other environmental covariates on the abundance of *A. orbigera* and *Diomus* sp. adults and larvae. Poisson distributions are especially appropriate for count data, as these datasets are discrete, positive, and often exhibit a strong mean-variance relationship (Royle et al 2002). Additionally, as our surveys included several 'zero' occurrences (bushes where no beetles were detected), we incorporated a bimodal, zero-inflated distribution using a Bernoulli variable (Burton et al 2012). These distributions are especially useful for modeling rare species (Wenger and Freeman 2008). The Bernoulli variable allows for a simple modeling of two distinct processes which adhere to fundamentally different distributions: presence or absence from a particular site and species abundance (and covariates that predict abundance) where present (Wenger and Freeman 2008). Zero-inflated distributions, therefore, can be envisioned as a two-part model pertaining to both processes.

In addition to accounting for environmental covariates (fixed effects; see below), our model incorporated an error term for spatial location as a random effect (Thogmartin et al 2004). Our model likelihood was:

$K_i \sim \text{Poisson}(\lambda_{1i})$

where K_i is the number of beetle species *i* per coffee bush. We added a term, v_i, to account for zero-inflation according to the following:

$$\lambda_{1i} = v_i * \lambda_{2i}$$

where $vi \sim \text{Bernoulli}(\Psi_i)$ and where Ψ_i was drawn from a uniform distribution, ranging between 0 and 1 (Burton et al 2012). Non-zero values (i.e. where $v_i = 1$) of beetle abundance were therefore assigned to λ_{2i}, which was estimated according to the function:

$$\log(\lambda_{2i}) = \alpha + \beta * X_i + \omega_{\text{nest}(i)}$$

where α denotes the intercept, β is the vector of fixed effect coefficients associated with each covariate, X_i denotes the matrix of covariates for species *i*, and ω is the spatial random effect of each *A. sericeasur* nest.

The distribution ω was estimated using a function with exponential decay for each *A. sericeasur* nest to control for spatial relatedness (Thogmartin et al 2004; Thomas et al 2006). This spatial consideration was important, as *C. viridis* and *A. orbigera* are known to cluster around *A. sericeasur* nests (Vandermeer et al 2010; Liere et al 2012). To define the spatial random effect, ω, we utilized the parameter *φ*, which represents the rate of decline of correlation between covariates with increasing distance between points (Thomas et al 2006). The parameter *φ* was drawn from a uniform distribution ranging from 0.1 to 20 m, the latter approximating the average distance between *A. sericeasur* nests. The precision (inverse of variance) of ω was drawn from a gamma distribution with shape 0.5 and rate 0.0005. For α and β we used non-informative, or flat, priors following a normal distribution with mean 0 and variance 100,000.

In addition to including the influence of the other beetle species on each beetle life stage, we incorporated the effect of time (2 sampling events), as well as the abundance of the following organisms, as covariates in our model: *A. sericeasur* ants, total arboreal ants (excluding *A.*

sericeasur), and *C. viridis*. These variables were chosen as they represent the primary food source for the beetles (*C. viridis*) and the mutualists of their food source (ants). We based abundance values on the individuals recorded per coffee bush. Ant abundance data were recorded as categorical variables, but were estimated as abundance numbers from a uniform distribution between the upper and lower bounds of each category as part of the overall model. We fit models using Markov Chain Monte Carlo (MCMC) methods in OpenBUGS 3.2.1 (Thomas et al 2006), a statistical package that utilizes MCMC for Bayesian inference. Each beetle species and life stage (i.e. larvae, adults) was modeled separately. For each model, we excluded variables where posteriors did not properly converge. To obtain posterior parameter results, we ran 40,000 iterations of each model with a burn-in of 10,000 iterations.

Results

Resource partitioning, functional response, and intraguild predation

We observed some degree of resource partitioning between species of adult beetles. *Diomus* sp. adults only consumed the smallest size class (nymphs) of *Coccus viridis*, whereas *A. orbigera* adults tended to prefer the medium and large size classes, although this preference (by *A. orbigera*) was only marginally significant (P=0.063 for difference between large and small class preference) (Fig. 1.2). When medium and large *C. viridis* were pooled together, *A. orbigera* did show a significantly greater preference for the pooled larger sizes over the small size (P=0.016). Neither species of beetle larvae demonstrated a preference for any particular class (A.L. Iverson, pers. obs.). Our assessment of the functional response of *Diomus* sp. adults to small *C. viridis* showed that the response approached an asymptote (i.e. satiation) at around 30 *C. viridis* after 24 h (Fig. S1.1).

When we tested whether intraguild predation could occur between *A. orbigera* and *Diomus* sp., we found that of the 38 *Diomus* sp. larvae placed with *A. orbigera* adults, eight (21.1%) were consumed by *A. orbigera*. Thus, although not a preferred food resource, *A. orbigera* adults will engage in intraguild predation, at least in laboratory settings.

Beetle spatial distributions

From our field survey data, we observed that *Diomus* sp. adults were located significantly farther from *A. sericeasur* nests than were *A. orbigera* adults, whereas larvae of the two species showed no difference (Table 1.1). *Diomus* sp. larvae were also located farther from nests than *A. orbigera* adults, although this was only significant in the second survey. Similarly, *Diomus* sp. adults were located farther from nests than *A. orbigera* larvae, but only in the first survey. When we plotted the abundance of *A. orbigera* and *Diomus* sp. as a function of distance from the nest, we found that *A. orbigera* adults and larvae were negatively associated with distance from *A. sericeasur* nests (linear regression, $P<0.05$). *A. orbigera* was primarily distributed within a radius of 3-4 m of the nest and then sharply decreased in density (Fig. 1.3). The distribution of *Diomus* sp., on the other hand, increased with increasing distance from *A. sericeasur* nests, although this trend was not significant for adults ($P=0.12$).

Predictors of beetle abundance

When we modeled how arboreal ants, the other beetle species, space, and time were associated with *A. orbigera* and *Diomus* sp. abundance, we found that both *A. orbigera* larvae and adults were positively associated with scales and *A. sericeasur* ants, yet negatively associated with other arboreal ants (Table 1.2). These arboreal ants consisted of a total of 20 species, the most abundant being *A. sericeasur*, *Pheidole synanthropica, Solenopsis picea, Crematogaster nigropilosa*, *Pseudomyrmex simplex, Camponotus brettesi, Camponotus textor,* and *Brachymyrmex* spp. *Diomus* sp. larvae and adults, on the other hand, exhibited the opposite trend and were negatively associated with *A. sericeasur*, albeit not significantly so with *Diomus* sp. adults, and positively associated with other arboreal ants. *Diomus* sp. larvae were also negatively associated with both *A. orbigera* larvae and adults, and increased over time (from survey 1 to survey 2).

Myrmecophily in Diomus sp.

When we tested for *A. sericeasur* ant aggression towards five different insects, we found that ants showed no aggression towards *C. viridis* or *Diomus* sp. larvae, but exhibited a high degree of aggression towards the other insects (Fig. 1.4). In fact, all other insects were eventually removed from the open petri dish by the ants, and were likely taken back to the nest as a food resource. When we tested for whether Diomus was employing morphological or chemical mimicry by covering crickets with a *Diomus* sp. paste, we found that no *Diomus* sp.-covered crickets were removed by *A. sericeasur,* whereas 75% of the control crickets were removed. However, this avoidance of *Diomus* sp.-covered crickets did not appear to be due to a repelling chemical, as there was no significant difference between treatments when we recorded how many times *A. sericeasur* passed directly over a *C. viridis* individual, a *Diomus* sp. larva, or a dot (Fig. S1.2). Furthermore, the *A. sericeasur* ants that were fed a sugar solution containing *Diomus* sp. had a lower mortality rate than those fed a plain sugar solution (Fig. S1.3).

Discussion

Competitive coexistence

Despite competing for a shared resource, our research suggests that *A. orbigera* and *Diomus* sp. do spatially niche partition. The spatial clustering of *A. orbigera* appears to exclude

Diomus sp. larvae and adults, and only outside of the ring of *A. orbigera* influence can the *Diomus* sp. reach their highest numbers. The cutoff where *A. orbigera* populations drop and *Diomus* sp. populations rise occurs approximately 3-4 m from the *A. sericeasur* nest (Fig. 1.3). This distance corresponds with the distance where *C. viridis* abundance drops (Fig. S1.4), so it appears that the *A. orbigera* are clustering around their food source. The tendency for *Diomus* sp. larvae to exist outside of the influence of *A. orbigera* may reflect intraguild predation by *A. orbigera*, although it could also result from preferred ovipositioning in those areas by *Diomus* sp. adults. Adult *A. orbigera* and *Diomus* sp. beetles may avoid intense competition through their preference for differently sized *C. viridis*, where *Diomus* sp. consume only the smallest class of *C. viridis* and *A. orbigera* prefer the larger sizes. However, as the strongest signal of competitive exclusion was observed between adults of *A. orbigera* and *Diomus* sp., it appears that the difference in food size preference does not allow *Diomus* sp. to fully escape intense competition, as *A. orbigera* still feeds on the smallest *C. viridis*.

Although our results for *A. orbigera* larvae follow a similar pattern found by Liere et al. (2014), also at *Finca* Irlanda, strikingly, our results of *A. orbigera* adult distributions show an opposite pattern. Liere et al. (2014) found that *A. orbigera* adults were concentrated around *A. sericeasur* nests, but were found in highest numbers 3-5 m from the nest, theoretically to avoid *A. sericeasur* aggression. We believe the difference in outcomes is not contradictory, but is likely related to the *A. orbigera* adults' preference to be near *C. viridis*, while avoiding contact with the aggressive *A. sericeasur*. The particular location of the highly mobile *A. orbigera* at any point in time may reflect the degree to which they are tending *C. viridis*, which is reflective of the size of the *C. viridis* colony. Therefore, in years with smaller colonies, *A. orbigera* adults may be able to persist closer to the *A. sericeasur* nests due to less *A. sericeasur* aggression. The year of our survey, 2010, was characterized by overall low *C. viridis* numbers (J. Vandermeer, pers. comm.). We thus envision a situation where *A. orbigera* populations may expand and contract relative to an *A. sericeasur* nest and in each situation *Diomus* sp. exists on the periphery.

Alternatively, our differing results may reflect the activity of *Pseudacteon* spp. (Diptera: Phoridae) parasitoids of *A. sericeasur* ants, whose larvae slowly decapitate the *A. sericeasur* ants and whose presence cause the *A. sericeasur* to enter a catatonic state (Philpott et al 2004). When this occurs, *A. orbigera* beetles detect the *A. sericeasur*'s alarm pheromone and take advantage of the motionless ants to consume or oviposit under the less-defended scales (Liere and Larsen 2010; Mathis et al 2011; Hsieh et al 2012). For our study, we selected relatively large *A. sericeasur* nests, whereas Liere et al. included colonies of all sizes. As larger clusters, and theoretically larger nests, of *A. sericeasur* nests generally attract more *Pseudacteon* spp. (Vandermeer et al 2008), our study may have included nests where *A. orbigera* could persist closer to *A. sericeasur* due to lower aggression resulting from the presence of these parasitoids.

The results from our models of important environmental predictors of *A. orbigera* and *Diomus* sp. abundance generally corroborated our results from analyses of spatial distribution. As predicted, *A. orbigera* adults and larvae were most commonly found in areas where *A. sericeasur* were most abundant, and less commonly found where other arboreal ants were more abundant. This finding reflects the higher concentration of *C. viridis* close to *A. sericeasur* nests (Fig. S1.4) and the competitive exclusion of other ants in areas of high *A. sericeasur* activity (Vandermeer et al 2010). *Diomus* sp. adults and larvae, in contrast, were most abundant where other arboreal ants were more abundant, likely reflecting their own competitive exclusion around *A. sericeasur* nests. The *Diomus* sp. were likely capitalizing on the smaller colonies of *C. viridis* that are typically tended by these arboreal ants, such as *Crematogaster* spp. and *Pheidole* *synanthropica* (Vandermeer et al 2010). Indeed, 73% and 85% of all *Diomus* sp. larvae were on bushes with *P. synanthropica* and any arboreal ant other than *A. sericeasur*, respectively (Fig. S1.5).

Diomus sp. larvae negatively covaried with *A. orbigera* adults and larvae, perhaps reflecting intraguild predation of *A. orbigera* on *Diomus* sp. Although we did not observe *A. orbigera* larvae predating *Diomus* sp. larvae, it is likely plausible given the similarity in morphology between *Diomus* sp. larvae and *C. viridis*.

Myrmecophily in Diomus sp.

Our results suggest that *Diomus* sp. larvae escape aggression and predation from *A. sericeasur* ants predominately through chemical camouflage or mimicry, likely mimicking *Coccus viridis*. We support this claim through a four-step experimental process. First, we determined that *A. sericeasur* does not demonstrate any aggression towards *Diomus* sp. larvae, equal to their response to the *C. viridis* but different from the three other insect groups, which received a high amount of aggression. From this experiment we concluded that *Diomus* sp. larvae chemically or morphologically mimic *C. viridis*, chemically camouflage themselves, or produce a noxious substance that serves as a repellent. We ruled out the repellent option, as *A. sericeasur* did not avoid contact with *Diomus* sp. compared to *C. viridis* or compared to random contact. Additionally, *A. sericeasur* were not harmed by consuming *Diomus* sp., and in fact they trended towards a lower death rate on the *Diomus* sp. solution, likely due to improved nutrition. Finally, *A. sericeasur* did not show aggression towards crickets covered in a *Diomus* sp. paste, whereas they demonstrated high levels of aggression towards uncovered crickets, thus demonstrating a chemical, rather than morphological, effect. Although these results allow us to conclude that chemical mimicry or camouflage is likely the primary mechanism being employed, we cannot rule out the role of morphological mimicry, as the shape of *Diomus* sp. larvae and *C. viridis* are strikingly similar. These mechanisms may function synergistically.

Without directly analyzing the chemicals used by *Diomus* sp., we cannot know with certainty if *Diomus* sp. is mimicking *C. viridis* or another organism towards which *A. sericeasur* is not aggressive. Nor can we know whether the mimicked chemical(s) is of exogenous or endogenous origin. In the former case, *Diomus* sp. could sequester mimetic compounds from its prey rather than directly biosynthesizing them (Völkl 1995; Majerus et al 2007). If *Diomus* sp. is native to Mexico, an exogenous origin may be more plausible, given that *C. viridis* appears to have originated in East Africa. However, there is not a clear consensus on the origin of *C. viridis*, nor do we know for sure if *Diomus* sp. is native to Mexico (Bach 1991; Murphy 1997). An alternative scenario is that *Diomus* sp. does not mimic the *C. viridis*' cuticular hydrocarbons but rather is chemically 'camouflaged' with uninteresting compounds from the ants' perspective (Dettner and Liepert 1994). However, chemical mimicry is a strong candidate, especially since it has been described with *Diomus thoracicus* in French Guiana. There, the *D. thoracicus* larvae function as inquilines within colonies of the ant *Wasmannia auropunctata* through chemical mimicry of the ants' species- specific cuticular hydrocarbons (Vantaux et al 2010). In our case, we are fairly certain that *Diomus* sp. is not mimicking *A. sericeasur* cuticular hydrocarbons, as we observed similar unaggressive behavior in other *C. viridis*-tending ants (e.g. *Pheidole synanthropica* and *Crematogaster* spp.) towards *Diomus* sp. larvae.

Coccinellid myrmecophily has arisen convergently in a few different subfamilies (Majerus et al 2007) and appears to have arisen as a response to coccinellid beetles' predilection for the Hemipteran families Aphidae and Coccidae, which themselves are often myrmecophilous (Holldobler and Wilson 1990; Hodek and Honek 1996). However, myrmecophilous behavior among coccinellid beetles is not well documented among tropical species (Vantaux et al. 2010). Therefore, the discovery of this behavior in two coexisting and theoretically competing tropical coccinellids from separate subfamilies (Giorgi et al 2009) is surprising.

Implications for biological control

A. orbigera and *Diomus* sp. appear to play important and complementary roles in the stability and effectiveness of conservation biological control of *C. viridis* in the studied coffee agroecosystem. We show that the beetles, although dependent upon the same resource, do appear to spatially niche-partition. *A. orbigera* is the primary predator of *C. viridis* near large *C. viridis* aggregations, which are facilitated by *A. sericeasur* ants. However, outside of these nuclei, *C. viridis* may not be sufficiently concentrated to sustain large *A. orbigera* larvae populations due to their high resource needs, on average eating 20 mature *C. viridis* per day (Liere et al. 2014). It is precisely in these smaller *C. viridis* patches where the *Diomus* sp. larvae thrive. Although *Diomus* sp. adults feed on similar numbers of *C. viridis* as *A. orbigera* adults (20-30 per 24 h) (Fig. S1.1; Liere & Larsen 2010), *Diomus* sp. consumes *C. viridis* that are a fraction (~1/5th) of the length of the mature *C. viridis* that *A. orbigera* prefers, which allows *Diomus* sp. adults to persist around these smaller colonies. Although *A. orbigera* adults are highly mobile and have been shown to persist in high numbers slightly beyond where we observed their highest numbers (i.e. up to 5 m), even in that situation, *A. orbigera* are still highly dependent on *A. sericeasur*-tended *C. viridis* colonies (Liere et al 2012; Liere et al 2014).

We therefore believe that *Diomus* sp. is a critical suppressor of *C. viridis* populations beyond the zone of *A. sericeasur* influence. Theoretical models predict enhanced biocontrol from multiple predators in situations where the predators have a broader habitat range than the pest, where the predators' domains do not completely overlap, and where the predator hunting mode is identical (Schmitz 2007). These criteria were met by *A. orbigera* and *Diomus* sp. *Diomus* sp. appears to be a weaker competitor, yet because its domain extends to areas that are not as suitable for *A. orbigera*, it can persist in the agroecosystem and predate the *C. viridis* in these *A. orbigera*-unsuitable areas. Furthermore, although we did observe intraguild predation between *A. orbigera* adults and *Diomus* sp. larvae, theoretical evidence suggests that this scenario, rather than restricting biocontrol, could actually improve it through greater stability of predator and pest populations (Ong and Vandermeer 2015).

Additionally, the coexistence of *A. orbigera* and *Diomus* sp. may be beneficial under seasonal fluctuations of *C. viridis* populations. In the Soconusco region of Chiapas, *C. viridis* tend to decrease in the dry season (Dec-April) and *A. orbigera* populations drop considerably at this time, as well (H. Liere, pers. comm.). Although we do not have year-round abundance data on *Diomus* sp., it is highly plausible that *Diomus* sp. is an important control of *C. viridis* populations as they emerge from low-density periods. At this critical time of population growth, *C. viridis* densities may not be high enough to sustain significant *A. orbigera* populations, as there would likely not be many *C. viridis* colonies of sufficient size to sustain *A. orbigera* larvae. *Diomus* sp. larvae, on the other hand, could persist within much smaller, incipient *C. viridis* colonies. This scenario could thus be especially important in preventing early pest outbreaks. Additionally, the presence of both *A. orbigera* and *Diomus* sp. in this agroecosystem likely provides a further ‘insurance’ in the face of disturbance, in which one species may be more effective than the other under different conditions (Philpott and Armbrecht 2006; Tscharntke et al 2007).

Conclusions

We provide one of only a few documented cases of myrmecophilous coccinellids in the tropics (see also Liere & Perfecto 2008, Orivel et al. 2004, Vantaux et al. 2010) and, to our knowledge, the first example of chemical mimicry or camouflage by a coccinellid predator of an agricultural pest in the tropics. Although relatively few examples of myrmecophilous coccinellids are known (Majerus et al 2007), our research plus that of others (Liere and Perfecto 2008) has demonstrated the presence of two such species in coffee farms in the Soconusco region of Chiapas. This suggests the potential for many more to be discovered worldwide, especially in the tropics. Our findings related to the behavior and spatial distributions of *Diomus* sp. and *A. orbigera* have important implications for biological control. Larval forms of both beetle species are not capable of much movement, largely restricted to a single plant during their larval life. Thus they require local concentrations of food, supplied largely by the mutualistic effects of ants. Adult beetles, by contrast, fly long distances and can thus forage on sparsely dispersed food sources. The clumping of the adults near ant nests is thus largely for the purpose of oviposition (and perhaps mating itself) in a high quality site (abundant food source and protection against natural enemies indirectly supplied by the ants) for the larvae. The two species of beetles, sharing so many life history characteristics, are thus likely candidates for strong competition with one another. Spatial partitioning seems to reduce competition between them, perhaps enhancing the biological control effectiveness of the system as a whole.

We suggest that the *A. orbigera* are important predators of the *C. viridis* when in large aggregations, whereas the *Diomus* sp. provide complementary control of *C. viridis* when in smaller aggregations, often beyond the influence of *A. sericeasur* ants. These complementary effects may be enhanced under spatio-temporal fluctuations, such as when are at low population densities overall. Predation by the beetles, compounded with other natural enemies, including the fungus *Lecanicillium lecanii* (Jackson et al 2012; Ong and Vandermeer 2014), appear to keep the *C. viridis* populations at low, non-pest densities at *Finca* Irlanda. Our work supports the growing evidence that multiple natural enemies, under certain conditions, can be an important means to achieve more effective and stable autonomous biological control (Schmitz 2007; Vandermeer et al 2010; Cardinale et al 2012; Ong and Vandermeer 2015).

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Table 1.1. Pairwise comparisons of median distance to nest (m) for *A. orbigera* and *Diomus* sp. beetles. Significant values ($P<0.05$, in bold) represent *Diomus* sp. beetles existing farther from nest than *A. orbigera* beetles according to trial permutation test (resampling with replacement).

	Survey 1			Survey 2		
Comparison	*A. orbigera*	*Diomus* sp.	P	*A. orbigera*	*Diomus* sp.	P
A. orbigera adults-*Diomus* sp. adults	1.91	2.91	**0.0027**	2.00	2.80	**0.0085**
A. orbigera larvae-*Diomus* sp. larvae	3.32	3.39	0.4593	5.34	4.68	0.2373
A. orbigera adults-*Diomus* sp. larvae	2.46	3.38	0.1181	3.20	6.94	**0.0004**
A. orbigera larvae-*Diomus* sp. adults	2.27	2.89	**0.0095**	1.97	2.05	0.2524

Table 1.2. Posterior distributions of Poisson regression models with 95% credibility intervals for *A. orbigera* and *Diomus* sp. adults and larvae. Means are considered significant if credibility intervals do not cross zero (significant values in bold).

	A. orbigera adults			*A. orbigera* larvae			*Diomus* sp. adults			*Diomus* sp. larvae		
Parameter	β_{mean}	2.5%	97.5%	β_{mean}	2.5%	97.5%	β_{mean}	2.5%	97.5%	β_{mean}	2.5%	97.5%
A. orbigera adults	-	-	-	-	-	-	-	-	-	**-1.1290**	**-2.3290**	**-0.1228**
A. orbigera larvae	-	-	-	-	-	-	-	-	-	**-1.3750**	**-3.1370**	**-0.2085**
Diomus sp. adults	-	-	-	-	-	-	-	-	-	-	-	-
Diomus sp. larvae	-	-	-	-	-	-	-	-	-	-	-	-
Arboreal ants[a]	**-0.0082**	**-0.0200**	**0.0024**	**-0.0284**	**-0.0372**	**-0.0201**	**0.0153**	**0.0082**	**0.0224**	**0.0150**	**0.0051**	**0.0249**
Azteca sericeasur	**0.0107**	**0.0033**	**0.0183**	**0.0173**	**0.0053**	**0.0276**	0.0031	-0.0091	0.0139	**-0.2397**	**-0.4324**	**-0.0850**
Coccus viridis (scales)	**0.0004**	**0.0002**	**0.0005**	-0.0002	-0.0005	0.0001	0.0000	-0.0008	0.0022	0.0041	-0.0003	0.0087
Time	-	-	-	-	-	-	-	-	-	**1.5090**	**0.9991**	**2.0750**

[a] Arboreal ant category does not include *Azteca sericeasur*

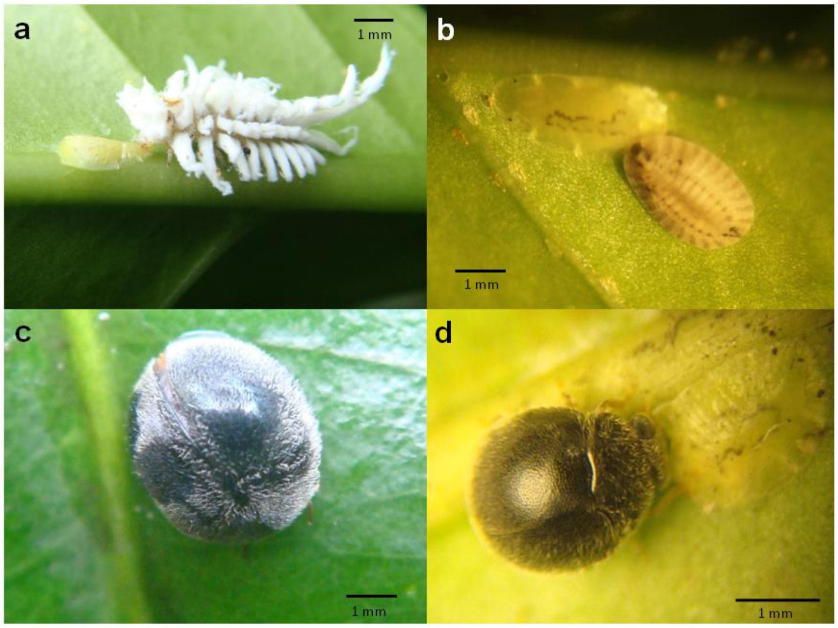

Figure 1.1. Images of larval a) *Azya orbigera* and (b) *Diomus* sp. feeding on mature *C. viridis*, as well as adult (c) *A. orbigera* and (d) *Diomus* sp. Photo credits: Ivette Perfecto (a) and (c); H. Liere (b); A. Iverson (d)

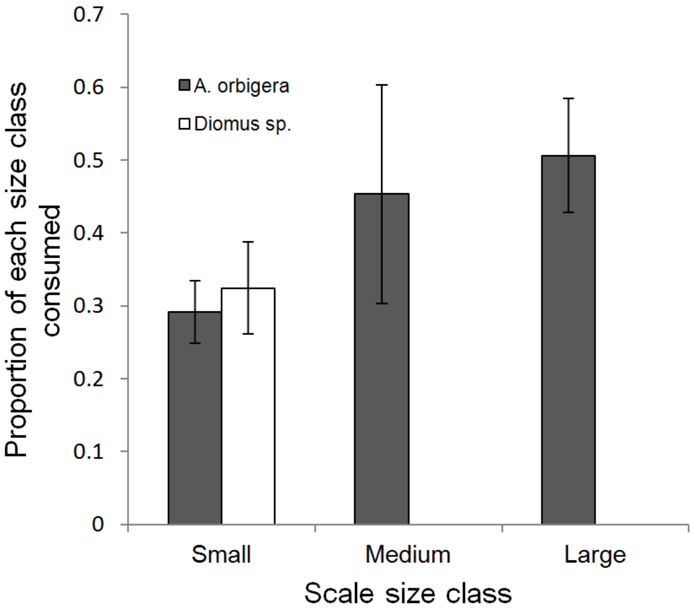

Figure 1.2. Proportion (mean ± SE) of each *Coccus viridis* size class eaten by *A. orbigera* and *Diomus* sp. adults when presented with all size categories. Tukey's HSD test: small-medium, N.S.; small-large, P=0.063. N= 21 for *Diomus* sp., 25 for *A. orbigera*

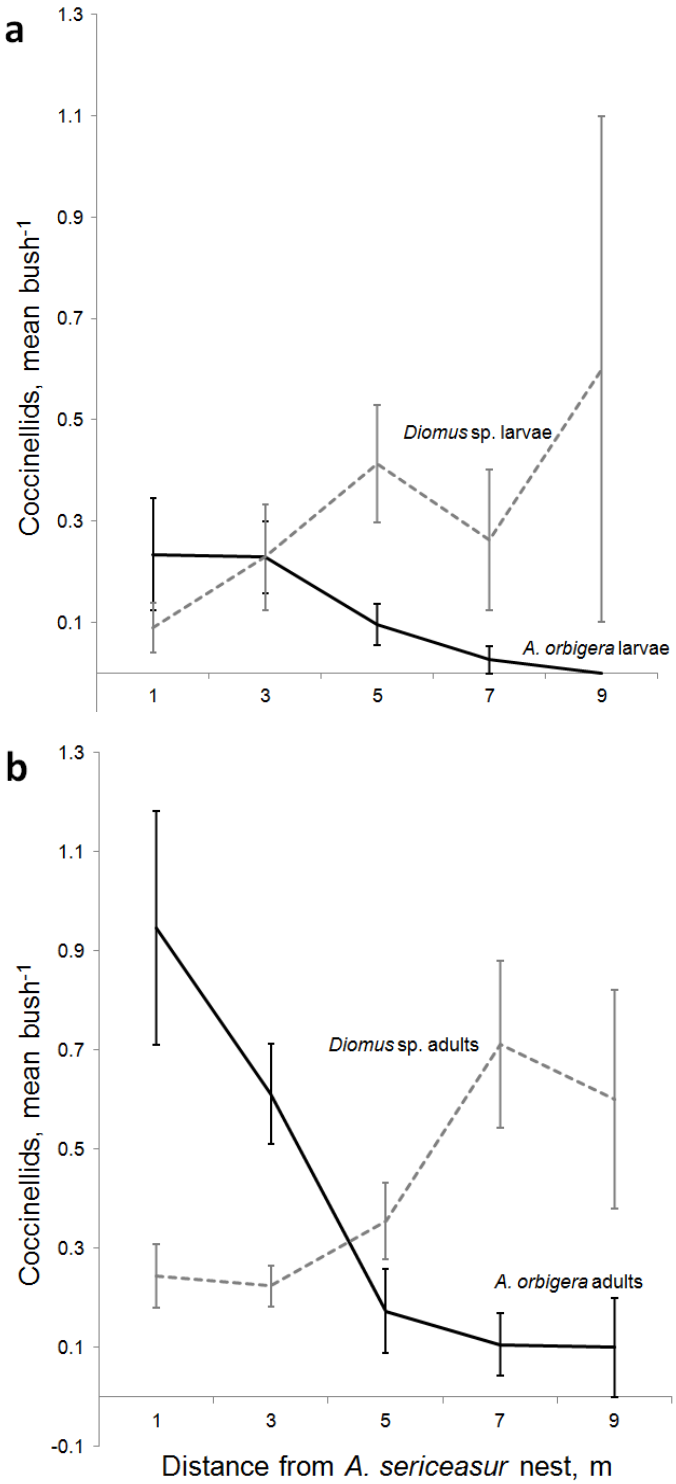

Figure 1.3. Number (mean ± SE) of *Azya orbigera* and *Diomus* sp. (a) larvae and (b) adults per coffee bush at increasing distances from *Azteca* ant nests. Linear regressions: *A. orbigera* adults and larvae significantly negative (P=0.0010 and P=0.034, respectively); *Diomus* sp. larvae significantly positive (P=0.020); *Diomus* sp. adults, N.S. (P=0.12). N=73 *A. orbigera* larvae, 179 *A. orbigera* adults, 118 *Diomus* sp. larvae, 130 *Diomus* sp. adults

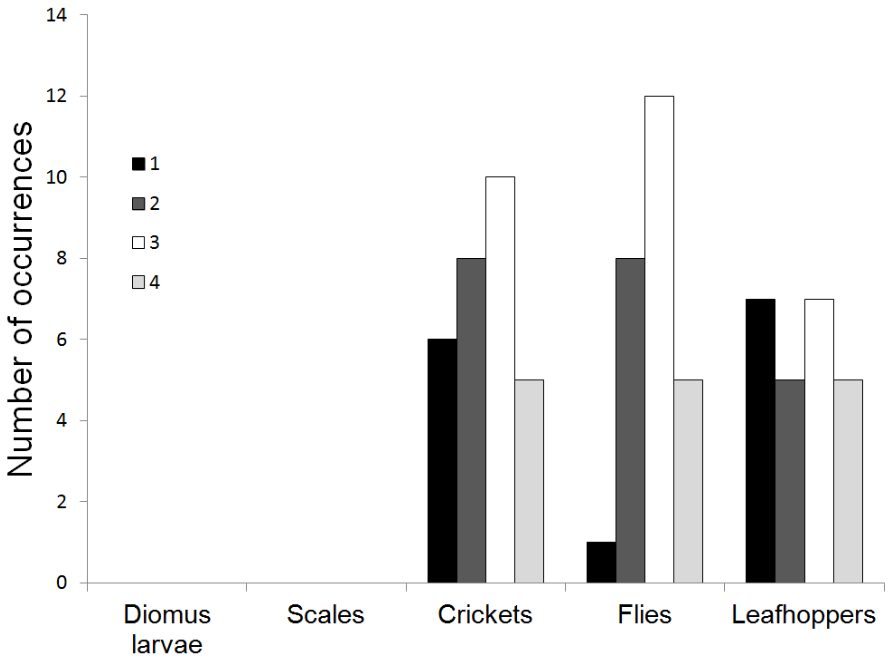

Figure 1.4. *Azteca* aggression towards different insect groups. Aggression levels are as follows: 1) tapped antennae on insect, 2) pinched with mandibles, 3) grabbed insect and carried for short distance (within petri dish), 4) removed insect from petri dish. Values are recorded as total occurrences in N=5 replicates

Supplementary material

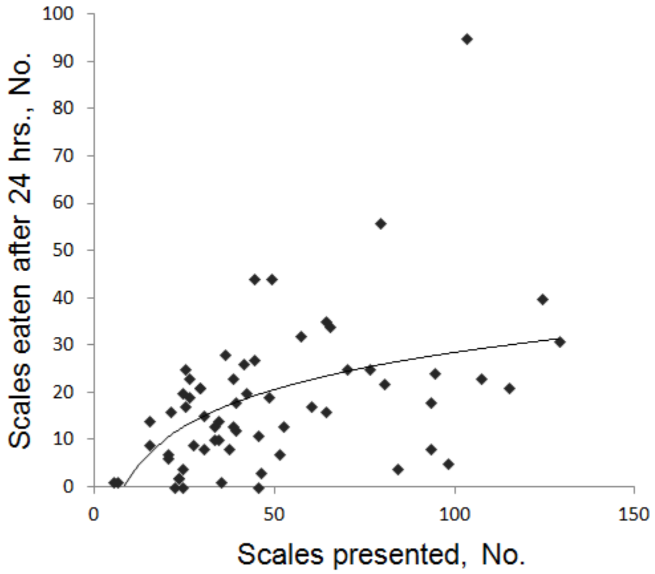

Figure S1.1 Functional response of *Diomus* sp. adults: Number of small *Coccus viridis* consumed after 24h.

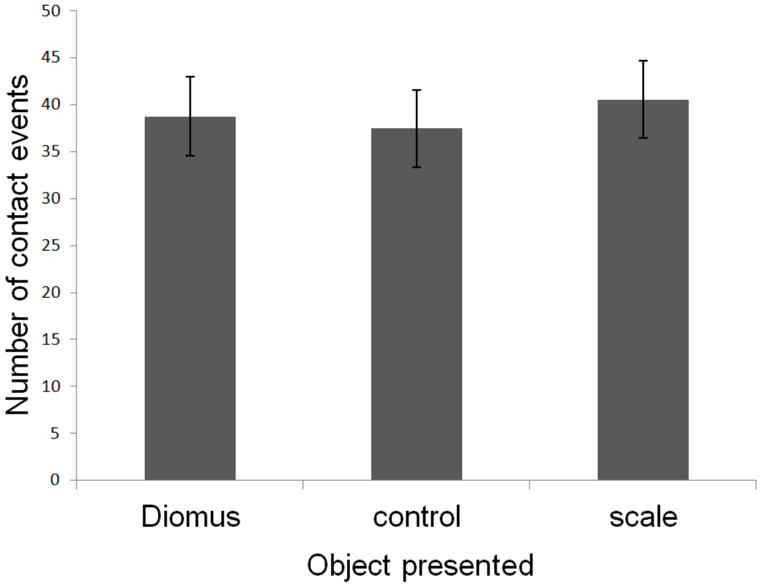

Figure S1.2. Number of times *Azteca sericeasur* ants passed over *Diomus* sp. larvae, *Coccus viridis*, or a control dot when placed/drawn on a petri dish. One-way ANOVA=N.S.

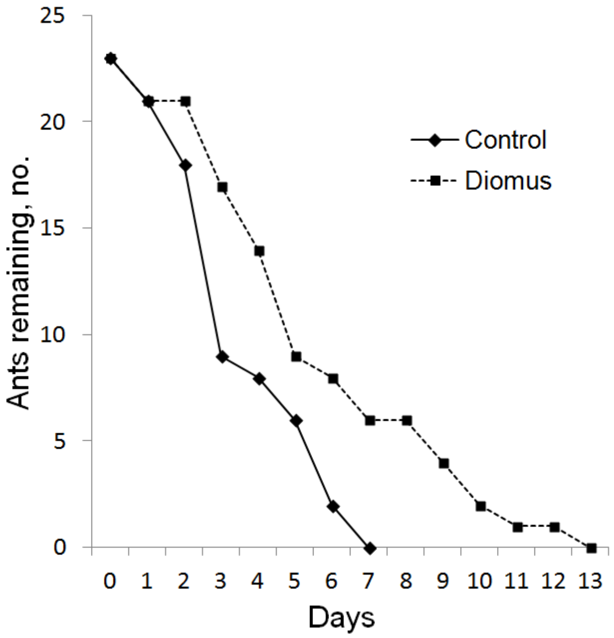

Figure S1.3. Death rate in *Azteca sericeasur* while being fed a sugar solution (control) or a sugar solution containing smashed *Diomus* sp. larvae.

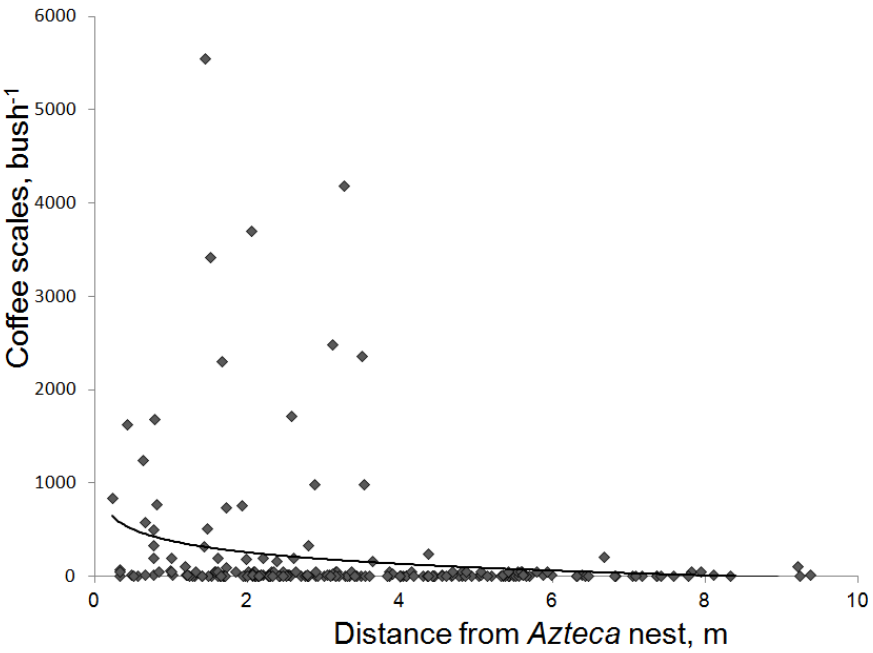

Figure S1.4. Number of *Coccus viridis* as a function of distance from an *Azteca* nest. r^2=0.0321, P=0.005.

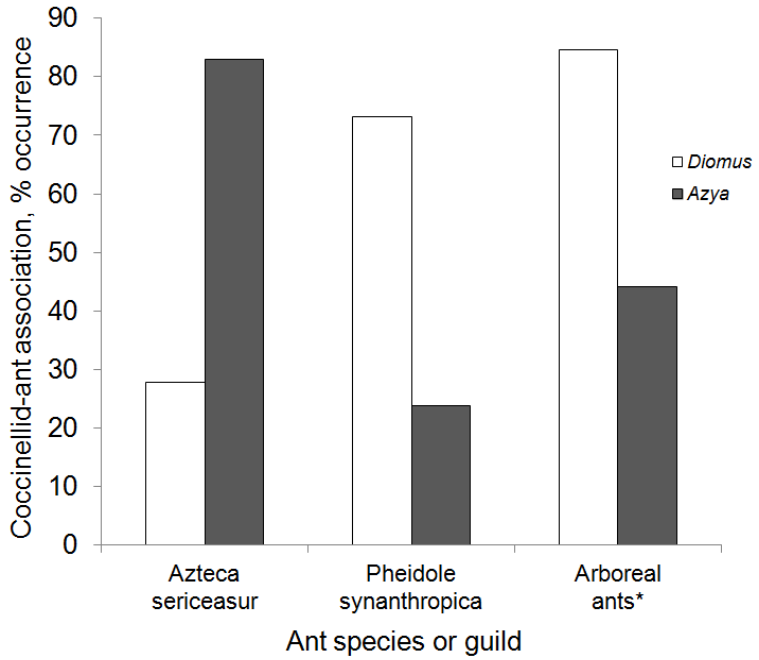

Figure S1.5. Overall percent of *Azya orbigera* and *Diomus* sp. beetles recorded on same coffee bush as *Azteca sericeasur*, *Pheidole synanthropica*, or any arboreal ant. *excluding *A. sericeasur* but including *P. synanthropica*.

CHAPTER II

Is local or landscape-scale heterogeneity more beneficial to arthropod natural enemies?[2]

Abstract

Context. Arthropod natural enemies play an important role in controlling herbivore pests in agroecosystems. Naturally occurring predator and parasitoid populations are well known to respond to multiple scales of environmental heterogeneity, yet our understanding of which scales are most important for different functional guilds of enemies is limited.

Objectives. We sought to determine how natural enemies (collectively and by guild) differentially respond to changes in local-, intermediate-, and landscape-scale heterogeneity.

Methods. We synthesized the results from 40 empirical studies that observed how natural enemy richness, diversity, or parasitism rate are affected by environmental heterogeneity. Using a vote-tally method we recorded the number of positive, negative, and neutral responses of natural enemies to heterogeneity at three scales.

Results. Heterogeneity at all scales was important, and overall we observed a 14-fold higher number of positive responses to heterogeneity than negative responses. However, natural enemy diversity and abundance responded most strongly to broader-scale, and particularly landscape-level, heterogeneity. When three different natural enemy guilds were considered separately, parasitoids and especially plant-foraging predators more often benefitted from environmental heterogeneity than ground-foraging predators.

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[2] Co-authors are Burnham, R. & Vandermeer, J.

Conclusions. These results highlight the importance of maintaining environmental heterogeneity especially in the areas immediately surrounding farm fields and beyond to augment the natural enemy community. Both regional management programs or incentives and collective individual landholder decisions, which can scale up to create higher-level heterogeneity, would likely be effective for maximizing natural enemy populations at scales larger than individual farms.

Keywords: agroecosystem, arthropod guild, management, parasitoid, predator

Introduction

The push towards agricultural intensification over the past several decades has resulted in simplified cropping systems and landscapes, as is highlighted by an increase in monocultures and a decrease or degradation of natural and semi-natural habitats in surrounding areas. This environmental simplification is generally found to result in compromised ecosystem services, such as decreased pollination, eroded soil nutrient and water supplies, and diminished pest control (Millenium Ecosystem Assessment 2005; Tscharntke *et al.* 2005; Foley *et al.* 2011). With pest control, environmental simplification can decrease the abundance, diversity, or effectiveness of natural enemies of crop pests (Root 1973; Andow 1991; Perfecto *et al.* 2003; Bianchi, Booij & Tscharntke 2006; Vandermeer & Perfecto 2007; Letourneau *et al.* 2011). This deterioration of the natural enemy community may push farmers to be increasingly reliant on synthetic pesticides. However, a growing demand for organic produce, coupled with increasing costs of synthetic inputs, generates an increasing interest in controlling pests through biological control (Lewis *et al.* 1997; Landis, Wratten & Gurr 2000; Simon *et al.* 2010).

Understanding how natural enemies are influenced by their environment is crucial for developing strategies to augment the efficiency of biological control. Through much research over especially the past two decades, we have come to appreciate that heterogeneity at both a local and landscape scale in agricultural areas commonly correlates with higher levels of natural enemies (Andow 1991; Bengtsson, Ahnstrom & Weibull 2005; Bianchi, Booij & Tscharntke 2006; Letourneau *et al.* 2011; Chaplin-Kramer *et al.* 2011). The importance of landscape heterogeneity on biological control is evident, as the home range of many arthropod individuals extends far beyond the scale of a crop field (Tscharntke *et al.* 2007; Rusch *et al.* 2010), and may reach up to several kilometers (Roschewitz *et al.* 2005). Studies investigating the role of landscape heterogeneity often focus on the habitat composition extending from a couple hundred meters to several kilometers (e.g., percent of non-crop area within a given radius of a study field). Many studies point toward the importance of providing natural, semi-natural, or perennial habitat that natural enemies can inhabit when conditions in the agricultural area make survival difficult (Thies & Tscharntke 1999; Clough *et al.* 2005; Attwood *et al.* 2008; Rusch *et al.* 2010). These refuges--forests, hedgerows, field margins, fallows, meadows, or wetlands—may function as source habitats for predator or parasitoid populations and provide alternative resources (e.g. prey, pollen, nectar), permanent vegetation for reproduction and overwintering, or protection during disturbances (Rusch *et al.* 2010; Morandin & Kremen 2013). Landscapes with more natural/semi-natural areas may also provide benefits in terms of connectivity, allowing organisms a conduit for migration (Benton, Vickery & Wilson 2003).

Similarly, local (within-field) heterogeneity has repeatedly been shown to positively influence the natural enemy community in agroecosystems (Andow 1991; Simon *et al.* 2010; Letourneau *et al.* 2011; Iverson *et al.* 2014). Studies of local heterogeneity usually compare fields of different planned (e.g., crop species) or sometimes associated (i.e. weeds) diversity, such as monocultural cropping vs. polycultural cropping. Two dominant theories are postulated to help explain why pest regulation in agroecosystems often results in areas of higher local floristic diversity: the resource concentration hypothesis and the natural enemies hypothesis (Root 1973). The resource concentration hypothesis proposes that specialized herbivores will be better able to locate their host plant and persist in areas where their food source is concentrated (i.e., monocultures) compared to polycultures, where they will be less efficient at locating acceptable food plants (Root 1973). This phenomenon results from one or more different mechanisms: (1) polycultures may cause chemical interference by collectively containing more plant volatiles which confuse or repel herbivores relying on olfactory cues in their search for host plant species; (2) herbivores may be visually confused when navigating through multiple plant species to reach their host; (3) a difference in host quality between polyculture and monoculture systems may result from changes in inter-plant competition; (4) the increased amount of non-host surface area in polycultures may inhibit herbivores through increasing search times for locating host plants; and (5) abiotic factors, such as differences in shade, humidity, wind, and mid-day temperatures between the two cultural practices (Andow 1991). Although these same mechanisms could also decrease the efficiency of natural enemies, evidence suggests natural enemies may not be as inhibited, and some even have enhanced search efficiencies in polycultures (Perfecto & Vet 2003).

The natural enemies hypothesis (Root 1973) proposes that natural enemies will be present in higher numbers in more complex habitats via at least two mechanisms. First, complex habitats will likely host a greater diversity of prey due to a greater diversity of host plants and microhabitats. Second, complex habitats offer other food resources, such as nectar and pollen, which are especially important for enemies (e.g., parasitoid wasps) whose different life stages require different foods. Both of these mechanisms result in increased temporal stability and availability of resources for the natural enemies.

It is worth noting that the alternative proposed mechanisms fall into a long-standing framing of the trophic process and whether herbivores are limited by the trophic level below them (bottom-up regulation), above-them (top-down regulation), or simply from abiotic factors. Because higher densities or biomass of potential energy sources are thought to support higher densities of natural enemies that, in turn, attack the pests, the situation is one of a trophic cascade. Herbivores, some of which are pests, eat the plants in the system, those herbivores are eaten by the predators, some of which are natural enemies of the pests, with energy streaming from the plants to the herbivores to the predators. Contrarily, if volatiles from an intercropped species attract parasitoids and predators, or if the presence of one crop reduces the foraging efficiency of a potential natural enemy, some trait of that natural enemy has been altered. Thus, the natural enemies hypothesis winds up fitting nicely into the contemporary category of density-mediated indirect interaction (trophic cascade) (Abrams 1995), while the resource concentration hypothesis fits within the confines of the category of trait-mediated indirect interaction (Abrams 1995). Inevitably, no single factor is likely the sole player in predicting arthropod population densities across space and time, and likely there are additive, synergetic, or even neutralizing interactions among them (Russell 1989).

The response of arthropods to environmental simplification at different scales undoubtedly varies by organism, and depends on the organism’s trophic position and dispersal ability, which are often a function of body size (Tscharntke *et al.* 2005; Gabriel *et al.* 2010; Gonthier *et al.* 2014). Higher trophic-level organisms, and especially specialists (i.e., many parasitoids), are often more susceptible to habitat fragmentation than herbivorous pests (Kruess & Tscharntke 2000; Tscharntke *et al.* 2005; Klein, Steffan-Dewenter & Tscharntke 2006). Natural enemy species that have high dispersal potential, such as ballooning spiders, might be less influenced by local habitat heterogeneity and more influenced by landscape heterogeneity (Schmidt & Tscharntke 2005; Clough *et al.* 2005). Many parasitoids, on the other hand, may be particularly sensitive to local heterogeneity due to their often limited dispersal abilities and narrow host ranges (van Nouhuys 2005; Shaw 2006). Furthermore, the effect of scale may be highly context-dependent, where the interaction between local and landscape heterogeneity is important, such as when local heterogeneity is more important for organisms in simple rather than in complex landscapes (Thies & Tscharntke 1999; Tscharntke *et al.* 2005; Gabriel *et al.* 2010; Geiger *et al.* 2010; Winqvist *et al.* 2011; Batáry *et al.* 2011; Concepción *et al.* 2012; Tuck *et al.* 2014).

In the present review, we synthesize the work of 40 studies to determine how three scales of environmental heterogeneity differentially affect natural enemy diversity, abundance, and parasitism rate. We also explore whether the response to scale varies depending on the natural enemy functional guild.

Methods

Literature search and study selection

In August 2010 we extracted publications from Google Scholar using combinations of the following keywords: agroecosystem, agriculture, biodiversity, local, landscape, management, intensification, natural enemies, predator, parasitoid, parasitism, scale, biocontrol, and biological control. We selected only studies that consisted of field experiments or surveys and that investigated how natural enemy abundance, richness, diversity, size, or parasitism rate differed between agricultural areas of differing management intensities at a local, intermediate, or landscape scale. Our search yielded 40 studies (Kruess & Tscharntke 1994, 2000; Marino & Landis 1996; Murphy, Rosenheim & Granett 1996; Bommarco 1998; Elliott *et al.* 1998; Murphy *et al.* 1998; Menalled *et al.* 1999, 2003; Carmona & Landis 1999; Thies & Tscharntke 1999, 2010; Nicholls, Parrella & Altieri 2001; Ostman *et al.* 2001; Elliott, Kieckhefer & Michels 2002; Armbrecht & Perfecto 2003; Kruess 2003; Thies, Steffan-dewenter & Tscharntke 2003; Weibull, Ostman & Granqvist 2003; Harmon *et al.* 2003; Pfiffner & Wyss 2004; Tylianakis, Didham & Wratten 2004; Costamagna, Menalled & Landis 2004; Prasifka, Heinz & Minzenmayer 2004; Purtauf, Dauber & Wolters 2005; Thies, Roschewitz & Tscharntke 2005; Schmidt & Tscharntke 2005; Schmidt *et al.* 2005; Bianchi *et al.* 2005; Purtauf *et al.* 2005; Roschewitz *et al.* 2005; Clough *et al.* 2005; Klein, Steffan-Dewenter & Tscharntke 2006; Wilby *et al.* 2006; Gianoli *et al.* 2006; Aroga & Ambassa-Kiki 2007; Cai, Li & You 2007; Gardiner *et al.* 2009; Meyer, Jauker & Steffan-Dewenter 2009).

Data compilation

Multiple observations were possible within a given study. If a study assessed multiple metrics (e.g., both richness and abundance) for a single species or a single group, each metric was considered as a separate observation. If a study considered multiple scales, only one observation was recorded for each of our three scale categories (see below) per natural enemy metric. For example, if a study calculated landscape diversity at 1km, 2km, and 3km radii, all of which fit into our category of ‘landscape diversity’, we distilled the information into one observation. To do so, if the response to at least one scale was positive and there were no negative responses, we recorded the observation as positive. If all responses were neutral or if there were discordant responses (positive and negative), we recorded the observation as neutral. We did the same if there were multiple measures at the same scale (e.g. percent non-crop area and landscape diversity at 1km). If studies reported natural enemy responses for individual species and for larger groupings (e.g. by guild or for all natural enemies), we used the most inclusive grouping available.

We grouped observations into three distinct environmental heterogeneity scales: local, intermediate, and landscape. Local-scale heterogeneity was characterized by within-field differences among planned or associated diversity of plants. Most often, these studies compared monoculture to polyculture cropping systems, but some included weed diversity (Purtauf *et al.* 2005; Roschewitz *et al.* 2005; Clough *et al.* 2005). Intermediate-scale heterogeneity pertained to the immediate surroundings of a field, such as proximity to field edges, presence of refuge strips, or field perimeter-to-area ratios. For example, fields with higher perimeter-to-area ratios had relatively more field margins per unit area, and were considered more complex. Landscape-scale heterogeneity pertained to regions incorporating multiple fields and/or habitat patches or larger (minimum 200m radius but up to 6km radius from sampling location). More heterogeneous landscapes had a greater diversity of habitat types or a larger proportion of non-crop area.

We then categorized the observations according to the functional guild of the natural enemy, permitting comparisons of the relative importance of environmental heterogeneity to each functional guild. In one analysis, we coarsely divided the observations into parasitoids or predators. In another analysis, we further divided the predators into either ground-foraging species or plant-foraging species (Appendix 2.1). Although most species of natural enemies are capable of foraging on both the ground and on plants, we separated them by the habitat in which they spend the majority of their foraging time (if known), or by where they were captured in the study. Plant-foraging species included primarily enemies that are strong fliers (e.g., coccinellid beetles, pompilid and sphecid wasps, and most beneficial insects in the orders Neuroptera and Hemiptera) or species that are almost exclusively plant-dwelling (e.g., syrphid fly larvae). Ground-foraging species included primarily ground-foraging ants (Formicidae), ground-foraging beetles (Carabidae and Staphylinidae), and ground-dwelling spiders (Araneae). Although they are often plant-foragers too, spiders were grouped as ground-foraging because nearly all researchers in our included studies collected these using ground-based pitfall traps (Weibull, Ostman & Granqvist 2003; Harmon *et al.* 2003; Pfiffner & Wyss 2004; Schmidt *et al.* 2005; Clough *et al.* 2005). The category of parasitoids included several families of Hymenoptera and, to a lesser extent, Diptera.

Data analysis

Each observation was recorded as positive, negative, or neutral depending on whether natural enemy diversity, abundance, size, or parasitism rate significantly ($p<0.05$) increased (positive), decreased (negative), or showed no significant effect (neutral) in the more heterogeneous environment. Using these tallies, we calculated the effect on the natural enemy community of 1) environmental heterogeneity (all three scales combined), 2) scale of heterogeneity (each scale considered separately), and 3) functional guild of natural enemy. Furthermore, we observed if the type natural enemy metric (i.e., abundance, diversity, or parasitism rate) affected these outcomes. We grouped the metrics of species richness and diversity indices under the category ‘diversity’. Therefore, when we mention natural enemy ‘diversity’ throughout the paper it includes species richness. Only two studies compared the sizes of the natural enemies (Bommarco 1998; Östman, Ekbom & Bengtsson 2001), so we included these observations in the ‘abundance’ category. Functional guilds were first separated into predator or parasitoid, and later into parasitoid, ground-foraging predator, or plant-foraging predator. For all analyses, we determined whether the observed frequency of positive responses compared to the combined neutral and negative responses was significantly different from the expectation of a binomial distribution.

Results

Benefits of environmental heterogeneity: Overall effect and effect by scale

Our literature search yielded 40 pertinent studies and 130 observations. Overall, we found that the number of positive responses (54.6% of observations) by natural enemies to a heterogeneous environment with all scales combined far outweighed the number of negative responses (3.8% of observations; Table 2.1, Fig 2.1). However, the number of neutral responses was also relatively large (41.5% of all observations). The extent of the benefit of heterogeneity on natural enemies depended on the scale at which the heterogeneity was observed, and only at the landscape scale were the positive responses (61.9%) of natural enemies significantly larger than the combined neutral (34.9%) and negative responses (3.2%; Table 2.1, Fig 2.1). Intermediate-scale heterogeneity still had a majority (55.6%) of observations returning a positive response, while local-scale heterogeneity had the lowest at 38.7% positive responses.

Effect by natural enemy guild

When we subdivided results by natural enemy guild, we found that positive outcomes from increased heterogeneity were also always significantly greater than negative outcomes for all guilds. However, the positive outcomes were only significantly greater than the combined neutral and negative effects for plant-foraging predators at intermediate and landscape scales and for parasitoids at a landscape scale (Table 2.1, Fig 2.1). No guild showed a significant positive response at the local scale.

Effect by natural enemy metric

When the results were dissected according to the reporting metric (i.e., abundance, diversity, or parasitism rate), parasitism rate at a landscape scale was the only metric where positive outcomes (68.2%) were significantly higher than neutral and negative outcomes combined, although species diversity was marginally significant (P=0.055) at a landscape scale.

Discussion

Overall response to environmental heterogeneity

In this synthesis, we show that the natural enemy community consistently benefits from environmental heterogeneity within and around agroecosystems. In all scale and natural enemy functional guild categories, we observed a much greater number of positive than negative responses (on average by a factor of 14). We thus corroborate the growing body of evidence showing beneficial responses of natural enemy communities from environmental heterogeneity at multiple scales (Landscape scale: Bianchi, Booij & Tscharntke 2006; Chaplin-Kramer *et al.* 2011. Local scale: Andow 1991; Langellotto & Denno 2004; Poveda, Gomez & Martinez 2008; Simon *et al.* 2010; Letourneau *et al.* 2011. Both scales: Gonthier *et al.* 2014).

Scales of heterogeneity

Although positive responses far outweighed negative responses at all scales, beneficial effects were especially pronounced at landscape scales (e.g. heterogeneity at >200m radius from sample plot). These results provide support for a density-mediated mechanism (the enemies hypothesis), i.e. a top-down mechanism of control (Hairston, Smith & Slobodkin 1960). Here, natural enemies likely benefit from increased food and habitat resources in non-crop areas surrounding farm fields. However, trait-mediated effects (the resource concentration hypothesis) often functions simultaneously and complementarily to the enemies hypothesis and is likely still an important factor in many of these studies (Russell 1989). These results suggest that broad-scale heterogeneity is extremely valuable in maintaining natural enemy populations, supporting the idea that many arthropod species interact with their environment at a larger-than-local level (Thies & Tscharntke 1999), with important implications regarding regional planning and management processes.

Although the effects of local heterogeneity can often be as important or more important than effects of landscape heterogeneity in agroecosystems (Puech *et al.* 2014), our results support the findings of other vote-tally reviews that have observed single scales, where higher positive responses of natural enemies to environmental heterogeneity appear to be found at landscape scales. For instance, Bianchi, Booij & Tscharntke (2006) found that 74% of their observations returned a positive response to landscape heterogeneity, whereas other studies of local heterogeneity showed positive responses to (surprisingly consistently) barely exceed 50% of all responses for natural enemies (52.7% in Andow 1991; 52% in Poveda, Gomez & Martinez 2008; 53.3% in Simon *et al.* 2010).

Arthropod guilds and environmental heterogeneity

The significantly positive responses to increasing broad-scale heterogeneity observed in the parasitoid and especially plant-foraging predator communities may reflect their particular sensitivity to environmental disturbance at these scales. Parasitoids and many plant-foraging predators, such as syrphid flies, predatory wasps, and some predatory beetles, are reliant on alternative food sources, such as pollen and nectar, at some point in their life cycles (Langellotto

& Denno 2004). Although some crops or weeds within crops may provide these resources, they are often most abundant in non-crop areas. Furthermore, the small size and high prevalence of prey specialization in parasitoids may also contribute to lower dispersal abilities and increased sensitivity to environmental heterogeneity (Roland & Taylor 1997).

On the other hand, ground-foraging predators, such as ground spiders and carabid beetles, are often less reliant on floral resources and rather are particularly sensitive to soil management practices (e.g., tilling), which may mask any differences in vegetation diversity or structure. Many of these species rely on high-quality soil habitats for protection (e.g., overwintering) or for oviposition (Rusch *et al.*, 2010). For instance, Langellotto and Denno (2004) observed a large impact, especially on spiders, from enhancing the structural complexity of soil detritus. Other studies have shown that structural diversity, rather than vegetation species diversity, in the landscape physically inhibits carabid movement between fields (Frampton *et al.* 1995; Mauremooto *et al.* 1995). Additionally, some spiders are able to avoid size-dispersal limitations through long-distance windborne dispersal (ballooning), which may allow them to be less affected by intensively managed landscapes (Weyman, Sunderland & Jepson 2002).

When the metrics of parasitism rate, abundance, and diversity were considered independently, parasitism rate at a landscape scale was the only category where observations of enemies benefitting from heterogeneity were significantly higher than the combined neutral and negative observations. These results may again reflect how the small and specialized parasitoids may be more sensitive to environmental disturbance than other natural enemies (see above).

Management implications

The response of natural enemies to environmental heterogeneity is undoubtedly context-dependent. However, the strong trends apparent from our review support the value of increasing environmental heterogeneity for promoting natural enemy presence in agroecosystems. Our results suggest that individual farmers can enhance the local natural enemy community through increases in within-field diversity (e.g., polycultures), but more often through improvements in broader-scale, and especially landscape-scale, heterogeneity. Intermediate-scale enhancement may be provided, for example, by increasing the size of field margins, decreasing the size of fields, or including vegetation strips within fields. These vegetation strips may be especially effective if specific plants that provide resources for natural enemies but do not simultaneously attract pests are included (Pfiffner & Wyss 2004). Furthermore, vegetation strips or field margins offer additional benefits, such as pollination, erosion control, or biodiversity conservation (Wratten *et al.* 2012; Morandin & Kremen 2013).

The benefit of landscape-scale heterogeneity emphasizes the importance of region-wide land management or collective, community-scale planning initiatives. Currently, many management suggestions that seek to enhance biological control have focused solely on increasing local diversity (Gurr, Wratten & Barbosa 2000). We suggest, therefore, that governments and organizations should not only encourage farmers to make local-scale changes for biodiversity enhancement but should provide incentives for individual landholders and communities to make landscape-level management decisions that will positively impact biodiversity (*sensu* Tscharntke *et al.* 2007). Government-supported or certification-based (*sensu* Tscharntke *et al.* 2014) economic incentives could play an important role in promoting landscape heterogeneity. Although planning at large geographic scales is challenging, additive effects are common, where the land-use changes of individual farmers scale up to landscape-level effects (Holzschuh, Steffan-Dewenter & Tscharntke 2008; Gabriel *et al.* 2010).

To some degree, pest management could focus on the most detrimental type of pest present in a given agroecosystem. For example, if the dominant pest spends at least part of its life cycle in the soil, ground-foraging predators, such as ground spiders and carabid beetles may be the most effective biocontrol agents. As these predators appear to respond less to the environmental heterogeneity studied here and likely more to direct soil management, it may be important to vary a farm's cultural techniques to optimize the survival and growth of the predators. However, caution must be exercised to avoid a "one-problem, one-species" approach, as it is clear that the consortium of natural enemies is important for biological control given the inherent complexities of food webs in even simplified agroecosystems (Altieri 1999; Tscharntke *et al.* 2007; Vandermeer, Perfecto & Philpott 2010).

Limitations and further research

Enhancements in the natural enemy community may not necessarily translate into enhanced crop health (Symondson, Sunderland & Greenstone 2002). However, although we did not include the effect of environmental heterogeneity on direct biocontrol, crop yield, or pest abundance, other studies have shown that natural enemy species often respond more strongly to heterogeneity than do pest species (Langellotto & Denno 2004; Chaplin-Kramer *et al.* 2011). Furthermore, Risch, Andow & Altieri (1983) show in a review of 150 studies that herbivores respond in an opposite manner to local heterogeneity, where 53% of herbivore species were significantly less abundant on more diverse farms. These studies suggest that an improved natural enemy community would translate into improved crop health.

Further research is needed to clarify the complex ecological interactions that underpin effective biocontrol and the influence of spatial scale. This is especially important in tropical agroecosystems, as the vast majority of studies carried out to date involve temperate cereal crops

(Matson 1997). Additionally, research on biocontrol should report the dispersal abilities, if known, of each of the organisms studied, allowing for a clearer consensus on the role of dispersal in an organism's sensitivity to environmental heterogeneity. Also of critical importance are the growing number of studies noting indirect effects, both density- and trait-mediated (Werner & Peacor 2003), and the potential effects of land management, as well as intrinsic (self-organized) factors, in structuring them (Vandermeer & Perfecto 2008; Hsieh *et al.* 2012; Liere, Perfecto & Vandermeer 2014). We furthermore urge researchers to include yield or net profit and other relevant data (e.g., potential for sustainability) in their analyses.

Conclusions

The simplification of agricultural lands threatens the health of many of the human-inhabited ecosystems worldwide, while future food-demand projections predict agricultural land area and intensity to increase (Ramankutty *et al.* 2008; Tilman *et al.* 2011). While other studies note that this projected demand may be satisfied through an improved socio-political structure (IAASTD 2008), it is nevertheless critical to understand how we can plan and implement agroecosystems that provide important services, such as pest control, with an eye on reducing reliance on pesticides. Boosting natural enemy populations is one way to achieve this goal. We found that environmental heterogeneity especially at broader (i.e. intermediate and particularly landscape) scales, is important for increasing the diversity and abundance of natural enemies. Both individual and collective land planning and management will likely be important for maximizing potential biocontrol services from natural enemies.

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Table 2.1. Proportion positive, neutral, and negative responses of natural enemies (including separate guilds and reporting metrics) to increasing environmental heterogeneity.

	Positive	Neutral	Negative	P[a]	N
All scales combined					
All natural enemies combined	0.55	0.42	0.04	0.127	130
Parasitoids	0.58	0.40	0.02	0.084	53
Predators combined	0.52	0.43	0.05	0.324	77
Ground-foraging predators	0.38	0.55	0.08	0.923	40
Plant-foraging predators	0.67	0.30	0.03	**0.018**	33
Species diversity	0.57	0.37	0.07	0.181	30
Species abundance	0.52	0.44	0.05	0.356	66
Parasitism rate	0.59	0.41	0.00	0.115	34
Landscape scale					
All natural enemies combined	0.62	0.35	0.03	**0.021**	63
Parasitoids	0.66	0.34	0.00	**0.031**	29
Predators combined	0.59	0.35	0.06	0.115	34
Ground-foraging predators	0.47	0.47	0.07	0.500	15
Plant-foraging predators	0.72	0.22	0.06	**0.015**	18
Species diversity	0.70	0.20	0.10	0.055	10
Species abundance	0.55	0.42	0.03	0.237	31
Parasitism rate	0.68	0.32	0.00	**0.026**	22
Intermediate scale					
All natural enemies combined	0.56	0.39	0.06	0.203	36
Parasitoids	0.50	0.43	0.07	0.395	14
Predators combined	0.59	0.36	0.05	0.143	22
Ground-foraging predators	0.42	0.50	0.08	0.613	12
Plant-foraging predators	0.80	0.20	0.00	**0.011**	10
Species diversity	0.60	0.30	0.10	0.172	10
Species abundance	0.58	0.37	0.05	0.180	19
Parasitism rate	0.43	0.57	0.00	0.500	7
Local scale					
All natural enemies combined	0.39	0.58	0.03	0.859	31
Parasitoids	0.50	0.50	0.00	0.377	10
Predators combined	0.33	0.62	0.05	0.905	21
Ground-foraging predators	0.23	0.69	0.08	0.954	13
Plant-foraging predators	0.20	0.80	0.00	0.813	5
Species diversity	0.40	0.60	0.00	0.623	10
Species abundance	0.38	0.56	0.06	0.773	16
Parasitism rate	0.40	0.60	0.00	0.500	5

[a] bold refers to values where the frequency of positive responses compared to the combined neutral and negative responses was significantly different from a binomial distribution ($P<0.05$).

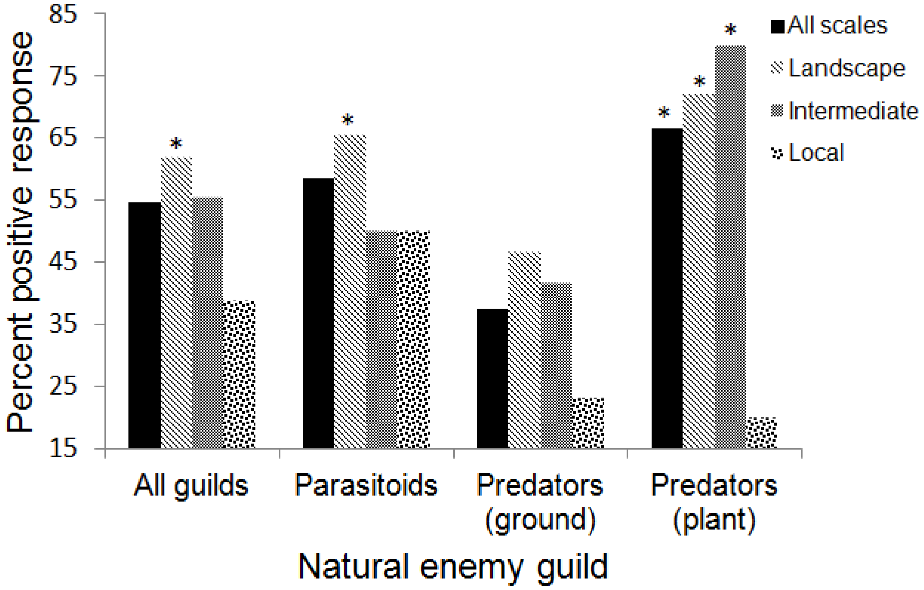

Figure 2.1. Percent of all observations returning a positive response of natural enemy abundance or diversity as a result of environmental heterogeneity at local, intermediate, and landscape scales, as well as at all scales combined. *refers to values where the frequency of positive responses compared to the combined neutral and negative responses was significantly different from a binomial distribution ($P<0.05$).

Appendix 2.1. Summary of included articles including natural enemy groups, guild types, and number of observations showing a positive, neutral, or negative response to environmental heterogeneity.

			Landscape			**Local**			**Medium**			
Author	**Guild***	**Natural enemies**	**Pos**	**Neu**	**Neg**	**Pos**	**Neu**	**Neg**	**Pos**	**Neu**	**Neg**	**Metric#**
Armbrecht & Perfecto 2003	Ground-foraging	Hymenoptera: Formicidae				1			1		1	D
Aroga & Ambassa-Kiki 2007	Plant-foraging	Forfilculidae, Coccinellidae, Blattelidae, Araneidae, Formicidae, various Hemiptera					1					A
Bianchi et al. 2005	Plant-foraging, Parasitoids	Coleoptera: Staphylinidae, Carabidae; Hymenoptera: Braconidae, Trichogamma; Neuroptera: Chrysopidae	3	3								A, P
Bommarco 1998	Ground-foraging	Coleoptera: Carabidae	1									A (size)
Cai et al. 2007	All	Various				3	1					A, D
Carmona & Landis 1999	Ground-foraging	Coleoptera: Carabidae								1		A
Clough et al. 2005	Ground-foraging	Araneae: various	1	1			2		2			A, D
Costamagna et al. 2004	Parasitoids	Hymenoptera: Braconidae		1								A
Elliot et al. 1998	Plant-foraging	Coleoptera: Coccinellidae; Neuroptera: Chrysopidae; Hemiptera: Nabidae	6									A
Elliot et al. 2002	Plant-foraging	Coleoptera: Coccinellidae; Neuroptera: Chrysopidae; Hemiptera: Nabidae	2	3								A
Gardiner et al. 2009	All	Various	1									A
Gianoli et al. 2006	All	Various					1					A
Harmon et al. 2003	Ground-foraging	Coleoptera: Carabidae, Staphylinidae; Araneae					2	1				A
Klein et al. 2006	Plant-foraging	Hymenoptera: Eumenidae, Pompilidae, Sphecidae					2		1	1		A, D
Kruess & Tscharntke 1994	Parasitoids	Hymenoptera: Braconidae, Eulophidae, Eupelmidae, Pteromalidae, Torymidae	2						3			A,D,P
Kruess & Tscharntke 2000	Parasitoids	Hymenoptera: Pteromalidae, Eupelmidae, Braconidae, Ichneumonidae	2						1	1		A,D,P
Kruess 2003	Parasitoids	Hymenoptera: Braconidae, Pteromalidae, Eucoilidae	2	2		3	1					A, D, P
Lundgren et al. 2009	Plant-foraging	Hemiptera: Anthocoridae				1						A

Marino & Landis 1996	Parasitoids	Diptera: Tachinidae; Hymenoptera: Braconidae, Ichneumonidae	1						1		A
Menalled 2003	Parasitoids	Hymenoptera: Braconidae	3	2							P
Menalled et al. 1999	Parasitoids	Hymenoptera: Braconidae, Eulophidae, Ichneumonidae	1	2							P
Meyer et al. 2009	Plant-foraging	Diptera: Syrphidae	1		1						D, A
Murphy et al. 1996	Parasitoids	Hymenoptera:Mymaridae						1			A
Murphy et al. 1998	Parasitoids	Hymenoptera:Mymaridae				1					P
Nicholls et al. 2001	Plant-foraging, Parasitoids	Coleoptera: Coccinellidae; Neuroptera: Chrysopidae; Hemiptera: Nabidae; Diptera: Syrphidae; Hymenoptera: Mymaridae						4	4	1	A, P
Ostman 2001	Ground-foraging	Coleoptera: Carabidae		1		1		1			A (size)
Pfiffner & Wyss 2004	Ground/plant-foraging	Coleoptera: Carabidae; Araneae; various other predators						2	4		A, D
Prasifka et al. 2004	Ground/plant-foraging	Araneae; Coleoptera: Coccinellidae; Hemiptera: Anthocoridae, Nabidae, Geocoridae	1					1			A
Purtauf et al. 2005a	Ground-foraging	Coleoptera: Carabidae	1	1							A, D
Purtauf et al. 2005b	Ground-foraging	Coleoptera: Carabidae	1	1			2				A, D
Roschewitz et al. 2005	Parasitoids	Hymenoptera: various	1				1				P
Schmidt et al. 2005	Ground-foraging	Araneae: various	1	1		1	1				A, D
Schmidt & Tscharntke 2005	Ground-foraging	Araneae: various	2	1							A
Thies 1999	Parasitoids	Hymenoptera: Ichneumonidae	1				1	1			P
Thies et al. 2003	Parasitoids	Hymenoptera: Ichneumonidae	1								P
Thies et al. 2005	Parasitoids	Hymenoptera: Aphidiidae	3								P
Thies and Tscharntke 2010	Parasitoids	Hymenoptera: Ichneumonidae	1								P
Tylianakis et al. 2004	Parasitoids	Hymenoptera: Aphidiidae						1	1		P
Weibull et al. 2003	Ground-foraging	Araneae; Coleoptera: Carabidae, Staphylinidae		1	1		2	1	1		D
Wilby et al. 2006	All	Various		2		1	1				A, D

*all refers to studies containing all mentioned guilds (parasitoids, ground foragers, and plant foragers)
#A=abundance, D=diversity (species richness or diversity index), P=parasitism rate

CHAPTER III

Parasitoid wasps benefit from shade tree size and landscape complexity in Mexican coffee agroecosystems[3]

Abstract

Increased agricultural intensification has led to a decrease in biodiversity and the deterioration of important agricultural ecosystem services, such as biological control. Parasitoid wasps are important biological control agents for many crop pests, and augmenting their abundance and diversity may confer significant economic and environmental benefits. We investigated how management practice, landscape composition, and biotic and abiotic environmental components affect the parasitoid community in coffee farms of Chiapas, Mexico. Local variables pertaining to vegetation structure and diversity, *Azteca sericeasur* (keystone ant species) presence, and abiotic factors such as synthetic chemical usage and altitude were quantified. Additionally, the landscape composition was assessed for different land uses at both 250m and 500m radii. Utilizing generalized linear mixed-effect models (GLMM), we found that both local and landscape factors affected the parasitoid community. At the local scale, the proximity of *A. sericeasur* nests benefited parasitoid abundance and diversity, whereas different measures of vegetation structure had both positive and negative effects on parasitoid richness,

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*these authors contributed equally to this work

abundance, and diversity. At the landscape scale, we found neighboring intensively managed farms to have an adverse impact on parasitoids. Surprisingly, parasitoids were also positively influenced by increasing altitude and the use of synthetic pesticides. Our findings indicate that the studied agricultural matrix supports a diverse parasitoid community, and that properly managed vegetation structure and increased landscape complexity may augment natural parasitoid communities. Thus, conservation management should take into account environmental complexity at multiple scales.

Keywords:

Agricultural management, Biodiversity, Biological control, Coffee agroecosystem, Landscape complexity, Parasitoid wasps

Introduction

In addition to providing food, fiber, and fuel, agroecosystems provide various supporting, regulating, and cultural ecosystem services, such as nutrient cycling, soil formation, pollination, biological control, and landscape aesthetics (Iverson et al., 2014; Millenium Ecosystem Assessment, 2005). However, agricultural intensification over the past several decades has maximized crop production to the exclusion of various other services, with considerable negative consequences to ecosystem health and biodiversity (Perfecto and Vandermeer, 2008). We are now faced with the challenge of maintaining food production, but reversing the trend of increasing negative impacts of agricultural intensification on humans and the environment (Tscharntke et al., 2012). Designing agroecosystems that decrease the need for pesticides through autonomous biological control is one approach to accomplish this goal (Vandermeer et al., 2010).

Multiple studies have been devoted to understanding patterns of biocontrol provision in agroecosystems, yet results vary as to if and at which scale environmental heterogeneity is beneficial. On a local scale, organic or diversified farm practices can have a positive impact on natural enemy abundance and diversity (Bengtsson et al., 2005; Döring and Kromp, 2003; Hole et al., 2005), but not always (Clough et al., 2005; Pfiffner and Luka, 2003; Weibull et al., 2000). The structure of the landscape is also often, but not always, highly influential on natural enemies (Batáry et al., 2011; Bianchi et al., 2006; Chaplin-Kramer et al., 2011; Gonthier et al., 2014; Perfecto and Vandermeer, 2008). Structurally complex landscapes can support more species through resource provision and diversity, refuges during crop disturbance or seasonality, and migration facilitation (Rusch et al., 2010; Tscharntke et al., 2012).

The effect of environmental heterogeneity on natural enemies is commonly context-dependent, and there are often strong interactions between local and landscape management (Tscharntke et al., 2005). For example, a growing body of research suggests that the benefits of diversified local management are highly contingent on the landscape, where improvements in ecosystem service provision are maximized under intermediate levels of landscape complexity (Tscharntke et al., 2012). At high levels of landscape complexity, species pools of natural enemies may be high enough in the surrounding habitat fragments to maintain relatively high rates of biocontrol even in intensively managed farms (Batáry et al., 2010; Batáry et al., 2011; Concepción et al., 2007). On the other hand, in areas where the landscape is very homogeneous (i.e. cleared of natural vegetation), alternative resources may not be sufficient to support a regional species pool of natural enemies, however locally diverse a farm may be (Perfecto et al., 2009).

Parasitoid wasps are important biological control agents of many crop pests. Some studies show parasitoid-mediated mortality rates averaging above 25% and reaching up to 80% on serious agricultural pests, such as the coffee leafminer (Pereira et al., 2007; A.L. Iverson, unpublished data). Augmenting naturally occurring parasitoid populations may therefore have a considerable economic importance (Heraty, 2009). To do so may be challenging in agroecosystems, as parasitoids are particularly sensitive to agricultural intensification due to their small size, low dispersal ability, high-host specificity, and their dependence on diverse habitats to provide both arthropod hosts and floral resources (Landis et al., 2000; LaSalle and Gauld, 1991).

Parasitoid community dynamics do not occur in isolation, and are highly affected by other organisms through direct or indirect interactions (Muller et al., 1999; Völkl, 1992). In farms located in the same region as the present study, the arboreal-nesting ant, *Azteca sericeasur* (Hymenoptera: Formicidae), serves as a keystone species regulating the abundance of other arthropods, including the green coffee scale *Coccus viridis* (Hemiptera: Coccidae), other ant species, and the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae) (Gonthier et al., 2013; Jimenez-Soto et al., 2013; Vandermeer et al., 2010). In terms of parasitoids, *A. sericeasur* is influential through two opposing mechanisms. On one hand, the ant exhibits a negative effect on parasitoids as it attacks potential enemies of the green coffee scale, which benefits the ant through its honeydew production (Liere and Perfecto, 2008). However, large populations of scales often occur in the vicinity of *A. sericeasur* nests due to the mutualism between the two organisms, leading to a larger host population that may attract more parasitoids. Therefore, the effects of *A. sericeasur* on parasitoids may not be unidirectional.

We surveyed the parasitoid wasps in coffee farms of differing agricultural intensities in Chiapas, Mexico to determine how their distribution is impacted by local- and landscape-level environmental characteristics. At the local scale, we evaluated several habitat characteristics including vegetation structure and diversity, as well as *A. sericeasur* presence. We also examined the landscape composition at 250 and 500m radii. We addressed the following questions: (1) Do parasitoid wasps benefit from local and/or landscape complexity? (2) Is the local or landscape scale more influential? (3) Is vegetation structure or vegetation diversity more influential? (4) Does the presence of *A. sericeasur* affect local parasitoid populations? The various levels of structure and diversity naturally present in these coffee farms allowed us to uniquely analyze the relative influence of these variables, in addition to landscape complexity, to provide us with important insights on ecosystem management for biodiversity conservation and ecosystem service provision. This approach is especially necessary in tropical agroecosystems, where less is known about parasitoid community dynamics and on local-landscape effects on organisms in general.

Methods

Site description

We selected 38 sites spanning 11 contiguous, large (~300 ha.) coffee farms in the Soconusco region of Chiapas, Mexico ($15^0$20’N, $90^0$20’W), near the border with Guatemala (Fig. 1). Site elevations ranged from 600 to 1300m above sea level, and rainfall averages ca. 4500mm annually (Philpott and Bichier, 2012). Farms were selected to cover a range of management intensity, from low shade to high shade, corresponding approximately to shaded monoculture to traditional polyculture, respectively (Moguel and Toledo, 1999). Low shade farms averaged approximately 20% shade cover, whereas high-shade farms averaged 80%. No-shade farms were not common in the study area, and therefore all farms contained a moderate diversity and abundance of shade trees (>100 individuals/ha.). On the most intensively managed farms, most (>80%) of the shade trees were *Inga* spp. (predominantly *I. micheliana* and *I. vera;* Fabaceae). Within plantations, most coffee bushes were arranged in rows, although this structure was not as pronounced in the most shaded farms. Sites within farms were selected based on: 1) maximizing the distance between sites (minimum distance of 350m) and 2) spanning a range of distances to forest fragments. We marked a point within the coffee plantation that satisfied these requirements, and this became the center of each site where we sampled wasps and from which we based the measurements of environmental variables (see below).

Parasitoid wasp sampling

From 25 May to 23 June 2012, we collected parasitoid wasps using pan traps (Abrahamczyk et al., 2010; Nuttman et al., 2011). Traps consisted of blue, yellow, and white 355-ml plastic bowls and were glued to a 1” PVC coupling. In addition, the blue and yellow bowls were sprayed on the interior with a thin layer of blue and yellow ultra-violet paint, respectively (Clearneon, Inc.: Wichita, Kansas). The ultra-violet paint is effective at attracting many Hymenoptera that are capable of sensing short-wavelength irradiation (Briscoe and Chittka, 2001). Different colors are more effective at capturing different families; therefore, having multiple colors decreased the likelihood of biasing the results towards certain families that are especially attracted to certain colors (Abrahamczyk et al., 2010; Nuttman et al., 2011). At the center of each site we placed two sets of traps, one with traps attached to a PVC pipe (1” diameter) set at 0.25m and the other at 1m above ground level. Each set consisted of 3 traps, each of a different color, clustered together and separated by approximately 1m from the other set. Traps were filled with 180ml of solution containing water, soap (blue Dawn® dish soap; Procter and Gamble, Cincinnati, OH, USA), and honey (solution: 1l water, 10ml soap, 15ml honey). This solution is effective both at attracting (via honey) and capturing Hymenoptera, as the soap decreases the surface tension of the solution, causing the arthropods to sink more quickly (Abrahamczyk et al., 2010; Nuttman et al., 2011). Traps were placed in each site one time for approximately 5 h between the hours of 7am and 2pm on sunny, windless days. Approximately 75% of days were acceptable for trapping, and in most cases, sites within the same farm were sampled on the same day.

Field measurements

At each site, we surveyed various environmental characteristics. First, we marked a 56m radius circle (area 1.0ha) around the center of each site using a GPS device (Garmin GPSmap76CSx). We counted and identified every tree >10 cm at diameter at breast height (dbh) within this hectare to obtain tree richness and abundance. If a tree species was not known, samples were collected and brought to camp for keying. Most trees were identified to species, whereas some sterile specimens were only identified to genus and morphospecies. Tree species richness estimates were calculated using the diversity estimator Incidence Coverage-based Estimator (ICE) in EstimateS (Colwell et al., 2012). We also recorded the number of *A. sericeasur* nests within the hectare, as well as the distance to the nearest *A. sericeasur* nest from the plot center. Within a 15m radius of the plot center we measured the dbh and estimated the height of every plant > 1cm dbh. We then calculated the aboveground plant biomass of this 15m-radius section of the plot according the formula:

$$\log(B) = -3.375 + 0.948 \log(D^2H)$$

where B is aboveground biomass, D is dbh, and H is plant height (Brown and Iverson, 1992). We recorded shade cover using a convex spherical densiometer (Forestry Suppliers, Inc., Jackson, MS, USA) at three spots in each site, 5m away from the center of the site at 0, 120 and 240^o. In each of the three spots we measured the shade cover in all four cardinal directions. We also measured groundcover extent (% coverage) and groundcover species richness in five $0.5m^2$ quadrats set at 5m from the plot center every 72^o. Cover was estimated using the following ranges: 0-1, 2-5, 6-20, 21-40, 41-60, 61-80, and 81-100%. Additionally, we measured the abundance of floral resources by counting the number of flowers present in the groundcover species (two 15x2m transects) and number of flowering trees within the sampled hectare. For coffee cover, we measured the number of coffee bushes located within a 15m X 15m square centered on the middle of the plot. Synthetic chemical usage on farms was determined based on knowledge of the farm's practices and certifications. Non-organic farms used glyphosate as an herbicide and often endosulfan as a pesticide for the coffee berry borer (Curculionidae: *Hypothenemus hampei*).

To determine the landscape configuration, we used ArcGIS 10.1 (ESRI, Redlands, CA, USA) to delineate forest and farm boundaries using our knowledge of the area and basemaps provided within ArcGIS. We manually digitized polygons and assigned them to one of 6 categories depending on the intensity of land use: forest, low intensity farm (i.e., high shade), medium intensity farm, high intensity farm, or developed (i.e. semi-urban) (Fig. 1). We then created 250 and 500m buffers around the center of each plot and calculated the amount of each land use category within the buffer. These radii were chosen so as to be large enough to encapsulate the surrounding landscape but small enough so neighboring buffers did not greatly overlap each other. However, although some buffers did have some degree of overlap, there is theoretical and empirical evidence that overlapping landscapes does not contribute to spatial autocorrelation (Zuckerberg et al., 2012). We also determined the nearest distance to a forest fragment from the plot center as well as the elevation of the plot center. Finally, we calculated the Shannon-Weiner Diversity Index (i.e., habitat diversity index) using the amount of each land use type within each buffer.

Identification and statistical analyses

Specimens were stored in 90% ethanol and identified to the family level and assigned to morphospecies using the keys *Hymenoptera of the World* (Goulet and Huber, 1993) and *Hymenoptera of Costa Rica* (Hanson and Gauld, 1996). To ensure consistency, the same individual keyed all samples to morphospecies. We analyzed the parasitoid community according to species richness, abundance, and Shannon's diversity (i.e. Shannon-Wiener Diversity Index). We did not use species richness estimators (e.g. Chao 1) due to the high presence of singleton-only samples (N=11), where species accumulation curves do not asymptote and therefore estimates cannot be computed (Chao et al., 2005). To observe the effects of local and landscape variables on parasitoid abundance and diversity, we created generalized linear mixed-effect models (GLMM), using each farm (N=11) as a random effect. We fit the parasitoid richness and abundance data to a Poisson distribution and the Shannon-Weiner Diversity Index to a log-normal distribution.

Local- and landscape-scale environmental variables (Table 3.1) were included in the GLMM and unimportant variables were removed stepwise through manual backward elimination until we reached a model that maximized the model fit according to the number of significant variables and AIC values. We first calculated the variance inflation factor (VIF) (Graham, 2009) with all variables and eliminated those which were highly collinear (causing VIF values to be above 10), leaving the ones which had the most ecological relevance. The excluded variables were, therefore, ecologically or methodologically redundant. For example, the amount of high-intensity farmland and amount of low-intensity farmland strongly covaried (negatively); therefore, we only included amount of high-intensity farmland in the analysis. After performing backward elimination, we verified that the variables in the final model were not highly collinear by again calculating the VIF. Additionally, if competing models had negligibly different AIC values, we selected the model that returned the highest number of significant predictor variables.

We also analyzed whether the influence of local environmental variables on the parasitoid community was dependent upon the landscape context. To do so, we divided sites categorically into a low or high intensity landscape context based on the amount of neighboring high-intensity farm lands at a 500m radius. Within each of the two landscape intensity levels, we divided the local variables equally on either side of the median value for that variable and determined if parasitoid abundance, richness, and diversity were significantly different within that intensity level according to a Welch two sample t-test (if normally distributed) or the non-parametric Wilcoxon rank sum test (if not normally distributed). All statistical analyses were performed in R for Windows (Version 3.0.2), using the packages “car”, “lme4”, and “lmerTest”.

Results

Parasitoid sampling

We identified 164 wasp morphospecies representing 27 families and 422 individuals (Table 3.2 and Appendix 3.1). Wasps from the family Encyrtidae were the most abundant with

132 individuals and 34 unique morphospecies. All families observed except Vespidae are parasitoid families. However, Vespidae are still important predators of agricultural pests and we, therefore, retained them in our analysis (Hanson and Gauld 1996). For ease of reporting, we refer to all wasps in this study as parasitoids.

Influence of local and landscape variables

Both local and landscape variables influenced the parasitoid community for all three metrics of wasp abundance, species richness, and Shannon's diversity (Table 3.3). Landscape composition, vegetation structure, and *A. sericeasur* presence, but not vegetation diversity, played important roles in determining the parasitoid community. Results were relatively consistent for landscape variables whether calculated at a 250 or 500m radius (Table 3.3).

In terms of local habitat variables, tree biomass, having a positive impact, was the most influential variable on the parasitoid community for all measures and scales (Table 3.3). Being nearer to an *A. sericeasur* ant nest also benefited parasitoid abundance and Shannon's diversity. Additionally, farms at higher altitudes and that utilized synthetic chemicals (pesticides and herbicides) had increased parasitoid abundance, species, and Shannon's diversity. Organic (N=5) and non-organic (N=6) farms were distributed relatively evenly throughout the study region. Utilizing logistic regression, we found that organic farms did have higher shade, less groundcover (due to higher shade), and a higher abundance and richness of tree species than non-organic farms. Additionally, we found a significant negative relationship between the distance to the nearest forest patch and the use of chemicals. Other environmental variables did not differ between organic and non-organic farms.

For landscape composition, the presence of intensively managed (i.e., low-shade) farms had an adverse impact on parasitoid abundance, richness, and Shannon's diversity (Table 3.3). Additionally, the quantity of forest area in the landscape had a positive impact on parasitoid abundance. In contrast, higher habitat diversity, as defined by Shannon's habitat diversity index, was associated with decreased parasitoid abundance and richness.

Dependency of local variables on landscape context

Interestingly, parasitoid abundance benefitted from local shade tree cover only in the context of an intensively farmed (low-shade) landscape (Fig. 2). However, this outcome was observed only when one outlier site (where parasitoid abundance=3) was omitted. This site may have been an outlier due to a particularly large and recent disturbance (shade tree removal) prior to the sampling. We found that shade tree cover did not influence parasitoid abundance in the context of a less intensively farmed landscape. No other local variables were observed to be dependent on the landscape context.

Discussion

Agricultural lands are commonly, and often correctly, regarded as biodiversity deserts. However, high-quality agricultural matrix may be an extremely important habitat for the migration and persistence of biodiversity (Perfecto et al., 2009; Vandermeer et al., 2010). We found a high diversity of parasitoid wasps in the studied coffee agroecosystems, all of which were relatively structurally complex and diverse compared to monoculture cropping. Although we did not sample in complete monocultures, our landscape results, plus other research, suggests that parasitoid diversity would likely be lower in these simplified systems (Altieri et al., 1978; Andow, 1991) .

Understanding how to best enhance the naturally occurring populations of biocontrol agents, such as parasitoid wasps, may allow farmers to rely less on synthetic insecticides. In the present analysis we show that parasitoid wasps responded to both their local environment and to the landscape in which they are embedded in a diverse, tropical moist forest setting. Interestingly, we also show that wasps responded more to plant structure, and specifically tree biomass, than to plant diversity variables. Additionally, we found the presence of *A. sericeasur* ants to be an influential predictor of parasitoid composition.

The importance of tree biomass for the parasitoid community likely reflects the resources and habitat space that trees provide. Many tropical trees are insect-pollinated and provide floral resources, such as nectar and pollen, that are important nutritional resources for adult parasitoids (Lee and Heimpel, 2008; Stamps and Linit, 1998). Extra-floral nectaries may also be important resources, and these are common in tropical trees, including *Inga* spp., which are ubiquitous on the studied coffee farms. Furthermore, larger trees provide parasitoids with a greater quantity and diversity of habitat, including potentially important microhabitats (Stamps and Linit, 1998). This greater habitat space may also harbor more abundant and diverse arthropod host species. Other studies show that arthropod diversity is positively correlated with increased plant structural diversity and complexity (Lawton, 1983; Niemela et al., 1982; Perfecto et al., 1997). Although the negative relationship between the diversity of parasitoids and tree height seems to contradict the positive influence of tree biomass, we believe this finding is ecologically relevant. Short, robust trees may provide resources and habitat space in the branches that are located close to the ground, near to where the parasitoids were trapped. We therefore caution that this finding may not reflect there being a higher diversity of parasitoids around shorter trees, but rather that the relative vertical distribution of parasitoids shifts towards lower strata.

We did not see an influence of plant diversity, both in terms of the shade trees and groundcover species, on the parasitoid community. This result contradicts other studies that do show benefits from increased plant diversity due to additional and more stable resources for adult parasitoids (Landis et al., 2000; Randlkofer et al., 2010). However, many of these studies are based in relatively low-diversity settings in temperate regions, where the presence of resource-heavy plants is a limiting factor in parasitoid demographics. Our study system, on the other hand, is embedded within a highly diverse tropical moist forest ecosystem. Therefore, even the most species-poor sites may have sufficient resources such that those resources are not a primary limiting factor. Furthermore, the overall landscape of the studied coffee farms is quite complex, as shade trees are relatively common even in the most intensively managed farms. This heterogeneous landscape backdrop may have diluted any effects from local differences in plant species diversity through mass effects (species spillover) (Leibold et al., 2004)

We found that parasitoids benefitted from nearby *A. sericeasur* nests, likely as a result of the ant's mutualism with the green coffee scale, a potential host of several different parasitoid species (Uno, 2007). Although it may seem that the benefits of *A. sericeasur* to parasitoids would not translate into net benefits to coffee due to the harmful effects from the scale, paradoxically *A. sericeasur* appears to facilitate the effectiveness and stability of the biocontrol of the coffee scale (Vandermeer et al. 2002; Vandermeer et al., 2010). Here, the primary predator of the scale, *Azya orbigera* (Coleoptera: Coccinellidae), is dependent on areas with a high density of scales, where its slow-moving larvae can encounter sufficient food. Without the clustering caused by *Azteca* ants, *A. orbigera* likely could not persist in the system.

The abiotic factors of altitude and pesticide usage also positively influenced the parasitoid community. Both of these results were contrary to our expectations. The preference for higher elevations may reflect the parasitoids' response to the availability and density of hosts. In our study system, the green coffee scale trends towards being more abundant at higher elevations (A.L. Iverson, unpublished data), and this may hold true for other hosts, as well. Our finding that parasitoid abundance, richness, and diversity are higher on non-organic farms is perplexing. We believe that this outcome may have resulted from the fact that our sites on non-organic farms were statistically closer to forests than they were on organic farms. Although distance to forest was not a significant predictor in our overall model, the amount of forest in a landscape was important. Additionally, it is possible that the insecticides had a disproportionate effect on predators or hyperparasites of the parasitoids we trapped, resulting in enemy release of parasitoids in non-organic farms.

We found that the parasitoid abundance, richness, and Shannon's diversity were negatively impacted by landscape simplification, corroborating other research (Bianchi et al., 2005; Gagic et al., 2011; Gonthier et al., 2014; Marino et al., 2010). Intensively managed farms likely provide a lower quantity, quality, and temporal stability of resources (e.g. hosts and nectar) due to fewer or less stable microclimates, or from decreased habitat space (Lin, 2010; Rojas et al., 2001). The benefits of less-intensively farmed landscapes for parasitoid abundance are further enhanced by increased forest cover in the landscape, likely due to the greater abundance and/or diversity of hosts, floral resources, and refugia (Bianchi et al., 2006; Boccaccio and Petacchi, 2009). Low-intensity farmland negatively co-varies with the Shannon diversity of the landscape, which explains the negative effect of this type of diversity on the parasitoids.

Finally, much research shows strong interactive effects between local and landscape scales (Batáry et al., 2011; Thies and Tscharntke, 1999; Tscharntke et al., 2005; Tuck et al., 2014). We found that the influence of shade cover on parasitoid abundance was dependent on the landscape context, where only in simplified landscapes did parasitoids benefit from more shade cover. This result suggests that in a complex landscape, the added benefits of resources and habitat from higher tree cover may not be as important when parasitoids can utilize the resources present in the surrounding environment. Our inability to detect local-landscape interactions with other variables may again reflect that even in the most intensively managed sites there were sufficient resources and habitat to sustain viable populations of parasitoids.

Conclusions and management implications

As social, environmental, and economic costs for synthetic pesticides continue to rise, controlling pests through biological control is increasingly important. Proactive management of farms and landscapes to benefit important natural enemies is thus an important strategy for cost-effective pest control with multiple additional environmental and social benefits (Kremen and Miles, 2012). Our results suggest that to enhance parasitoid populations for biocontrol benefits on coffee farms in diverse, moist-forest settings, management should focus on both local- and landscape-level environmental characteristics. On a local level, where individual farmers are most practically involved, fostering a shade tree canopy that includes large biomass trees may be most effective. For farms in our study region, maintaining tree cover will also allow habitat space for *A. sericeasur* ants, which benefit parasitoid communities through increased host availability and stability. Individual farmer decisions can scale up to create landscape-level complexity, and landscapes with lower amounts of intensively managed farmland and higher amounts of forests are most beneficial for parasitoids. Although farmers will need to consider the cost-benefit scenario between biocontrol efficacy and coffee production, studies that have recorded yield in Chiapas coffee farms show that intermediate levels of shade or tree biomass are often most productive, and therefore these services do not necessarily trade-off (Soto-Pinto et al., 2000; A.L. Iverson, unpublished data). Finally, if coffee farms are embedded within a simplified landscape, increasing the shade cover may be useful for augmenting parasitoid numbers.

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Table 3.1. Local and landscape variables included in analysis.

	Variable	Description
Local		
Vegetation structure	Groundcover extent	Percent cover of groundcover in five 0.5m^2 quadrats
	Plant biomass	Biomass of plants >1cm dbh within 15m of plot center
	Tree density	Number of trees in 1 ha. surrounding plot center
	Tree shade cover	Percent shade at three equidistant locations 5m from plot center
	Flowering trees	Number of trees (in 1 ha.) in flower during period of trapping
	Groundcover flowers	Number of flowers in two 2x15m transects centered at plot center
Vegetation diversity	Total groundcover species	Species richness of groundcover plants in five 0.5m^2 quadrats
	Tree species richness (ICE)	Incidence coverage estimates (ICE) of tree species richness in 1 ha. surrounding plot center
Azteca sericeasur	Distance to *Azteca* nest	Distance (m) to *Azteca* nest from plot center
	Azteca nest density	Number of *Azteca* nests in 1 ha. surrounding plot center
Miscellaneous	Altitude	Elevation (m asl)
	Chemicals	Binary of whether farm practiced organic or conventional management
Landscape	Forests	Area (m^2) of forest land within 250 or 500m radius from plot center
	High intensity farms	Area (m^2) of land under high-intensity management within 250 or 500m radius from plot center
	Shannon habitat diversity	Shannon diversity index of land uses within 250 or 500m radius from plot center
	Nearest distance to forest	Distance (m) of plot center to nearest forest fragment

Table 3.2. Parasitoid wasps (number of individuals and morphospecies) by family collected in study.

Families	Individuals	Morphospecies	Relative Abundance	Relative Richness
Aphelinidae	3	2	0.71%	1.22%
Bethylidae	8	3	1.90%	1.83%
Braconidae	23	17	5.45%	10.37%
Cephidae	1	1	0.24%	0.61%
Ceraphronidae	21	7	4.98%	4.27%
Chalcidae	5	3	1.18%	1.83%
Chrysididae	7	2	1.66%	1.22%
Diapriidae	53	17	12.56%	10.37%

Elasmidae	1	1	0.24%	0.61%
Encyrtidae	132	34	31.28%	20.73%
Eucoilidae	34	8	8.06%	4.88%
Eulophidae	5	2	1.18%	1.22%
Eupelmidae	7	3	1.66%	1.83%
Evaniidae	1	1	0.24%	0.61%
Ichneumonidae	40	16	9.48%	9.76%
Liopteridae	2	1	0.47%	0.61%
Megalyridae	2	1	0.47%	0.61%
Megaspillidae	11	7	2.61%	4.27%
Myrmaridae	21	14	4.98%	8.54%
Perilampidae	1	1	0.24%	0.61%
Plumariidae	1	1	0.24%	0.61%
Pompiidae	3	1	0.71%	0.61%
Proctotrupidae	6	5	1.42%	3.05%
Scelionidae	22	8	5.21%	4.88%
Trichogrammatidae	5	3	1.18%	1.83%
Trigonalyidae	5	4	1.18%	2.42%
Vespidae	2	1	0.47%	0.61%

Table 3.3. GLMM estimates for parasitoid abundance, richness, and Shannon's diversity with landscape variables measured at 250m and 500m radii.

	At 250m Radius			**At 500m Radius**		
	Estimate	z	p	Estimate	z	p
Parasitoid abundance						
Local						
Altitude	0.192	2.87	0.005**	0.206	2.95	0.003**
Distance to *Azteca* nest	-0.109	-1.99	0.046 *	0.144	-2.45	0.014*
Tree biomass	0.163	2.87	<0.001**	0.194	3.25	0.011**
Chemical usage	0.107	1.65	0.098.	0.152	2.32	0.020*
Landscape						
Forests	0.221	2.31	0.021*	0.139	1.95	0.051.
High intensity farms	-0.240	-3.75	<0.001***	-0.257	-4.12	<0.001***
Shannon habitat Index	-0.275	-3.02	0.002**	-0.142	-2.09	0.037*
Parasitoid richness						
Local						
Altitude	0.287	3.20	0.001**	0.234	2.50	0.009 **
Tree biomass	0.161	2.15	0.031*	0.126	1.72	0.084.
Chemical usage	0.216	2.88	0.004 **	0.180	2.34	0.019*
Landscape						
High intensity farms	-0.207	-3.01	0.003 **	-0.196	-2.60	0.009 **
Shannon habitat index	-0.101	-1.72	0.085.	-	-	-
	Estimate	t^a	p	Estimate	t	p
Parasitoid Shannon Index						
Local						
Altitude	0.106	2.21	0.034 *	0.107	2.15	0.039*
Azteca nest density	-0.084	-1.75	0.089.	-	-	-

Distance to *Azteca* nest	-0.123	-2.57	0.015*	0.090	-2.18	0.036*
Chemical usage	-	-	-	0.105	2.04	0.049*
Tree biomass	0.121	2.38	0.023 *	0.133	2.60	0.014*
Tree height	-0.110	-2.19	0.035*	-0.11	-2.29	0.029*
Landscape						
Forests	-	-	-	0.09	1.82	0.079.

*P <0.05; **P < 0.01; ***P < 0.001; - omitted (not significant)

[a]T statistics and corresponding significance values are given for Shannon's diversity, as it was log transformed. Previous to running the models, variables were converted into z-scores so that estimate magnitude may be compared across variables.

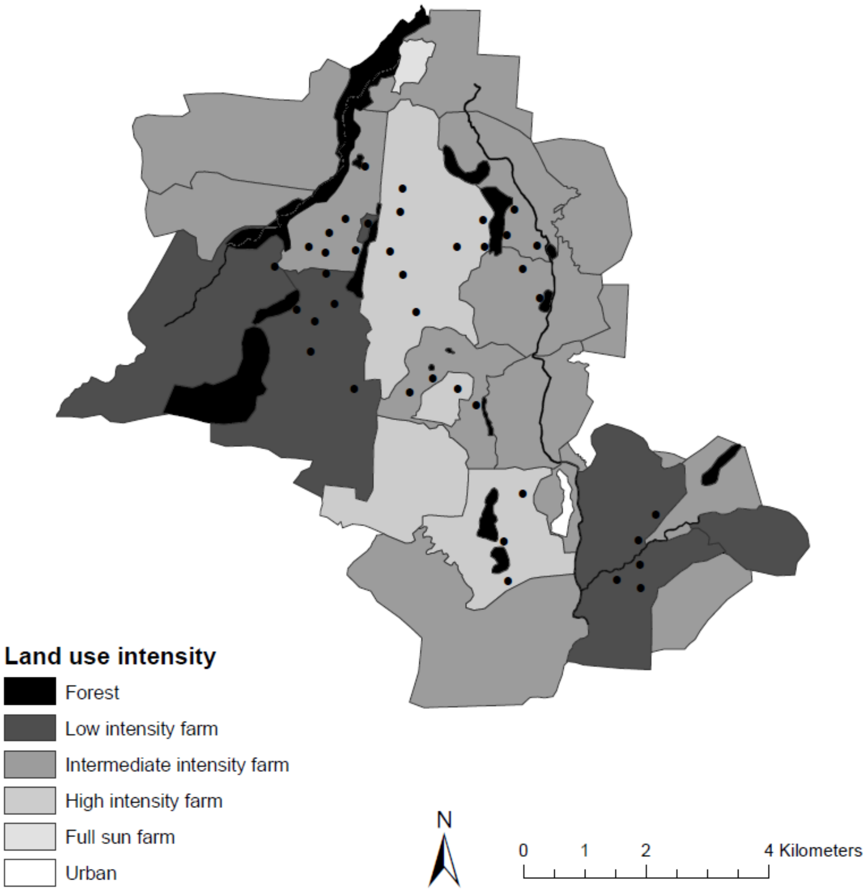

Figure 3.1. Map of farms, forests, and town (polygons), as well as plot sites (points), included in the study. All farms were located in the Soconusco region of Chiapas, Mexico.

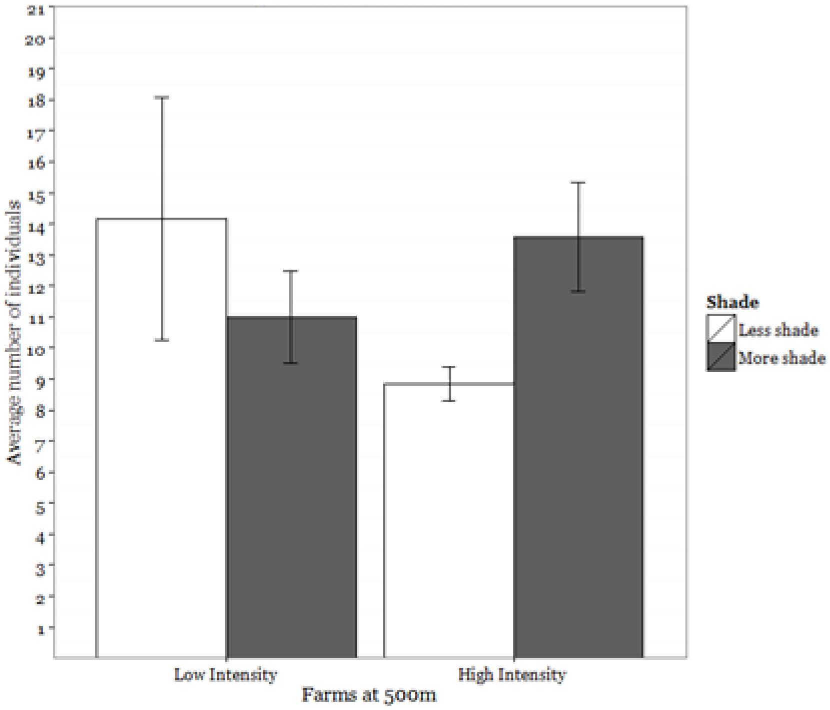

Figure 3.2. Effect of shade on parasitoid abundance in low-intensity and high-intensity landscape. Wilcoxon rank sum test with continuity correction for high intensity farms: W=73, p=0.031. Parasitoid abundance did not differ between shade levels in low intensity farms (W=36.5, p=0.76). Outlier site (where parasitoid richness=3) was excluded for this analysis.

Appendix 3.1. Parasitoid Wasps collected in Chiapas, Mexico

Family	Individuals	#Sites*	Relative Abundance (Individuals)	Relative Abundance (Species)
Aphelinidae			**0.71 %**	**1.22%**
Aphelinidae sp.1	2	2		
Aphelinidae sp.2	1	1		
Bethylidae			**1.90%**	**1.83%**
Bethylidae sp.1	2	2		
Bethylidae sp.2	2	2		
Bethylidae sp.3	4	3		
Braconidae			**5.45%**	**10.37%**
Braconidae sp.1	1	1		
Braconidae sp.2	1	1		
Braconidae sp.3	4	2		
Braconidae sp.4	1	1		
Braconidae sp.5	1	1		
Braconidae sp.6	1	1		
Braconidae sp.7	1	1		
Braconidae sp.8	1	1		
Braconidae sp.9	1	1		
Braconidae sp.10	2	2		
Braconidae sp.11	1	1		
Braconidae sp.12	3	1		
Braconidae sp.13	1	1		
Braconidae sp.14	1	1		
Braconidae sp.15	1	1		
Braconidae sp.16	1	1		
Braconidae sp.17	1	1		
Cephidae			**0.24%**	**0.61%**
Cephidae sp.1	1	1		
Ceraphronidae			**4.98%**	**4.27%**
Ceraphronidae sp.1	1	1		
Ceraphronidae sp.2	5	5		
Ceraphronidae sp.3	1	1		
Ceraphronidae sp.4	11	6		

Ceraphronidae sp.5	1	1		
Ceraphronidae sp.6	1	1		
Ceraphronidae sp.7	1	1		
Chalcididae			**1.18%**	**1.83%**
Chalcididae sp.1	3	2		
Chalcididae sp.2	1	1		
Chalcididae sp.3	1	1		
Chrysididae			**1.66%**	**1.22%**
Chrysididae sp.1	4	4		
Chrysididae sp.2	3	2		
Diapriidae			**12.56%**	**10.37%**
Diapriidae sp.1	4	2		
Diapriidae sp.2	1	1		
Diapriidae sp.3	2	2		
Diapriidae sp.4	4	4		
Diapriidae sp.5	4	3		
Diapriidae sp.6	5	5		
Diapriidae sp.7	1	1		
Diapriidae sp.8	1	1		
Diapriidae sp.9	3	2		
Diapriidae sp.10	1	1		
Diapriidae sp.11	11	9		
Diapriidae sp.12	1	1		
Diapriidae sp.13	4	3		
Diapriidae sp.14	3	3		
Diapriidae sp.15	3	2		
Diapriidae sp.16	3	2		
Diapriidae sp.17	2	2		
Elasmidae			**0.24%**	**0.61%**
Elasmidae sp.1	1	1		
Encyrtidae			**31.28%**	**20.73%**
Encyrtidae sp.1	19	10		
Encyrtidae sp.2	5	4		
Encyrtidae sp.3	1	1		
Encyrtidae sp.4	4	3		
Encyrtidae sp.5	1	1		
Encyrtidae sp.6	2	2		
Encyrtidae sp.7	2	2		

Encyrtidae sp.8	1	1		
Encyrtidae sp.9	3	3		
Encyrtidae sp.10	3	2		
Encyrtidae sp.11	1	1		
Encyrtidae sp.12	1	1		
Encyrtidae sp.13	11	6		
Encyrtidae sp.14	19	9		
Encyrtidae sp.15	5	4		
Encyrtidae sp.16	8	8		
Encyrtidae sp.17	1	1		
Encyrtidae sp.18	1	1		
Encyrtidae sp.19	1	1		
Encyrtidae sp.20	7	6		
Encyrtidae sp.21	2	2		
Encyrtidae sp.22	1	1		
Encyrtidae sp.23	9	6		
Encyrtidae sp.24	1	1		
Encyrtidae sp.25	2	2		
Encyrtidae sp.26	7	5		
Encyrtidae sp.27	2	2		
Encyrtidae sp.28	3	2		
Encyrtidae sp.29	2	2		
Encyrtidae sp.30	1	1		
Encyrtidae sp.31	1	1		
Encyrtidae sp.32	3	2		
Encyrtidae sp.33	1	1		
Encyrtidae sp.34	1	1		
Eucoilidae			**8.06%**	**4.88%**
Eucoilidae sp.1	19	3		
Eucoilidae sp.2	1	1		
Eucoilidae sp.3	7	3		
Eucoilidae sp.4	3	3		
Eucoilidae sp.5	1	1		
Eucoilidae sp.6	1	1		
Eucoilidae sp.7	1	1		
Eucoilidae sp.8	1	1		
Eulophidae			**1.18%**	**1.22%**
Eulophidae sp.1	1	1		
Eulophidae sp.2	4	1		
Eupelmidae			**1.66%**	**1.83%**

Eupelmidae sp.1	3	3		
Eupelmidae sp.2	3	1		
Eupelmidae sp.3	1	1		
Evaniidae			**0.24%**	**0.61%**
Evaniidae sp.1	1	1		
Ichneumonidae			**9.48%**	**9.76%**
Ichneumonidae sp.1	1	1		
Ichneumonidae sp.2	1	1		
Ichneumonidae sp.3	6	2		
Ichneumonidae sp.4	4	4		
Ichneumonidae sp.5	2	2		
Ichneumonidae sp.6	3	3		
Ichneumonidae sp.7	9	6		
Ichneumonidae sp.8	1	1		
Ichneumonidae sp.9	2	2		
Ichneumonidae s. 10	1	1		
Ichneumonidae sp.11	1	1		
Ichneumonidae sp.12	3	2		
Ichneumonidae sp.13	1	1		
Ichneumonidae sp.14	3	2		
Ichneumonidae sp.15	1	1		
Ichneumonidae sp.16	1	1		
Liopteridae			**0.47%**	**0.61%**
Liopteridae sp.1	2	2		
Megalyridae			**0.47%**	**0.61%**
Megalyridae sp.1	2	2		
Megaspillidae			**2.61%**	**4.27%**
Megaspillidae sp.1	2	2		
Megaspillidae sp.2	2	2		
Megaspillidae sp.3	3	3		
Megaspillidae sp.4	1	1		
Megaspillidae sp.5	1	1		
Megaspillidae sp.6	1	1		
Megaspillidae sp 7	1	1		
Myrmaridae			**4.98%**	**8.54%**
Myrmaridae sp.1	1	1		

Myrmaridae sp.2	2	2		
Myrmaridae sp.3	1	1		
Myrmaridae sp.4	2	2		
Myrmaridae sp.5	1	1		
Myrmaridae sp.6	1	1		
Myrmaridae sp.7	2	2		
Myrmaridae sp.8	3	3		
Myrmaride sp. 9	1	1		
Myrmaridae sp.10	2	2		
Myrmaridae sp.11	2	2		
Myrmaridae sp.12	1	1		
Myrmaridae sp.13	1	1		
Myrmaridae sp.14	1	1		
Perilampidae			**0.24%**	**0.61%**
Perilampidae sp.1	1	1		
Plumariidae			**0.24%**	**0.61%**
Plumariidae sp.1	1	1		
Pompiidae			**0.71%**	**0.61%**
Pompiidae sp.1	3	3		
Proctotrupidae			**1.42%**	**3.05%**
Proctotrupidae sp.1	2	2		
Proctotrupidae sp.2	1	1		
Proctotrupidae sp.3	1	1		
Proctotrupidae sp.4	1	1		
Proctotrupidae sp.5	1	1		
Scelionidae			**5.21%**	**4.88%**
Scelionidae sp.1	1	1		
Scelionidae sp.2	5	3		
Scelionidae sp.3	3	3		
Scelionidae sp.4	3	2		
Scelionidae sp.5	2	2		
Scelionidae sp.6	5	3		
Scelionidae sp.7	2	2		
Scelionidae sp.8	1	1		
Trichogrammatidae			**1.18%**	**1.83%**
Trichogrammatidae sp.1	2	1		
Trichogrammatidae sp.2	2	1		

Trichogrammatidae sp.3	1	1		
Trigonalyidae			**1.18%**	**2.42%**
Trigonalyidae sp.1	1	1		
Trigonalyidae sp.2	1	1		
Trigonalyidae sp.3	1	1		
Trigonalyidae sp.4	2	2		
Vespidae			**0.47%**	**0.61%**
Vespidae sp. 1	2	2		

* refers to number of sites (out of 38) where each parasitoid species was found

CHAPTER IV

Do polycultures promote win-wins or trade-offs in agricultural ecosystem services? A meta-analysis[4]

Abstract

1. Agriculture comprises the largest global land use, make it a leading cause of habitat loss. It is therefore critical to identify how to best construct agricultural systems that can simultaneously provide food and other ecosystem services. This challenge requires that we determine how to maximize win-win relationships and minimize trade-offs between services.
2. Through meta-analysis, we tested whether within-field crop diversification (polyculture) can lead to win-win relationships between two ecosystem services: yield of a focal crop species and biocontrol of crop pests. We selected only studies that recorded both services (N=26 studies; 301 observations), allowing us to better determine the underlying mechanisms of our principal findings. We calculated log-response ratios for both ecosystem services in mono- and polycultures.
3. We found win-win relationships between per plant yield of the primary crop and biocontrol in polyculture systems that minimized intraspecific competition via substitutive planting. Additionally, we found beneficial effects on biocontrol with no difference in per unit area

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yield of the primary crop in polyculture fields at high cropping densities (additive planting) where legumes were used as the secondary crop. These results suggest there is a strong potential for win-win relationships between biocontrol and per unit area yield under certain scenarios. Our findings were consistent across geographical regions and by type of primary crop. We did not find evidence that biocontrol had an effect on yield but rather both were independently affected by polycultural cropping.

4. *Synthesis and applications*. We show that well-designed polycultures can produce win-win outcomes between per plant, and potentially per unit area, primary crop yield and biocontrol. Biocontrol services are consistently enhanced in polycultures, so polyculture management that focuses on yield optimization is likely to be the best strategy for maximizing both services. In doing so, we suggest that practitioners utilize polycultures that decrease plant–plant competition through a substitution of relatively large quantities of the primary crop for compatibly harvestable secondary crops. Additionally, if planting at high cropping densities, it is important that legumes be the secondary crop.

Keywords: additive design, agroecosystems, biological control, multifunctionality, polyculture, substitutive design, yield

Introduction

The Green Revolution was very successful at producing food on a scale that the world had never before seen. However, it also contributed significantly to the degradation of many of the other services that ecosystems provide to humanity - services like soil formation, nutrient cycling, water supply, climate regulation, pollination, and biological control of crop pests

(Costanza *et al.* 1997; Tilman 1999; Millenium Ecosystem Assessment 2005; Losey & Vaughan 2006; Foley *et al.* 2011). Now that ca. 40% of the Earth's terrestrial surface is covered by agricultural habitats, these represent the single largest land use globally (Foley *et al.* 2005; Ramankutty *et al.* 2008), and are arguably one of the most important focal areas for conservation of biodiversity and ecosystem services (Clay 2004; Perfecto, Vandermeer & Wright 2009). There is considerable evidence that agricultural practices differ in their impacts on ecosystem services and, therefore, there is growing interest in how agroecosystems might be managed not only as a source of a provisioning service (food, fuel, or fibre), but of other ecosystem services as well (Perfecto & Vandermeer 2008; Perfecto, Vandermeer & Wright 2009; Power 2010; Kremen & Miles 2012).

Increasing crop diversity through the use of polycultures has often been proposed as a means to achieve win-win scenarios among ecosystem services in agroecosystems (Power 2010). Yet, the vast majority of empirical studies performed to date have examined how crop diversity influences ecosystem services individually. For example, although there is evidence that increasing crop diversity can enhance pollination (Holzschuh *et al.* 2006; Kennedy *et al.* 2013), soil fertility (Mäder *et al.* 2002), disease regulation (Power & Flecker 1996), and biological control (Andow 1991; Simon *et al.* 2010; Letourneau *et al.* 2011), there is little work showing how these ecosystem services covary in response to crop diversity, especially with respect to crop yield (Steffan-Dewenter *et al.* 2007). With an improved understanding of how these services covary, we will better be able to optimize agroecosystems for both food production and other important services by maximizing synergies and minimizing trade-offs (Power 2010).

Using meta-analysis, we examine the effect of polycultural cropping on two agricultural ecosystem services: biocontrol of herbivorous pests (reduction of pest abundance or plant damage, increase in natural enemy abundance) and yield of a focal crop (grams of consumable product per plant). In so doing, we explore whether polycultural cropping promotes a trade-off or a win-win relationship between these two ecosystem services. We also separately examine the individual components of primary crop yield and biocontrol (e.g. separating by focal crop type, secondary vegetation type, biocontrol response metric) and analyse results according to the broad geographical region of the study (temperate vs. tropical). All studies included in this meta-analysis report both the levels of biocontrol of herbivorous pests and of yield of focal crop in the same experiment (same location and same seasons), allowing us to ascertain more directly the relationship between polycultural cropping and these ecosystem services. With these analyses we not only determine whether trade-offs or win-wins result between biocontrol and yield, but shed light on the mechanisms by which these relationships may result. Building upon the work of others (Poveda, Gomez & Martinez 2008; Power 2010; Letourneau *et al.* 2011; Kremen & Miles 2012; Cardinale *et al.* 2012), this is the first synthesis study, to the best of our knowledge, to directly assess how biocontrol and yield are simultaneously affected by polycultural cropping.

Materials and methods

Data collection

We conducted a literature search on 18 December 2011 in ISI Web of Science, returning 1,479 publications (for keywords see Appendix 4.1 in Supporting Information). To augment this search, we reviewed the bibliographies of two key reviews of intercropping and pest control (Andow 1991; Letourneau *et al.* 2011). We also surveyed co-authors for additional known papers. We selected papers from these searches using the following criteria:

1) The study was an empirical investigation that directly measured yield and at least one biocontrol variable in agricultural fields with at least two levels of plant species richness (e.g. monoculture and polyculture). We considered fields as polycultures only if the multiple species were grown in the same field. Species richness included both harvested crops and non-harvested plants (e.g. cover crops). Yield was defined as total biomass of the plant tissue for which the crop is grown (e.g. fruit, seed, fibre, or leaf weight), not overall plant biomass. Metrics of biocontrol were: i) abundance of arthropod herbivores, ii) abundance of natural enemies of pests, iii) degree of pest parasitism, or iv) amount of plant damage.

2) Crop species richness differed between treatments at a single point in time (i.e. crop rotations not included).

3) Experimental treatments varied based on plant species richness, rather than on other forms of diversity (e.g. genetic diversity).

4) The treatment (i.e. monoculture or polyculture) had more than one replicate.

Papers rarely included estimates of yield of the secondary crop(s), therefore we could only consider primary crop yield in the analysis (see ‘Experimental design’ below). Weeds were not included as a secondary species with the exception of the studies (N=2) that explicitly included associated plants as a diversity treatment and, therefore, excluded them from monocultures (Schellhorn & Sork 1997; Showler & Greenberg 2003). Although most of the secondary species were crops, not all were. Therefore, we refer to them collectively as ‘secondary vegetation’. In the rare cases where similar data on a biocontrol metric were reported using two or more different methods, we used only the data from the method that, in our expert opinion, would most likely have a direct impact on yield. For example, Belay *et al.* (2009) reported internode damage, exit holes, tunnelling, and cob damage on maize. In this case, we chose cob damage as the category that most likely directly affected the yield of the commercially important part of the crop. If the author reported damage on the above- and below-ground parts of the plant that reflected activity from different arthropod guilds (e.g. Sekamatte *et al.* 2003), we included both damage metrics as separate observations. If a study reported multiple biocontrol variables (e.g. natural enemy diversity and plant damage), each was considered as a separate observation. Percentage parasitism was pooled into the natural enemy abundance category because there were not enough observations to consider it individually (N=4). If a study investigated the effects of different combinations of crop ratios in polyculture (e.g. Weiss *et al*. 1994), each ratio treatment was compared to the monoculture values and included as a separate observation.

A total of 26 studies (Villamajor 1976; Nordlund, Chalfant & Lewis 1984; Letourneau 1986; Rodenhouse *et al.* 1992; Weiss *et al.* 1994; Williams *et al.* 1995; Schellhorn & Sork 1997; Hooks, Valenzuela & Defrank 1998; Ogol, Spence & Keddie 1999; Nabirye *et al.* 2003; Sekamatte, Ogenga-Latigo & Russell-smith 2003; Showler & Greenberg 2003; Hooks & Johnson 2004; Maluleke, Addo-Bediako & Ayisi 2005; Schader, Zaller & Köpke 2005; Skelton & Barrett 2005; Matama-Kauma *et al.* 2006; Arim *et al.* 2006; Gianoli *et al.* 2006; Rao 2007; Chabi-Olaye *et al.* 2007; Belay, Schulthess & Omwega 2009; Hummel, Dosdall & Clayton 2009; Lenardis *et al.* 2011; Ramalho *et al.* 2012; Nyasani *et al.* 2012) yielded 301 comparisons between monocultures and polycultures (see Table S4.1). Of these, 16 resulted from our ISI search, an additional six from two key review papers (Andow 1991; Letourneau *et al.* 2011), and a further three studies from surveying co-authors (Maluleke, Addo-Bediako & Ayisi 2005;

Belay, Schulthess & Omwega 2009; Ramalho *et al.* 2012). From these studies, we extracted data from tables or text, or used the program DataThief (Tummers 2006) to obtain data points from figures. If the data that were needed to calculate effect sizes were not available, we contacted the authors and requested the original datasets. Three datasets were contributed in this manner, whereas one could not be included due to lack of response.

Experimental designs

Of the 26 studies, 12 were designed as substitutive experiments, and 14 were designed as additive. Substitutive designs hold overall plant density constant in mono- and polycultures, whereas in additive designs, the primary crop density does not change and secondary species are added so that total crop density increases (see Vandermeer 1989). In the additive design, intraspecific interactions are held constant at a fixed density, even as interspecific interactions are added in polyculture. In the substitutive design, the addition of interspecific interactions in polyculture is coupled with the potential of reduced intraspecific interactions. Polycultural cropping systems can best be viewed in the framework of a continuous response surface, where the response (e.g. yield) is projected as a function of various combinations of densities of each crop (Law & Watkinson 1987), where optimal scenarios can be developed through modelling approaches (García-Barrios *et al.* 2001). Therefore, dividing cropping systems into a binary designation as additive or substitutive is not ideal, yet we did not see practical alternatives given the type of data our analysed studies included.

As it was not possible to include secondary crop yield in the analysis, we calculated yield as the mass of consumable product per individual plant of primary crop rather than per unit area for substitutive studies, as the former allowed us to better ascertain ecological mechanisms underlying yield increases or decreases. Any decrease in yield per unit area in a substitutive design can result from (a) a decrease in the per plant yield that results from the treatment, and/or (b) a decrease in plant density, which is imposed by, and inherent to the substitutive design. Per unit area calculations with these designs thus confound the explanation of observed relationships. By comparison, calculating yield on a per plant or per unit area basis makes no difference for studies performed using an additive design because the constant density of the primary crop from mono- to polyculture ensures that one achieves the same yield ratio (see 'meta-analysis' below).

Meta-analysis

Calculating overall trade-off or win-win relationships

To standardize results between studies and allow for meaningful comparisons, we calculated dimensionless effect sizes for the impact of polycultural cropping (as compared to monocultural cropping) on yield per plant of the primary crop and for biocontrol, measured as a decrease in herbivorous pests or plant damage, or an increase in natural enemies. We calculated log-response ratios for yield and biocontrol variables by taking the natural log of the mean value for polyculture over the mean value for monoculture for each observation (Hedges, Gurevitch & Curtis 1999). Because a beneficial effect of polycultural cropping on biocontrol differs for herbivore abundance and plant damage (negative log-response ratio is beneficial) as opposed to natural enemy abundance (positive log-response ratio is beneficial), we changed the sign of log-response ratios for herbivore abundance and plant damage so that all beneficial biocontrol effects were reflected in positive values.

When a biocontrol variable was zero in monoculture (e.g. no herbivores found), we used the lowest value found in the rest of that particular study's dataset for that variable (i.e. the lowest non-zero value). We chose this method as opposed to adding a constant, as there was a large variation in the magnitude of biocontrol values between studies and a constant would have considerably (and arbitrarily) changed the effect sizes for small values. In cases where biocontrol data were reported as a time series (e.g. bi-weekly measures of pest abundance) within a growing season, the mean of the individual ratios of an entire time series was used as an estimate of each biocontrol variable that was measured. We determined whether time had a significant effect on the log-response ratios by calculating the statistical significance ($P<0.05$) of the linear and quadratic regressions of the log-response ratios of each time-series. For time series that showed a significant trend (N=4 observations), data were plotted separately as a series in order to visualize the time effect (Fig. S4.1), but were still included in the other analyses.

We used the effect sizes to determine whether polycultural cropping leads to a negative or positive relationship between biocontrol and yield. To do so, we calculated the mean and 95% confidence intervals of the effect sizes using the estimated means generated from generalized linear mixed models (GLMM), using study as a random factor. We plotted these data on a Cartesian plane with the primary crop yield and biocontrol response ratios on the x- and y-axis, respectively. This plot allows an easy visualization of trade-off, win-win, and lose-lose relationships (Fig. 4.1).

Mechanisms 1: Role of plant competition and biocontrol

In order to determine the influence of inter- and intraspecific plant competition on per plant yield, we calculated the proportional change in density of the primary crop relative to the secondary vegetation for substitutive studies as:

$$\text{Proportional Density Change} = \frac{Density_{mono} - Density_{poly}}{Density_{mono}}$$

where $Density_{mono}$ and $Density_{poly}$ refer to the planting densities (per unit area) of the primary crop in monoculture and polyculture, respectively. This analysis was facilitated by the fact that studies varied in the ratio of primary crop to secondary vegetation when planted in polyculture (i.e. planting mixes in polyculture were not always 50:50). If we found a more positive effect on yield as the secondary vegetation's relative density increased, it would suggest that improvements in yield may have resulted from decreased intraspecific competition despite increased interspecific competition, or alternatively, because of facilitation. Note that a similar analysis could not be performed for additive studies where the focal crop density did not change from monoculture to polyculture.

To test if biocontrol influences yield through indirect effects of suppression of pests and decreased plant damage, we calculated the Spearman rank correlation between the effect sizes for biocontrol and primary crop yield, where a significant correlation would indicate covariance between the two services.

Mechanisms 2: Examining variation in yield and biocontrol

To understand what might drive variation in biocontrol or yield, we dissected each into the following categories: 1) biocontrol metric (e.g. herbivore abundance, predator abundance, and plant damage), 2) type of primary crop, 3) type of secondary vegetation, and 4) geographical region. Primary crops were categorized in a manner that allowed sufficient sample size for meaningful analysis according to the following groups: maize, legumes, and all others. For the secondary vegetation, we performed two separate analyses, first grouping as legumes or non-legumes and secondly as a harvested crop (e.g. produced for food or fibre) or a non-harvested plant (e.g. cover crops, weeds, or grass strips). For geographic region, we divided experiments by temperate (>23.5° N and S) and tropical (<23.5° N and S) latitudes. All analyses were separated by substitutive and additive designs.

Unless otherwise noted, all statistical analyses were conducted using R, version 2.13.1 (R Development Core Team, 2011). Studies were weighted by sample size according to the "weights" element within the glmer function (for GLMMs, lme4 package) and compared to the non-weighted values. As conclusions did not differ when values were weighted, here we present only non-weighted results. Because there can be a tendency to not publish non-significant or small-negative-result studies, we tested our results for this publication bias by calculating Rosenthal's fail-safe value (Rosenthal 1979) using the Fail-safe Number Calculator (Rosenberg 2005).

Results

Yield and Biocontrol: trade-off or win-win?

Our first goal was to understand how polycultural cropping impacts biocontrol and primary crop yield simultaneously. Plotting log-response ratios for both services on a Cartesian plane allowed for easy visualizations of win-win or trade-off relationships (Fig. 4.1). We found a significant win-win scenario for biocontrol and per plant primary crop yield in substitutive design experiments, which showed a 40% and 31% increase for yield and biocontrol, respectively, in poly- over monocultures (Table 4.1, Fig. 4.2a). In additive studies, on the other hand, we found a significant trade-off between biocontrol and per plant (*ergo* per unit area, see

‘Materials and methods’) primary crop yield, where the biocontrol effect was higher in polycultures compared to monocultures (36% increase), but yield of the primary crop was lower (24% decrease) (Table 4.1). When additive studies were split into those with legumes vs. without legumes as secondary vegetation, polycultures with legumes retained their biocontrol advantage and did not show reduced yields (Fig. 4.2b). These results are robust to publication bias, according to Rosenthal’s method for deriving a fail-safe value (Rosenthal 1979) (Table S4.2).

Mechanisms 1: Role of plant competition and biocontrol

When we analysed how primary crop yield effect sizes varied as a function of the relative density of the primary (in relation to secondary) crop in substitutive polycultures, we found a significant positive relationship (*P* <0.001; Fig. 4.3). This result indicates that as individuals of the primary crop are replaced with individuals of the secondary plant(s), the per plant yield of the primary crop increases. This trend appeared to be driven primarily by the presence of legumes; when we repeated the analysis separating studies into legume or non-legume polycultures, those with legumes remained highly significant (*P*<0.001), whereas those without legumes showed no trend (*P*=0.320). However, all regressions became non-significant when a single large study (Nordlund, Chalfant & Lewis 1984) (N=46 observations) was eliminated. When the biocontrol response was plotted in the same way against the proportion of the polyculture field in primary crop, the linear regression was non-significant (*P*=0.756), indicating that having relatively more secondary crop did not influence the degree of biocontrol.

To determine if primary crop yield covaries with biocontrol, we performed a Spearman rank correlation between the effect sizes of the two variables. This analysis resulted in a non-significant trend for additive designs and a marginally significant negative trend for substitutive designs (Fig. 4.2a and b, gray dots), showing that yield does not covary consistently with biocontrol. When we performed a Spearman rank correlation for effect sizes of each biocontrol metric separately (herbivore abundance, predator abundance, plant damage) with primary crop yield, results varied between negative, positive, and non-significant relationships, further suggesting that biocontrol does not consistently covary with primary crop yield (Table S4.3).

Mechanisms 2: Examining variation in yield and biocontrol

The studies used in this meta-analysis included 12 primary crops and 42 secondary crops (Table S4.1). We examined how the type of secondary crop influenced biocontrol and primary crop yield by calculating separate effect size means for two groupings of secondary crops: 1) legume vs. non-legume and 2) harvested vs. non-harvested (e.g. cover crop, grass corridor). In additive designs, polyculture yields did not differ from monocultures when the secondary crop was a legume but were significantly lower in polycultures when the secondary crop was a non-legume (Table 4.1, Fig. 4.2b, Fig. S4.2). In substitutive designs, primary crop yields were improved regardless of whether the secondary crop was a legume or a non-legume (Table 4.1, Fig. S4.2). Whether a secondary crop was a harvested crop or not did not affect the primary crop yield in substitutive studies. However, if a secondary crop was a non-harvested crop in additive studies, the negative effect on primary crop yield was not significant (Table 4.1). Biocontrol values did not vary substantially between secondary crop categories, although in additive studies the biocontrol benefit in polycultures was not significant with non-legumes or harvested crops as secondary crops (Table 4.1, Fig 2b).

When the primary crop was grouped according to crop type (maize, legumes, or all others), we found that the yield effect sizes for each of the groups followed the same trends as the corresponding overall values (overall additive or substitutive) (Fig. 4.4, Table 4.1). When we observed effect sizes for biocontrol when separated by primary crop type, we found that maize crops in additive studies and 'other' crops (non-maize/non-legume) in both additive and substitutive studies had the largest biocontrol benefit when in polyculture (Table 4.1). When we separated the biocontrol effect according to each of the three metrics (plant damage, predator abundance, and pest abundance), each metric was greater (more beneficial to farmers) in polycultures relative to monocultures (all significant ($P<0.05$), except for the predator category ($P=0.075$), Fig. 4.5).

Finally, we separated studies into tropical (<23.5° N and S latitudes) and temperate (>23.5° N and S) regions. Effects of polycultural cropping on biocontrol and yield had a similar pattern in both temperate and tropical regions, although some outcomes were not significant (Fig. S4.3). These results mirrored the trend observed in the overall results of additive or substitutive studies (Fig. 4.2a and b).

Discussion

Our study shows that while no universal relationship is apparent between biocontrol and primary crop yield, win-win outcomes may be achieved under certain scenarios. We found that win-win relationships between per plant yield and biocontrol can be attained by reducing intraspecific competition through replacing the primary crop with a secondary crop. Furthermore, by observing additive studies, we show that per unit area (= per plant) primary crop yields are enhanced most with legumes as a secondary crop, where they produce the same as their monoculture counterparts, even without including secondary crop yields. This polycultural scenario thus shows strong potential for overall win-win outcomes considering per unit area yields.

Biocontrol services were consistently enhanced by polycultural cropping in both additive and substitutive designs, and this effect was attained even at low densities of secondary crop relative to primary crop (Fig. 4.3). These results support the findings of other studies showing the benefits of biocontrol services provided by diverse cropping systems (Andow 1991; Simon *et al.* 2010; Letourneau *et al.* 2011). Biocontrol benefits may result from associational resistance, such as a decrease in food concentration for specialized pests (i.e. resource concentration hypothesis), or an increase in their natural enemies (i.e. enemies hypothesis) (Root 1973; Beizhou *et al.* 2012; Hamback, Agren & Ericson 2013).

Given the strong biocontrol effect found in diverse cropping systems, the overall outcome of a win-win or trade-off relationship was largely determined by the yield response. Our analysis suggests that the yield response is highly influenced by plant–plant competition as mediated by planting density. When total crop density was held constant (substitutive designs), more diverse cropping systems had higher per plant primary crop yield, thus resulting in a significant win-win relationship between biocontrol and per plant yield. When overall crop density increased in polycultures relative to monocultures (additive designs), more diverse cropping systems had a lower per plant (or per unit area, see ‘Materials and methods’) primary crop yield, leading to a trade-off between the two services. These results were relatively consistent by region, type of primary crop, and biological control metric, suggesting that the patterns observed in this meta-analysis are broadly applicable, despite variations in species and climate.

Our results provide important insights into the ecological mechanisms that may contribute to crop production in agroecosystems. In additive studies, the decrease in primary crop yield in polycultures probably reflects increased interspecific competition in these mixtures. However, notably, this loss in yield disappeared when the secondary crop was a legume, suggesting that legume facilitation minimized the negative effects of increased competition (Table 4.1, Fig. 4.2b, Fig. S4.1). Letourneau *et al.* (2011), in a meta-analysis sharing nine studies in common with ours, found a beneficial effect of polycultural cropping on primary crop yield in additive studies (also without including secondary crop yield), suggesting that win-win scenarios may not be uncommon with additive designs. For studies using substitutive designs, the beneficial effects of polycultural cropping on per plant yield suggest that interspecific competition is less costly than intra-specific competition, and/or that positive interactions, such as facilitation, enhance per plant yield. Further supporting this evidence, we found a significantly positive relationship between primary crop yield and the proportion of a plot made up of secondary crop (Fig. 4.3). However, the influence of one particular study (Nordlund, Chalfant & Lewis 1984) limits our confidence in the generality of this finding.

Our analysis suggests that the beneficial effects of polycultural cropping on yield may not result primarily from increased biocontrol effects of lower plant damage, suppression of pests, or augmented natural enemy populations. However, due to the diversity of herbivores and natural enemies recorded in these studies, it is possible that biocontrol could sometimes be influential on yield despite the lack of a significant correlation between the two services. For example, a small change in the biocontrol value could have a considerable benefit to yield in one study, whereas in another it could make no difference. This particular outcome could occur if the herbivore species in one study, but not another, were particularly damaging or if natural enemies in one study were more effective predators of relevant herbivores. As a result, we may not see a positive correlation between the biocontrol metric (e.g. number of herbivores) and yield, even if generally there is a biocontrol effect.

Limitations

We were unable to include overall yield data in our analyses due to the lack of secondary crop yield information reported in the majority of studies. It was therefore most logical to calculate yield on a per plant basis for substitutive studies. Additive studies were equivalent in log-response ratios irrespective of calculating by area or by plant. Focusing on per plant yield was, in the end, most useful for understanding the ecological interactions which underlie the relationships between yield and biocontrol, as these occur at the scale of an individual plant. However, per plant primary crop yield calculations may lead to overestimations and underestimations compared to total yields in substitutive and additive studies, respectively. Our results for additive studies are thus conservative. For substitutive studies, we believe that per plant yield is indicative of overall yield for two reasons. First, the great majority of the substitutive observations (N=97 out of 117) had a harvestable secondary crop that would have contributed to total yield. Second, of all secondary crop observations, 56% (N=65 out of 117) were species that were also primary crops in other studies, and therefore also showed an average benefit from polycultural cropping.

Undoubtedly, in some cases yield (or profit) per unit area will be lower under substitutive polycultural systems due to the combination of a lower density of primary crop and a less-productive or nonsaleable (or lesser-value) secondary crop. There are thus some concerns that more land area would be required to produce the same amount of food, resulting in a net loss of ecosystem services, such as biodiversity conservation, across the landscape (Green *et al.* 2005; Phalan *et al.* 2011). However, others contest that this viewpoint relies on various assumptions that are not always met, such as countries being able to protect land (which relies on a complex social and political interplay) and that ecosystem service provision and high yield are not compatible (Fischer *et al.* 2011; Tscharntke *et al.* 2012). Although we cannot assess this question directly in our study, our results support the notion that crop production and ecosystem service provision need not be inversely related. Indeed, many studies show that polyculture overyielding can be predominant when considering total yield, especially with monocot/non-monocot crop combinations (Trenbath 1974; Vandermeer 1989; Picasso *et al.* 2011), and that polycultures often benefit biodiversity (Kremen & Miles 2012).

Implications for management and policy

Our findings have important implications for scientists, farmers, policy-makers, and society at large. A critical issue facing our world today is how we can produce and justly distribute sufficient food for a growing population while simultaneously minimizing adverse impacts on other important ecosystems services (Foley *et al.* 2011). Current global levels of agricultural intensification reflect a trend of simplifying agricultural systems for increasing production and have resulted in monocultures dominating the agricultural landscape in many regions of the world (Glaeser 2011). This intensification, which is expected to continue given projections of food demands to double by 2050 (Tilman *et al.* 2011), has had several negative effects on the health of ecosystems and the life that depends on them (Tilman 1999). Our study shows that polycultures consistently enhance biocontrol services and, depending on the context, may provide yield benefits. Our results show that win-win relationships between per plant yield and biocontrol may be achieved by reducing intraspecific competition through partial substitution of primary crops for secondary crops. For per unit area yield, our results suggest that fields incorporating harvestable legumes as a secondary crop have the best potential for win-win relationships when fields are at high cropping densities (i.e. within an additive framework). However, several other considerations, including crop value, crop–crop compatibility, and farmer preference, will be important in determining the crops to plant and the proportions in which to plant them. Finally, we urge for a greater investment in researching the underlying relationships between multiple agroecosystem services so we can better achieve agroecosystem multifunctionality.

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Table 4.1. Log-response ratios for primary crop yield and biocontrol

		Yield			Biocontrol			
		Mean	%Δ*	*P*†	Mean	%Δ*	*P*†	N
Overall	Additive	-0.279	-24.3	**0.038**	0.306	35.7	**0.016**	184
	Substitutive	0.339	40.4	**0.000**	0.273	31.4	**0.017**	117
Biocontrol variable‡	Herbivore	-0.075	-7.2	0.438	0.390	47.6	**0.002**	149
	Damage	0.016	1.6	0.783	0.230	25.8	**0.032**	98
	Predator	0.001	0.1	0.853	0.256	29.2	0.075	54
Primary crop	maize (sub)	0.516	67.5	**0.001**	0.212	23.7	0.178	39
	legume (sub)	0.343	40.9	**0.007**	0.192	21.2	0.132	30
	other (sub)	0.453	57.3	**0.000**	0.336	40.0	**0.013**	48
	maize (add)	-0.235	-20.9	**0.046**	0.403	49.6	**0.011**	109
	legume (add)	-0.152	-14.1	0.061	-0.020	-2.0	0.880	40
	other (add)	-0.489	-38.7	0.055	0.445	56.0	**0.045**	35
Secondary crop: Legume or non-legume	non-legume (sub)	0.399	49.0	**0.000**	0.246	27.9	0.054	61
	legume (sub)	0.415	51.5	**0.026**	0.308	36.1	0.103	50
	non-legume (add)	-0.371	-31.0	**0.012**	0.064	6.6	0.344	73
	legume (add)	-0.166	-15.3	0.214	0.555	74.1	0.052	102
Secondary crop: Harvested or not	harvested (sub)	0.367	44.3	**0.000**	0.304	35.5	**0.032**	97
	not harvested (sub)	0.273	31.4	**0.034**	0.169	18.4	0.074	20
	harvested (add)	-0.190	-17.3	**0.002**	0.126	13.5	0.322	82
	not harvested (add)	-0.396	-32.7	0.075	0.398	48.8	**0.023**	101
Region	tropical (sub)	0.373	45.2	**0.000**	0.288	33.3	0.056	51
	temperate (sub)	0.301	35.1	**0.013**	0.257	29.3	0.155	66
	tropical (add)	-0.181	-16.6	0.081	0.282	32.5	0.082	124
	temperate (add)	-0.623	-46.4	0.054	0.389	47.6	0.113	60

all tropical	0.279	32.2	**0.022**	175
all temperate	0.360	43.3	**0.012**	126

*Bold indicates significance at *P*<0.05 level.

†Refers to the percent difference in log-response ratios between the monoculture and polyculture values.

‡Sign for plant damage and herbivore abundance values has been switched, such that a positive value for each of these reflects a beneficial biocontrol effect. Additive and substitutive studies were combined.

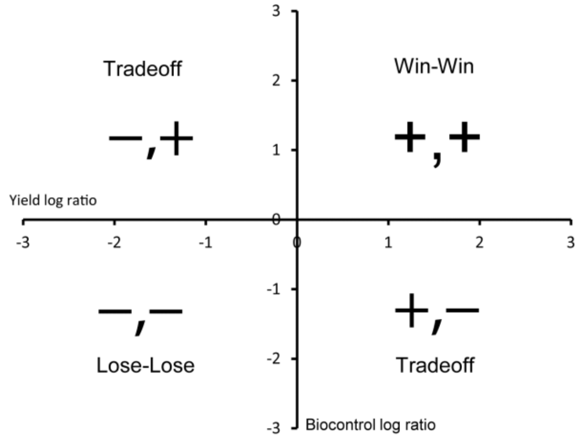

Figure 4.1. Graphical display of outcome scenarios for log-response ratios of yield and biocontrol.

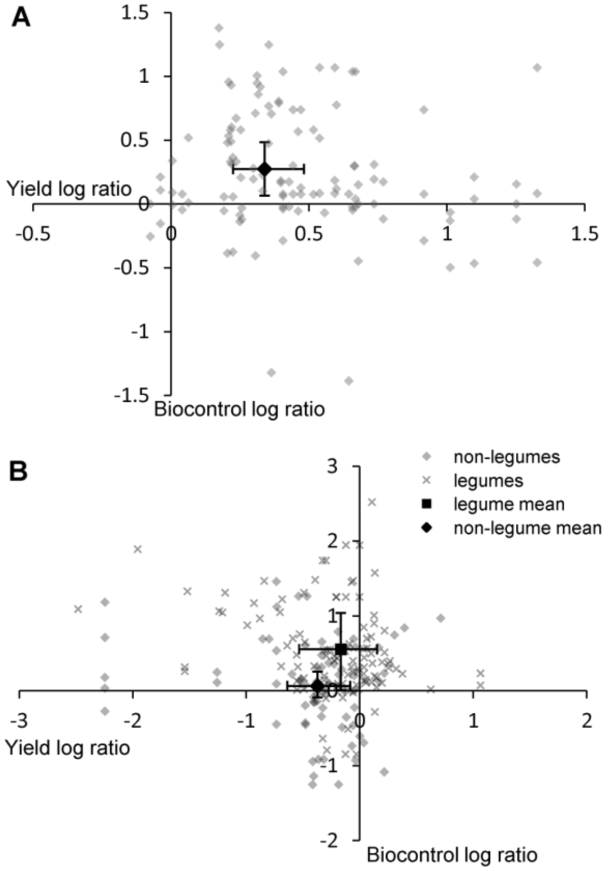

Figure 4.2. Log-response ratios of yield and biocontrol for a) substitutive and b) additive design experiments. In a), large diamond = model mean estimate for all studies ±95%CI. In b), large square = model mean for observations with a legume as secondary crop; large diamond = model mean for observations with a non-legume as secondary crop. Spearman rank correlation: a) all substitutive observations: ρ = -0.178 (N=117, *P*=0.055), b) all additive observations: ρ = -0.085 (N=184, *P*=0.252).

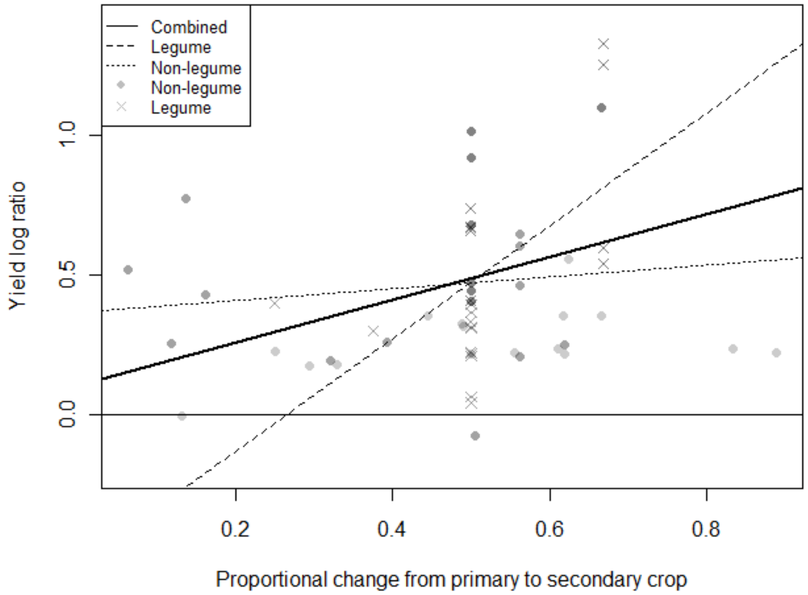

Figure 4.3. Proportion of primary crop substituted for secondary crop (legume, non-legume, and combined) vs. primary crop yield response ratio. Trendline for legume and non-legume combined: $R^2 = 0.1456$ ($P<0.001$, N=117); trendline for legume only: $R^2 = 0.310$ ($P<0.001$, N=50); trendline for non-legume only $R^2 = 0.0004$, ($P=0.31$, N=61). Sample size of combined values is larger than sum of subsets as some studies included a mix of both legumes and non-legumes as secondary crops.

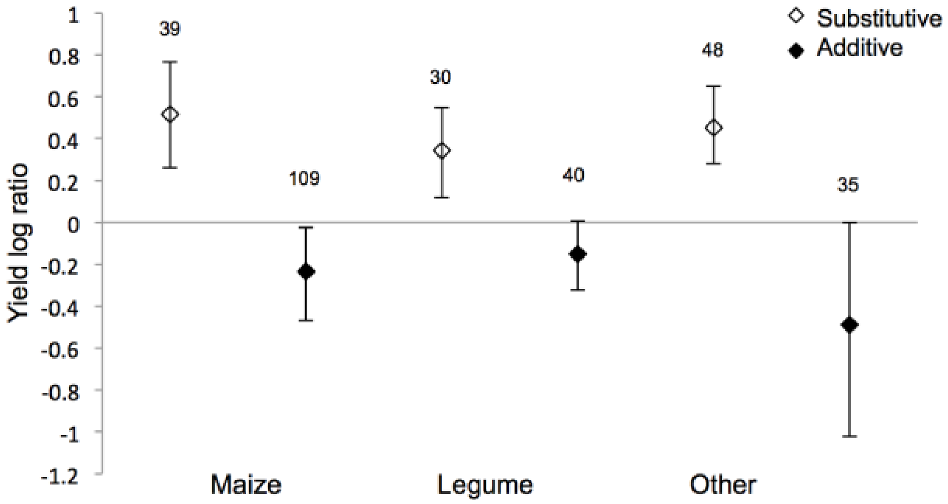

Figure 4.4. Yield log-response ratios (model mean estimate ± 95%CI) for different primary crop groups. 'Other' crops include wheat, cotton, tomato, zucchini, collards, broccoli, and oilseed rape. Numbers above points indicate sample size.

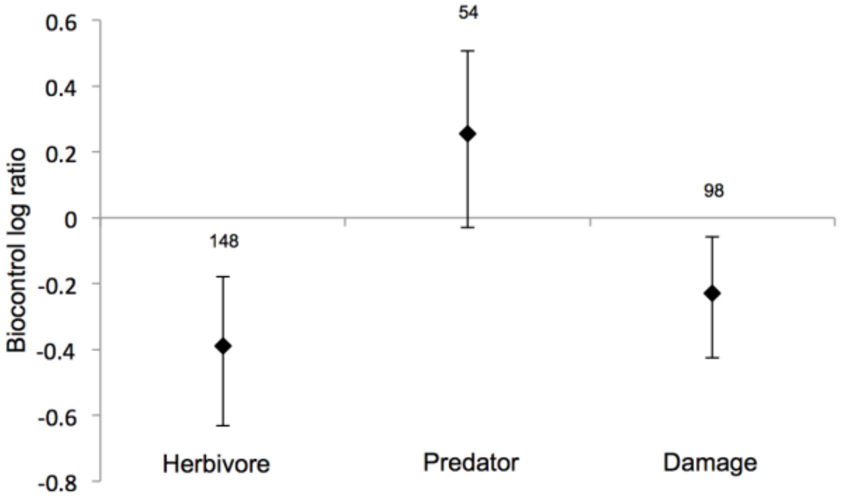

Figure 4.5. Biocontrol response ratios (model mean estimate ± 95%CI) for each metric of biocontrol. Numbers above points indicate sample size.

Table S4.1. Annotated list of all studies included in the meta-analysis.

Study	N^0 Obs*	Primary crop(s)	Secondary crop(s)	Secondary crop type	Biocontrol metric(s)	Design	Region	Country	N**
Arim *et al*. 2006	12	maize (*Zea mays*)	jack bean (*Canavalia ensiformis*)	cover crop	herbivore abundance, plant damage	add	tropical	Kenya	3
Belay *et al*. 2009	24	maize (*Zea mays*)	haricot bean (*Phaseolus vulgaris*)	crop	herbivore abundance, plant damage	add	tropical	Ethiopia	3
Chabi-Olaye *et al*. 2007	24	maize (*Zea mays*)	cassava (*Manihot esculenta*), cowpea (*Vigna unguiculata*), soybean (*Glycine max*)	crops	herbivore abundance, plant damage	sub	tropical	Cameroon	4
Gianoli *et al*. 2006	4	maize (*Zea mays*)	common bean (*Phaseolus vulgaris*)	crop	herbivore abundance, predator abundance	add	tropical	Peru	3
Hooks & Johnson 2004	6	broccoli (*Brassica oleracea*)	*Trifolium fragiferum, Trifolium repens, Melilotus officinalis*	cover crop	herbivore abundance, predator abundance	add	tropical	USA (Hawaii)	4
Hooks *et al*. 1998	7	zucchini (*Cucurbita pepo*)	buckwheat (*Fagopyrum esculentum*), yellow mustard (*Synapis alba*), *Chloris inflata, Hyptis pectinata, Amaranthus* sp., *Coronopus didymus, Commelina diffusa, Ipomoea* spp.	cover crops, weeds	herbivore abundance, predator abundance	add	tropical	USA (Hawaii)	4
Hummel *et al*. 2009	1	oilseed rape (*Brassica napus*)	wheat (*Triticum aestivum*)	crop	plant damage	sub	temp	Canada	4

Lenardis *et al*. 2011	6	Soybean (*Glycine max*)	*Artemisia annua*	crop	herbivore abundance	add	temp	Argentina	3
Letourneau 1986	1	maize (*Zea mays*)	squash (*Cucurbita moschata*), cowpea (*Vigna unguiculata*)	crop	herbivore abundance	add	tropical	Mexico	2
Maluleke *et al*. 2005	36	maize (*Zea mays*)	lablab bean (*Lablab purpureus*)	cover crop	herbivore abundance	add	temp	South Africa	4
Matama-Kauma *et al*. 2006	20	maize (*Zea mays*)	*Panicum maximum, Pennisetum polystachion, Sorghum arundinaceum*	trap crops	plant damage	add	tropical	Uganda	3
Nabirye *et al*. 2003	1	cowpea (*Vigna unguiculata*)	sorghum (*Sorghum bicolor*)	crop	plant damage	sub	tropical	Uganda	10
Nordlund *et al*. 1984	45	maize (*Zea mays*), common bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*)	maize (*Zea mays*), common bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*)	crops	herbivore abundance, predator abundance, plant damage	sub	temp	USA	6
Nyasani *et al*. 2012	18	common bean (*Phaseolus vulgaris*)	sunflower (*Helianthus annuus*), maize (*Zea mays*), potato (*Solanum tuberosum*)	crops	herbivore abundance, predator abundance	add	0	Kenya	4
Ogol *et al*. 1999	6	maize (*Zea mays*)	*Leucaena leucocephala*	hedgerow	herbivore abundance, plant damage	sub	tropical	Kenya	5
Ramalho *et al*. 2012	8	cotton (*Gossypium hirsutum*)	fennel (*Foeniculum vulgare*)	crops	herbivore abundance	sub	tropical	Brazil	4

Rao 2007	16	pigeonpea (*Cajanus cajan*)	blackgram (*Vigna mungo*), castor bean (*Ricinus communis*), greengram (*Vigna radiata*), peanut (*Arachis hypogaea*), sorghum (*Sorghum bicolor*), soybean (*Glycine max*), maize (*Zea mays*), sunflower (*Helianthus annuus*)	crops	herbivore abundance, plant damage	add	tropical	India	20
Rodenhouse *et al*. 1992	6	soybean (*Glycine max*)	*Festuca* sp., *Poa pratensis*, *Trifolium hybridum*, *Solidago canadensis*, *Setaria faberii*, *Aster pilosus*, *Festuca eliator*, *Poa compressa*	weeds (corridor)	herbivore abundance, predator abundance	sub	temp	USA	3
Schader *et al*. 2005	8	cotton (*Gossypium hirsutum*)	basil (*Ocimum basilicum*)	trap crop	predator abundance, plant damage	sub	tropical	Egypt	4
Schellhorn & Sork 1997	8	collards (*Brassica oleracea*)	*Barbarea vulgaris*, *Brassica nigra*, *Brassica kaber*, *Raphanus raphanistrum*, *Trifolium pratense*, *Polygonum persicaria*, *Taraxacum officinale*, *Phytolacca americana*	weeds	plant damage	add	temp	USA	4
Sekamatte *et al*. 2003	12	maize (*Zea mays*)	soybean (*Glycine max*), peanut (*Arachis hypogaea*), common bean (*Phaseolus vulgaris*)	crops	plant damage	add	tropical	Uganda	5

Showler & Greenberg 2003	10	cotton (*Gossypium hirsutum*)	*Amaranthus* spp., *Ambrosia artemisiifolia*, *Physalis heterophylla*, *Euphorbia* sp., *Urochloa texana*, *Croton capitatus*, *Portulacca oleracea*, *Verbesina encelioides*, *Cyperus rotundus*	weeds	herbivore abundance, predator abundance, plant damage	add	temp	USA	6
Skelton & Barrett 2007	4	wheat (*Triticum aestivum*)	alfalfa (*Medicago sativa*)	crop	herbivore abundance, predator abundance	sub	tropical	Philippines	3
Villamajor 1976	4	cotton (*Gossypium hirsutum*)	peanut (*Arachis hypogaea*), various weeds	crop, weeds	herbivore abundance	add	tropical	Philippines	3
Weiss *et al*. 1994	6	oilseed rape (*Brassica napus*)	pea (*Pisum sativa*)	crop	herbivore abundance	sub	temp	USA	5
Williams *et al*. 1995	8	soybean (*Glycine max*)	sorghum (*Sorghum bicolor*)	crop	parasitism rate, herbivore abundance	sub	temp	USA	2

*****Number of observations recorded from each study

**Sample size (i.e. number of paired crop fields assessed)

Table S4.2. Rosenthal's fail-safe numbers.

	Substitutive*	**N****	**Additive***	**N****
Yield	1,075	39	145,444	150
Biocontrol	299	35	6,251	79

*Fail-safe numbers refer to the number of non-significant or unpublished studies that would be required to change the results of the meta-analysis from significant to non-significant.

**Number of observations. Not all studies reported extractable variance data and therefore not all observations could be included in this calculation.

Table S4.3. Spearman rank correlation between effect sizes of primary crop yield and each of three biocontrol variables according to additive and substitutive designs.

Study Design	**Biocontrol Variable**	**Rho***	**p****
Additive	Herbivore abundance	0.180	0.075
	Plant damage	-0.228	0.075
	Predator abundance	-0.589	**0.003**
Substitutive	Herbivore abundance	0.143	0.322
	Plant damage	0.420	**0.011**
	Predator abundance	0.0137	0.942

*Values indicate correlation and direction.
** Bold indicates significance at $p<0.05$ level.

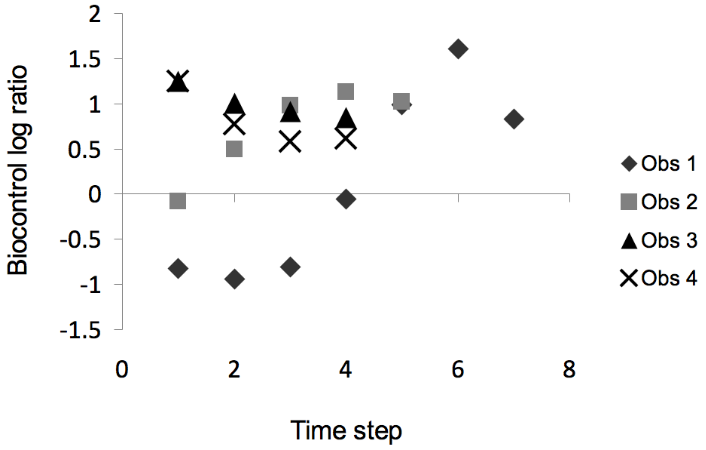

Figure S4.1. Observations with time series having a significant ($p<0.05$) linear or quadratic trend. Observation 1 and 2 have a significant linear and quadratic regression; observation 3 has a significant linear regression; observation 4 has a significant quadratic regression.

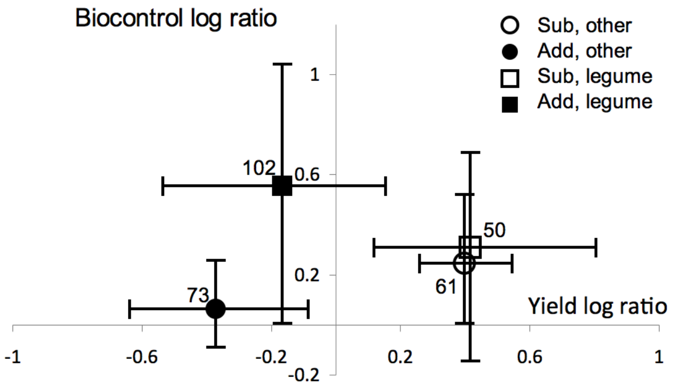

Figure S4.2. Yield and biocontrol log-response ratios (model mean estimate ± 95%CI) by type of secondary crop (legume vs. non-legume). Open symbol = substitutive studies, closed symbol = additive studies, squares=secondary crop is legume, circles=secondary crop is non-legume. Non-legumes include sorghum, basil, fennel, cassava, baby corn, sunflower, grass species, buckwheat, wheat, squash, castor bean, Brassicaceae species, wormwood, tomato, and weeds. Numbers above points indicate sample size.

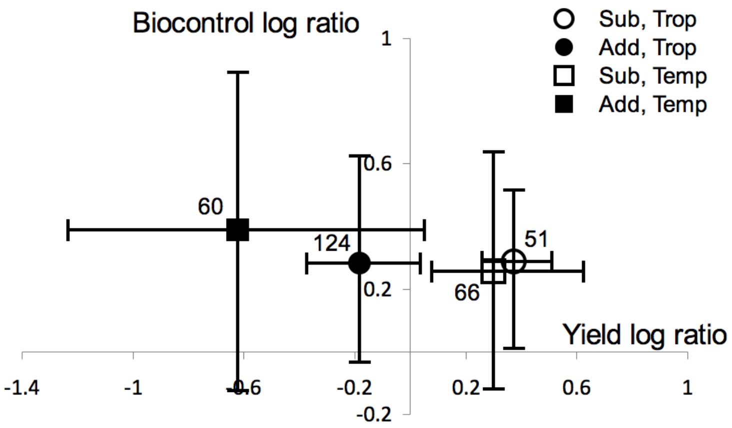

Figure S4.3. Yield and biocontrol log-response ratios (model mean estimate ± 95%CI) depending on geographical region. Open symbols = substitutive studies, closed symbols = additive studies, squares=temperate studies (>23.5^0 N and S latitudes), circles=tropical studies (<23.5^0 N and S latitudes). Numbers above points indicate sample size.

Appendix 4.1. Search terms for article selection.

We conducted a literature search on 18 December 2011 in ISI Web of Science using the keyword sequence: [Yield* AND (agr* OR agroecol*) AND (intensity OR monoculture* OR polyculture* OR intercrop* OR management OR diversity OR biodiversity) AND (“biological control” OR biocontrol OR pest* OR damage OR arthropod* OR insect* OR “natural enem*” OR predator* OR parasit* OR spider* OR beetle* OR wasp*)]. We accepted papers from any year.

CHAPTER V

Resolving a key dilemma in ecological complexity and farmer livelihoods: the question of agroecosystem multifunctionality[5]

Abstract

Agroecosystems are inherently multifunctional—providing multiple ecosystem services that maintain rural livelihoods and feed the world. Furthermore, the importance of ecological complexity within farms is increasingly being recognized for its importance in biodiversity conservation and sustainable land use. Today, more than ever before, society must address a critical challenge surrounding agriculture: can food production, livelihoods, ecological complexity, and biodiversity be simultaneously attained, or is multifunctionality prevented by tradeoffs? In coffee agroecosystems in Mexico and Puerto Rico, we assess both the provision of key agricultural ecosystem services that impact farm profit, and the biodiversity of several important taxonomic groups belonging to vertebrates, invertebrates, and plants. We then identify how ecological complexity influences the provisioning of multiple ecosystem services or multiple biodiversity clades through a multiple threshold approach. We show that ecological complexity is important for biodiversity, especially in the relatively ecologically simple island of Puerto Rico. However, ecological complexity is not important for farm income-related ecosystem services due to a lack of positive relationships between individual services. Therefore,

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[5] Co-authors are Burnham, R., Ennis, K., Fisher, K., Gonthier, D., Hajian-Forooshani, Z., Pak, D., Perfecto, I., Vandermeer, J.

attaining the highest levels of ecological complexity, leading to higher biodiversity and long-term sustainability, and highest levels of farm profit may be difficult without economic incentives. We show that several realistic scenarios which properly incentivize ecologically complex farming or disincentivize farming systems with low complexity can shift the balance in favor of win-win outcomes.

Introduction

Two evident facts color contemporary concern with food production. First, agriculture, in recent history, has homogenized previously heterogeneous environments, seeking and frequently attaining very high levels of productivity, but at the expense of ecological complexity (Matson *et al.* 1997; Tscharntke *et al.* 2005). Second, ecological complexity long has been appreciated for its functional utility in the long-term sustainability of agroecosystems and, more recently, for its role in maintaining biodiversity (Lewis *et al.* 1997; Perfecto & Vandermeer 2008; Perfecto, Vandermeer & Wright 2009; Tscharntke *et al.* 2011). Although frequently not acknowledged explicitly, these evident facts stand in contradiction. A key stumbling block in moving toward resolving this contradiction is the fact that the benefits provided by ecological complexity are multidimensional, yet they are commonly treated as individual and isolated issues (Bennett, Peterson & Gordon 2009; Gamfeldt *et al.* 2013). If benefits are isolated, we lose the ability to consider tradeoffs and synergies between services, leading us to improper conclusions or misguided management recommendations (Power 2010; Kremen & Miles 2012; Balvanera *et al.* 2014). Here, we explore some key functions of ecological complexity in agroecosystems, and calculate the consequences of recalibrating incentive/disincentive structures on the operation of those functions.

Given the precarious nature of farming and the relatively recent inclusion of many small-scale farmers into the cash economy, in addition to production-oriented agricultural policies, farmers are often pressured to consider short-term profits. However, for farmers the benefits from ecological complexity are realized on longer time scales. For example, soil carbon loss in an intensified farm may take as long as 20 years before its effects are discernible from background noise (Rasmussen *et al.* 1998). Other benefits of ecological complexity are only realized in uncommon, but probable over the long term, catastrophic events such as natural disasters or pest and disease epidemics (Philpott *et al.* 2008b; Avelino *et al.* 2015). Therefore, if short-term profits do not align with long-term sustainability, farmers may have to choose one at the expense of the other.

Here, we quantify the influence of ecological complexity on multiple ecosystem services that directly impact short-term farm profit, such as biocontrol and coffee yield. We approximate ecological complexity of an agroecosystem through an index consisting of structural and taxonomic diversity attributes. We then assess how economic incentives or disincentives could reconcile the potential conflict between short-term profit and long-term sustainability of farms. Furthermore, we investigate the impact of ecological complexity on multiple organism clades, and determine whether individual components of ecological complexity can sustain multiple clades simultaneously. We use the coffee agroecosystem as a model system. It is replicated around the world, managed in a variety of styles, provides a livelihood for millions of small-scale farmers in the developing world, and is the economic base for several tropical countries (Daviron & Ponte 2005; Perfecto, Vandermeer & Philpott 2014; Perfecto & Vandermeer 2015). We compare coffee farms in a diverse and ecologically complex setting (Chiapas, Mexico) with farms in a more species-poor and less ecologically complex setting (Puerto Rico) to understand how the ecological setting influences outcomes of multifunctionality.

Methods

Mexico

Site description

We selected 38 sites spanning 11 contiguous, large (~300 ha) coffee farms in the montane wet forest Soconusco region of Chiapas, Mexico. Sites ranged in elevation from 600 to 1300 m asl, and rainfall averages ca. 4500mm annually (Philpott & Bichier 2012). Farms spanned a range of management intensity, from low shade (2.5% canopy cover) to high shade (94% cover). All farms retained a moderate diversity and abundance of shade trees (>100 individuals/ha). On the most intensively managed farms, most of the shade trees (>80%) were *Inga* spp. (predominantly *I. micheliana* and *I. vera;* Fabaceae). Sites within farms were selected based on: 1) maximizing the distance between sites (minimum distance 350m) and 2) varying distance to forest fragments. A point within the coffee plantation that satisfied these requirements was identified, and this became the center of each site from which we based all surveys and experiments. Although “Robusta” coffee (*Coffea canephora*) was found on lower elevation farms, we focused our study in areas that were pure “Arabica” coffee (*Coffea arabica*).

Ecosystem service assessment

Five coffee bushes per site were selected as sampling locations for several of the ecosystem services measured. To select bushes, we located a point 5 m from the plot center in the following directions: 0^o, 72^o, 144^o, 216^o, 288^o, then selected the nearest bush to this point with >100 berries. We refer to the five bushes as ‘focal bushes’.

We assessed biocontrol by measuring the predation rate on two coffee pests: the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae), and the green coffee scale, *Coccus viridis* (Hemiptera: Coccidae) in June 2012. The coffee berry borer (CBB) is considered one of the most devastating pests of coffee, while the green coffee scale (GCS) can have significant impacts on coffee growth, but rarely reaches this pest status in Mexico (Vandermeer, Perfecto & Philpott 2010). To assess predation of CBB, we cut individual coffee branches with >25 berries, placed the branch into a 30 ml floral tube (Dakota Plastics Co., Watertown, SD, USA), and removed all berries already infested with CBB. Then, we deposited 20 CBB on top of the branches and allowed them to drill into the berries over the course of 12 h. We counted the total number of CBB that successfully infested berries, and placed five experimental branches per site, one on each of the five focal bushes. We left them in the field for 5 d, then collected the branches and dissected the berries to determine how many CBB were predated.

To measure biocontrol of the green coffee scale (GCS), we collected coffee leaves infested with GCS from the field and placed individual branches with two leaves each in a 10 ml floral tube. We then inspected each leaf under a dissecting microscope, removed any individuals showing any signs of predation (chew marks) or sickness (discoloration), and counted the total number of individuals > 2.0 mm in length. To avoid counting GCS that transitioned from <2.0 mm to >2.0 mm during the course of the experiment, we removed those that were slightly too small to be included in our count. We then placed one branch with a floral tube on each of the five focal bushes per site. After 3 d, we collected the branches and observed them under a stereoscope in order to count the number of GCS >2.0 mm that showed any sign of predation. This counting was possible because beetle predators of the family Coccinellidae often leave behind part of the larger GCS with obvious signs of predation.

To measure coffee yield, we divided each of the five focal bushes into three strata, each with approximately the same number of fruiting branches. In each stratum we randomly selected three branches and counted the number of berries on each branch (nine branches per bush). We then counted the total number of fruiting branches on the entire bush and multiplied this number by the average number of berries counted in the nine branches. We also assessed the naturally occurring prevalence of coffee leaf rust, *Hemileia vastatrix*, on the focal bushes by randomly selecting three branches from each stratum and counting the number of leaves and the number of leaves with coffee rust. These counts were performed in June 2012.

Biodiversity sampling

We selected seven clades that are beneficial to coffee through pest control or pollination. Clades were selected to represent a broad range of functional and taxonomic diversity.

We collected parasitoid wasps (once in May/Jun 2012), and bees (twice: in May/Jun 2012 and Mar 2013) using pan traps (Abrahamczyk, Steudel & Kessler 2010; Nuttman *et al.* 2011). Traps consisted of blue, yellow, and white 355-ml plastic bowls glued to a 1” PVC coupling. Each blue and yellow bowl was sprayed internally with a thin layer of blue or yellow ultra-violet paint, respectively (Clearneon, Inc.: Wichita, Kansas). We placed two sets of traps, all attached to a 1” PVC pipe, at the center of each site. One set was placed at a height of 0.25 m and the other at 1 m above ground level. Each set consisted of 3 traps, each of a different color, clustered together and separated by approximately 1 m from the other set. Traps were filled with 180 ml of solution containing water, soap (blue Dawn® dish soap; Procter and Gamble, Cincinnati, OH, USA), and honey solution (1 l water, 10 ml soap, 15 ml honey). Traps were placed in each site for approximately 5 h between 7am and 2pm on sunny, low-wind days.

From 31 Jan to 22 Feb 2013, we sampled birds at five locations per site in an approximate 'X' formation, with one point at the center of the plot and a point on each tip. All points were separated by ca. 100 m. One person recorded all birds heard and seen within 25 m of each point during a period of 10 min at each point. Birds flying overhead were not counted.

We sampled for ground-foraging and arboreal ants at each site by placing ca. 1 g of oil-based canned tuna fish at five locations on the ground and at five locations ca. 1 m from the ground on coffee bushes. After 20 min, all ant species on or immediately around the baits were identified or collected to assess species richness. To sample spiders, we removed two branches from each of three separate *Inga* spp. trees per site (for detailed methodology, see Hajian-Forooshani et al (2014)).To assess shade tree species richness, we counted and identified every tree >10.0 cm diameter at breast height (dbh) within a 1-ha area around each plot center. To sample groundcover plants, we recorded the groundcover species richness in five 0.5 m^2 quadrats established at 5 m from the plot center in the following directions: 0^o, 72^o, 144^o, 216^o, 288^o. Species lists of clades sampled are presented in Appendix 5.1.

Environmental covariates

At each site, we measured a 1-ha circular area (radius 56 m) surrounding the plot and identified every tree >10.0 cm dbh. We also recorded the number of *Azteca sericeasur* nests, and the distance to the nearest nest from the plot center. This arboreal-nesting ant is a keystone species that influences the biocontrol of green coffee scales, coffee berry borer, and coffee leaf rust in the Mexico study area (Vandermeer & Perfecto 2006; Vandermeer, Perfecto & Philpott 2010; Jackson, Skillman & Vandermeer 2012; Perfecto, Vandermeer & Philpott 2014). We measured tree shade density using a convex spherical densiometer (Forestry Suppliers, Inc., Jackson, MS, USA) at three points in each site, equidistantly spaced 5 m from the site center. In each of the three points, we measured the shade density in the four cardinal directions. We measured groundcover extent (% coverage) in five 0.5 m^2 quadrats set at 5 m from the plot center every 72^0. Groundcover extent was estimated using a 7-unit percentage scale of: 0-1, 2-5, 6-20, 21-40, 41-60, 61-80, and 81-100 percent. We counted the number of open flowers present on groundcover species (in two 15 x 2 m transects) and the number of flowering trees in the sampled hectare. To assess coffee cover, we measured the number of coffee bushes in a 15 m x 15 m square centered on the plot center. To quantify aboveground woody plant biomass, we measured the dbh and visually estimated the height of every plant $\geq$ 1.0 cm dbh within a 15 m radius of the plot center. The aboveground plant biomass of this area was calculated according to the formula:

$$\log (B) = -3.375 + 0.948 \log(D^2H)$$

where B is aboveground biomass (kg $tree^{-1}$), D is dbh, and H is plant height (Brown & Iverson 1992). This estimate is a rough proxy, as species-specific allometric equations for all but the most common tropical species have not been determined. As pesticide use could affect the abundance of various organisms, we included whether a farm was organic or conventional in our analysis. Synthetic chemical use on farms was determined based on knowledge of the farm’s practices and certifications. Non-organic farms used glyphosate as an herbicide and often endosulfan as a pesticide for the coffee berry borer (Curculionidae: *Hypothenemus hampei*).

Ecological complexity

We condensed vegetation richness and structure variables into an ecological complexity index (ECI) consisting of the following taxonomic and structural complexity values: tree species richness, groundcover species richness, groundcover extent (percent cover), groundcover height, shade tree biomass, shade density, and shade tree density. Although ecological complexity entails much more than what we could measure (e.g., non-linearities, indirect effects, networks, etc.) (Vandermeer, Perfecto & Philpott 2010), we consider our index to be an appropriate proxy for complexity. For analyses which incorporated individual clades or ecosystem services (see below), we considered the influence of three separate levels of complexity. These levels were taxonomic diversity (i.e., species richness), structural complexity (e.g., shade density, tree size, groundcover extent), or landscape complexity (e.g., percent forest cover).

Puerto Rico

Site description

We selected 36 coffee farms across the central eastern mountains of Puerto Rico, which receive ~1800-2300 mm of rainfall annually (NOAA National Weather Service) and are classified as submontane and lower montane wet forests (Helmer *et al.* 2002). Farms were located at least 1 km apart, ranging in elevation from 250 to 850 m asl. Farms represented an intensification gradient from no shade to high shade (84%). At each farm, we established one plot by choosing a representative area of 25 m x 25 m that contained coffee throughout. In each site, nine locations that were equidistantly spaced (9 m apart) were used for several of the biodiversity surveys and ecosystem service measurements. "Arabica" coffee was the sole coffee species in all plots.

Ecosystem service sampling

Two primary pests affect coffee in Puerto Rico: the coffee berry borer (CBB) and coffee leafminer (Lepidoptera: *Leucoptera coffeella*). We measured coffee leafminer parasitism rates by collecting 100 leafminer pupae from each plot, which often are attached to the underside of coffee leaves, and reared them in containers. We monitored pupae for 20 d and recorded the numbers of unparasitized leafminers and parasitoids that emerged. Collections occurred from 14

May to 22 Jun 2013. We sampled for CBB abundance by randomly selecting five coffee bushes per farm and counting the number of bored berries out of 100 total berries per bush from randomly selected branches. To measure coffee leaf rust, we selected the nearest non-rust resistant (i.e., no Arabica x Robusta crosses) coffee bush to each of the nine points (see above) in each plot. On each bush we randomly selected branches from each of three strata on the bush and counted all leaves until we reached a total of 100. Rust incidence was recorded as presence/absence per 100 leaves; surveys were carried out in May 2013.

To measure vertebrate predation of coffee pests, we constructed an exclosure experiment. In each plot we selected three sets of two similarly sized and similarly yielding coffee bushes. In each set we randomly selected one bush to be a bird/bat/anole exclosure, covering the top 1 m of the bush with 6.4 mm x 6.4 mm mesh netting, cinched at the base. The mesh size was chosen to exclude all but the smallest anoles but to allow pests and other arthropods to freely pass through. The other bush of each set was a control and was agitated similarly to the other bushes but received no mesh exclosure. Exclosures were placed in the field between 4 May and 18 May 2013 and were removed between 16 Aug and 30 Aug 2013. All berries infested with borers at the time of set-up were removed. Upon collection, we observed up to 300 berries on each bush and recorded borer incidence. On each bush we also sampled up to 50 new leaves, which had emerged since the time of exclosure construction, and recorded the number of leaves with leafminer damage or general herbivory (i.e., primarily herbivorous beetles and Orthopterans). Finally, we visually searched each bush for the planthopper *Petrusa epilepsis* (Hempitera: Flatidae) on each bush for a period of 3 min. This species is not known to be a significant pest in Puerto Rico, but can reach high numbers in some farms and could be a potential vector of plant diseases. The absence of leaf damage, rust, or planthoppers compared to other sites was considered a biocontrol benefit.

We assessed coffee yield through economic interviews with each farmer. As coffee yield is an incomplete picture of farm income, we also interviewed farmers to assess farm inputs and non-coffee outputs. Inputs included money spent on fertilizers, pesticides, and labor, and outputs included income gained from intercrops (e.g., plantains, citrus).

Biodiversity sampling

We selected seven different clades beneficial to coffee, representing a broad range of functional and taxonomic diversity. We sampled parasitoid wasps and bees from 29 Mar to 2 May 2013 using yellow, white, and blue pan traps. Pan trap construction and liquid solution were identical to those used in Mexico (see above). Blue and yellow-colored bowls were coated with blue and yellow invisible ultra-violet reactant paint, respectively (Black Light World; Cub Run, KY, USA). In each site we placed nine pan traps (three of each color), each separated by 9 m, beginning in the morning hours on a sunny day. Traps were left for 5 h, after which specimens were collected and identified to family and morphospecies in the lab.

To sample birds, two people performed a 30-min point count in each site from 16 Aug to 28 Aug 2013. We included any birds that were present within the 25 x 25 m plot plus a 10 m buffer area on all sides. If birds flew below the top of the shade tree canopy, or the equivalent height in sun farms (~20 m), they were included in the counts. From 16 Aug to 27 Aug 2013, we sampled ants in each site using oil-based tuna fish baits. We placed ~1 g of tuna fish at 25 locations per site (every 4.5 meters in a grid). Baits were allowed to sit for 20 min and then all ants were identified or collected for later identification. To sample anole lizards (*Anolis* spp.), we walked two transects measuring 25 m x 4 m along a row of coffee bushes in each plot. Within each transect, we searched the ground and all vegetation for anoles. Surveys were conducted from 28 Jun to 30 Aug 2013. For tree richness, we counted the number of species ≥1.0 cm dbh present in each plot. For groundcover plants, we measured the number of species within a 0.5 m^2 quadrat, placed on the uphill side of each of the nine points in the plot. Groundcover plant surveys were performed in May and Jun 2013. Species lists of clades sampled are presented in Appendix 5.1.

Environmental covariates

We measured the shade density provided be canopy trees at the nine points in each plot in the same manner as in Mexico. From a height of 60 cm we also separately measured the shade density resulting from the coffee bushes, as this can form a thick understory canopy. We measured the girth and estimated the height of all plants ≥1.0 cm dbh in the plot. We calculated tree biomass according to the same formula as in Mexico. For groundcover plants, we measured the percent cover of all plants within a 0.5 m^2 quadrat, placed on the uphill side of each of the nine points in the plot. As floral resources could influence the abundance of bees, wasps, and other arthropods, we counted the number of flowers present in the groundcover plants in three 25 m x 2 m transects in each plot. We also calculated an ecological complexity index (ECI) using several vegetation variables, as we did for Mexico data (see above).

Data analysis: Mexico and Puerto Rico

Richness estimators

If the species accumulation curves of a taxon satisfactorily approached an asymptote for individual sites, we calculated per-site species richness estimates for that taxon. This approach allowed us to assess the probable richness of a taxon had sampling measures permitted us to capture all species present in the area. In Mexico, we estimated species richness for trees (using incidence coverage-based estimation) (Colwell *et al.* 2012) and birds (using Chao 1 estimation) (Chao *et al.* 2005). In Puerto Rico, we estimated species richness for both parasitoid wasps and ants with Chao 1 estimation. All estimates were calculated using EstimateS (Colwell *et al.* 2012).

Landscape analysis

To quantify landscape composition in Mexico, we manually digitized all coffee farms and forests using the basemaps provided in ArcGIS 10.1 (ESRI, Redlands, CA, USA), and assigned farms to one of three levels of intensity according to the amount of shade over the coffee. In Puerto Rico, we manually digitized all coffee farms within a 1 km radius of each of our plots using ArcGIS basemaps. We then converted this coffee layer to raster format and joined it with a 15-m spatial resolution Puerto Rico landcover dataset (Gould *et al.* 2008). For both countries, we created 500 m buffers around the center of each plot and calculated the amount of each land use category within the buffer. The 500 m radii were chosen to reflect dispersal distances of insects and to limit overlap with neighboring sites. For Mexico, the land use categories were coffee farm, forest, or developed land. Because a very high proportion of the landscape in our Mexico study area was coffee plantation (mean = 93% cover), we also divided the coffee land use into three intensity levels. For Puerto Rico, the land use categories were coffee agriculture, forest, and open lands, including grasslands, non-coffee agriculture, pastures, and barren land. In addition to landscape composition, we also determined the nearest distance to a forest fragment from the plot center as well as plot center elevation.

Individual ecosystem services and clades

To observe the effects of variables relating to plant taxonomic diversity, vegetation structure, or landscape complexity on individual clades and ecosystem services (Table S5.1), we used generalized linear mixed-effect models (GLMM). We created mixed models by designating each farm (N=11) as a random effect if variables were measured at the plot level, with farm and plot as random effects if variables were measured at the individual bush or quadrat level in the plot. Response variables (ecosystem services and clades) that were not normally distributed were first sqrt- or log-transformed or were drawn from Poisson distributions in the GLMM, depending on the type of variable and its distribution. For binary response variables (e.g., proportion predation), we utilized logistic regression. Explanatory variables that had outliers were sqrt- or log-transformed.

Ecologically relevant local- and landscape-scale environmental variables that were not overly collinear (variance inflation factor <3) were included in each GLMM as explanatory variables to model each ecosystem service or clade. We then removed unimportant variables stepwise through manual backward elimination until we reached a model that maximized the model fit according to the number of significant variables and AICc values. If competing models had negligibly different AICc values, we selected the model that returned the highest number of significant predictor variables. All GLMM analyses were done in R version 3.1.2 (R Core Team) using the packages ‘lme4’, ‘AICcmodavg’, and ‘car’.

Multifunctionality analysis

To assess the provision of multiple ecosystem services and the ability to retain multiple biodiverse clades in a given site, we first performed a multiple threshold analysis (Byrnes *et al.* 2014). Threshold analysis determines which sites have values for a set of response variables that fall above a certain set percentage of maximum functioning (i.e. the threshold) for each variable. For biodiversity, the threshold was set as a given percentage of the maximum richness observed in any one site. For example, if a threshold was set at 80% and five ecosystem services were included, we would record the total number of ecosystem services (out of 5) that reached at least

80% of their maximum value in a given site. If a highly multifunctional site exists, it will have a value close to five (each ecosystem service is highly functioning and is counted). However, choice of threshold is arbitrary. Therefore, a multiple threshold analysis assesses the degree of multifunctionality across all thresholds (5-99%). From this analysis, we determine the range of thresholds for which there is a significant response of an explanatory on multifunctionality (i.e. sites which have multiple services or multiple clades 'functioning' above or being more speciose than a given threshold). This analysis was done in R version 3.1.2 (R Core Team) using the package 'multifunc' (Byrnes 2014).

Value-based weights and alternative economic scenarios

One disadvantage to using the multiple threshold approach is that weighting different services or functions is difficult. Therefore, to further visualize multifunctionality, we used a second method, which we call a weighted summation approach (i.e. functionally equivalent to an averaging analysis) (Byrnes *et al.* 2014), by first standardizing all biodiversity clades and ecosystem services into z-scores. For ecosystem services, because each one is not of equal value to the farmer, we weighted each service z-score according its potential to benefit farm income (i.e., short-term profit), mostly through the protection of coffee from pests and diseases (Table S5.2). These weights were based on our own field surveys or on published accounts of a pest or disease on coffee yield, and are intended to represent the potential harm possible of the pest/disease under high infestation rates. For example, in Puerto Rico we recorded berry borer infestation rates as high as 26% on the worst-infested farms. Thus, it is realistic to see infestation rates this high in any farm given the appropriate conditions. As borers directly affect yield by drilling into the coffee beans, we weighted the value of borer predation as 26% that of yield. As coffee yield is an incomplete picture of farm income, we incorporated net profit as a separate weight that penalized farms that had higher expenses per unit area. For biodiversity, we simply summed each of the z-scores for each taxon from each site, resulting in an unweighted overall ‘multi-biodiversity’ score. We regressed this score with the ecological complexity index, given that this was the most important of all variables for ‘multi-biodiversity’ (see results, multiple threshold).

Additionally, we adjusted the weights to reflect four different scenarios in Puerto Rico, in each case determining how much incentive would be necessary to create a situation where there was a significantly positive relationship between short-term farm profit and the ecological complexity (as measured by ECI) of the farm. First, we considered a scenario where farmers with ecologically complex farms (ECI>0.5) received a premium for their coffee, such as through certification. Second, we considered a scenario where farmers received a payment-for-ecosystem services (PES) for carbon storage. Third, we considered certification and PES jointly by setting the coffee price premium at a currently realistic level (50% premium) and determining how much PES would be necessary to result in a positive relationship between profit and ecological complexity. Finally, we considered a scenario in which externalities for environmental degradation (e.g. soil erosion, flooding, landslides, biodiversity loss, and contributions to localized and global climate change) were passed on to the farms that contribute most to the degradation. Alternatively, this scenario could be envisioned as a restructuring of agricultural subsidies, which most often benefit intensively managed farms. We tested the effect of using externality payments to fund a payment-for-ecosystem service program for carbon storage or simply removing subsidies favoring intensive farms and adding subsidies which incentivize ecologically complex farms. Here, we simultaneously penalized the most intense farms (ECI<0) while adding the same weight to the shade tree biomass component until the regression was significantly positive. For scenarios involving PES or penalties, we calculated the monetary value ha^{-1} or t^{-1} that would make the regression significant by multiplying the weight applied to either ECI or biomass by the average farm profit ($844 ha^{-1}). We used profit instead of yield, as money subtracted or added from PES or certification would theoretically be net profit/loss, and not just additional yield value (much of which is lost due to the cost of inputs, such as labor, fertilizers, and pest control).

Results

Individual ecosystem services and clades

When we considered ecosystem services individually, we found that all three categories of ecological complexity (local species richness, local structure, landscape) were important, except for landscape complexity in Mexico, and negative relationships predominated over positive ones (Table 5.1, Table S5.3). For biodiversity, we found that most individual clades are primarily positively influenced by increased ecological complexity, although some negative relationships were also present. Furthermore, although all three levels of vegetation complexity were influential on at least one taxon, structural and landscape complexity were more influential than plant species richness. Additionally, we found that clades in Puerto Rico were more commonly influenced by ecological complexity than clades in Mexico.

Considering multiple services and clades simultaneously

To determine how individual farms can best simultaneously provision multiple ecosystem services or retain multiple biodiverse clades, we first performed a multiple threshold analysis with the same clades and services represented in Table 5.1. Using this approach, we determined that an ecological complexity index, taking into account all vegetation structural and diversity measures, was the best predictor of ‘multi-biodiverse’ farms in both countries (Fig. 5.1, Table

S5.4). No other predictor (any individual component of the index) displayed a significant effect on retaining multiple clades in Mexico, and only shade and biomass displayed a weak relationship in Puerto Rico. The strength of the relationship between the ECI and ‘multi-biodiversity’, as well as the range of significant thresholds (Fig. 5.1, Table S5.4), was stronger in Puerto Rico than in Mexico.

When we performed a multiple threshold analysis with ecosystem services, neither the ecological complexity index nor any individual component of the index resulted in a significantly positive relationship for Mexico. For Puerto Rico, the only significant explanatory variable was the amount of cleared land in the landscape (including agriculture, grasslands, and developed land), which had a narrow range of negative influence on multifunctionality (Fig. S5.1). This lack of significant relationships for multiple ecosystem service provision is rooted in the presence of several tradeoffs between individual services (Fig. 5.2). Interestingly, although it is a common assumption that more productive farms will be less biodiversity friendly, we did not find many significant tradeoffs between individual biodiversity clades and coffee production. In fact, only one taxon out of seven from each country exhibited a significantly negative relationship, although the composite value of all biodiversity in Mexico was marginally negatively related (Fig. S5.2).

Value-based weights and alternative economic scenarios

We also used a weighted summation approach to quantify ‘multi-biodiversity’ and multiple ecosystem service provision in Puerto Rico. Multi-biodiversity was strongly positively correlated with ECI (Fig. 5.3a), corroborating results from the multiple threshold approach. For ecosystem services, this approach allowed us to weight each service according to its short-term economic value to the farmer. Therefore, the value of multiple service provision may be equated to hypothetical farm income. We found that if all coffee received the same (market) price, there was a non-significant negative trend between multiple provision and ECI (Fig. 3b). We found that if all coffee received the same (market) price, there was a non-significant negative trend between multiple provision and ECI (Fig. 4b). However, if ecologically complex farms received a 150% price premium for coffee, this negative trend would shift to a significantly positive relationship (Fig. 4c). If PES for carbon were used as the lone incentive, \$52 t^{-1} of tree biomass would be necessary to result in a positive relationship between multiple service provision and ECI (Fig. 4d). As current premiums for organic, fair-trade, or shade coffee range between 30-50%, we tested the scenario with a more realistic price premium (50%) for ecologically complex coffee production, along with PES. Here, the carbon payments would need to be \$30 t^{-1} of tree biomass to tilt the economic balance significantly in favor of increased ecological complexity (Fig. 4e).

Since a major point of many actual and proposed agro-environmental incentives is to counter disservices, such as erosion, flooding, landslides, biodiversity loss, and contributions to localized and global climate change, we also found it useful to construct a hypothetical scenario where externalities were paid by the intensively managed farms that contribute most towards these disservices. We found that by penalizing farms with a low ECI and applying those funds towards PES for carbon storage, an average penalty/payment of \$532 ha^{-1} was sufficient to favor ecological complexity on farms (Fig. 4f).

Discussion

These data, analyzed with a multifunctional approach, provide evidence that farms can realize significant multifunctionality, including farm profit, biodiversity conservation, and long-term sustainability, with a rational restructuring of incentives and disincentives. With payment schedules as currently applied, neither the number of high-functioning ecosystem services nor the short-term economic gain was related to ecological complexity, reflecting the many contradictory responses of different ecosystem services to ecological complexity (see Fig. 5.3). Thus farmers could be pushed in either direction along an ecological complexity gradient depending on local and/or regional political, social, and environmental context. Socioeconomic restructuring, along reasonable and achievable terms, is able to favor more ecologically complex farms. Although many farmers place a high value on ecological complexity, both through an understanding of long-term sustainability and for other aesthetic and cultural reasons (e.g. working under shade instead of hot sun), current agricultural policies tend to be aimed at farm intensification (Vandermeer, Perfecto & Philpott 2010). Thus, opposing forces may be at play in determining the level of ecological complexity within farms, and if a pre-emptive approach is lacking, the default appears to be pushing farmers in the direction of short-term investments in intensification and likely long-term ruin.

We find that alternative scenarios where ecologically complex farms are significantly more profitable are likely achievable under certain incentivized conditions. Coffee certification is one mechanism, although the necessary premium of 150% is larger than what is currently seen in the market for socially and environmentally certified coffee. However, the consumption of specialty coffee is growing fast, and consumers are increasingly aware of the social and environmental impacts of coffee production. Perhaps an even more effective strategy than certification is the scenario where externalities are internalized, or where agricultural subsidies are restructured. Here, a relatively modest value (\$532 ha^{-1}) compared to current agricultural subsidies in Puerto Rico (e.g. up to \$700 ha^{-1} for fertilizer credits), would be sufficient to achieve a significant, positive relationship between short-term farm profit and farm ecological complexity. Under this situation, short-term profit and long-term sustainability of the farm would not conflict.

If economically viable, the benefits of ecologically complex farms are multi-fold: they retain soil fertility, sequester carbon, protect against drought and disaster, prevent erosion, and shelter workers from the hot tropical sun (Tscharntke *et al.* 2011). Importantly, we found that ecologically complex farms also maintain more biodiverse clades. Although we did not gather baseline values from neighboring forests, other research has shown that biodiversity levels for some organisms, such as ants and birds, are as high or even higher in rustic shade coffee than in surrounding forests (Philpott *et al.* 2008a). The biodiversity we recorded in coffee farms are therefore very likely important in biodiversity conservation, especially given that many regions of coffee production are designated biodiversity hotspots (Myers *et al.* 2000).

We found that ecological complexity is difficult to quantify and that an index which integrates several structural and taxonomic components of the ecosystem is more meaningful and significant than the single components comprising the index (e.g. tree shade, tree diversity, groundcover extent). It is thus reasonable to suggest that coffee certification schemes with intentions of either biodiversity conservation or provisioning of ecosystem services, should consider these multiple dimensions of ecological complexity, rather than a simple ‘shade-threshold’ approach, as some do. Furthermore, conservationists interested in specific clades should take into account the shape (e.g. convex, concave, linear) of an organism’s response to disturbance (Perfecto *et al.* 2005). If clades are particularly sensitive to disturbance (e.g. some forest species), certification or other schemes will need to be structured so as to encourage a maximum amount of complexity, whereas the highest levels of complexity may not be required for other species. Additionally, a ‘triple certification’ of organic, fair trade, and ecological complexity may provide the greatest potential for farmer livelihoods and biodiversity conservation (Philpott *et al.* 2007).

Intriguingly, the strength of the effect of ecological complexity on biodiversity appears to be highly influenced by the regional ecological context. The island of Puerto Rico was almost completely deforested as recently as the late 1940s (Grau *et al.* 2003), and is both species-poor and lacks complex forest structure, despite currently high frequency of secondary forests. Increases in ecological complexity on Puerto Rican farms had a disproportionate positive effect on clades, compared to our Mexico farms. Although much of the landscape in this part of Mexico is coffee farms, fragments of primary forest are still relatively common. Even within farms, a high diversity of tree species, comprising multiple vertical strata, are present (e.g., ~100 spp. in one 300 ha. farm) (Philpott & Bichier 2012). Ecological complexity, *per se*, did not have an influence on multiple ecosystem service provision in either country, but only in Puerto Rico was there a variable (amount of open land in landscape) that showed a significant effect.

We currently sit at an important intersection that will determine our common future. We provide evidence for one important crop—the single most important agricultural product exported by the developing world (Talbot 2004)—where farmer livelihoods and ecological sustainability may be jointly achieved. However, the onus is on governments and consumers, as well as global trade, environmental, and development organizations to provide the economic structure that encourages ecological complexity on farms, thus allowing farmers to align short-term profits with long-term sustainability. We show that this is possible with the correct incentives, which are financially within the realm of currently existing or proposed certification schemes or PES. However, we recognize that compensations for ecosystem services do not always have their intended social and environmental benefits (García-Amado, Ruiz Pérez &

Barrasa García 2013). Therefore, an approach that accounts for externalities, which effectively give us the real price of producing coffee, may be the most efficient method to tilt the balance in favor of ecologically complex farms, which provide the best potential for sustained food production and biodiversity conservation.

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Table 5.1. Responses of ecosystem services or biodiversity clades to increased plant species richness, vegetation structure, or landscape complexity in Mexico and Puerto Rico.

	Mexico			Puerto Rico		
Ecosystem services[a]	Plant spp. richness	Vegetation structure	Landscape	Plant spp. richness	Vegetation structure	Landscape
Coffee yield	0	−	0	0	0	−
Farm profit	▨	▨	▨	0	0	−
Shade tree biomass	▨	+	▨	▨	+	▨
BC 1: Rust resistance	−	+	0	0	−	0
BC 2: Borer predation	0	−	0	▨	▨	▨
BC 3: Borer control	▨	▨	▨	0	−	0
BC 4: Scale predation	0	−	0	▨	▨	▨
BC 5: Leafminer control	▨	▨	▨	0	−	0
BC 6: Leafminer predation	▨	▨	▨	+	−	+
BC 7: Leafminer parasitism	▨	▨	▨	+, −	0	0
BC 8: Planthopper predation	▨	▨	▨	+	0	−
BC 9: Herbivore control	−	+, −	0	▨	▨	▨
Biodiversity						
Ants	0	0	+	0	−	0
Spiders	0	0	0	▨	▨	▨
Parasitoid wasps	0	0	+	+	+	0
Bees	0	−	0	0	−	+
Anoles	▨	▨	▨	0	+	−
Birds	+	+	0	0	+	0
Groundcover plants	0	+	0	0	+	−
Trees	0	0	−	0	+	0

[a] BC = Biocontrol-related ecosystem service. Positive and negative signs refer to significantly positive or negative effects, respectively, of increasing richness or complexity of each category on the organism.

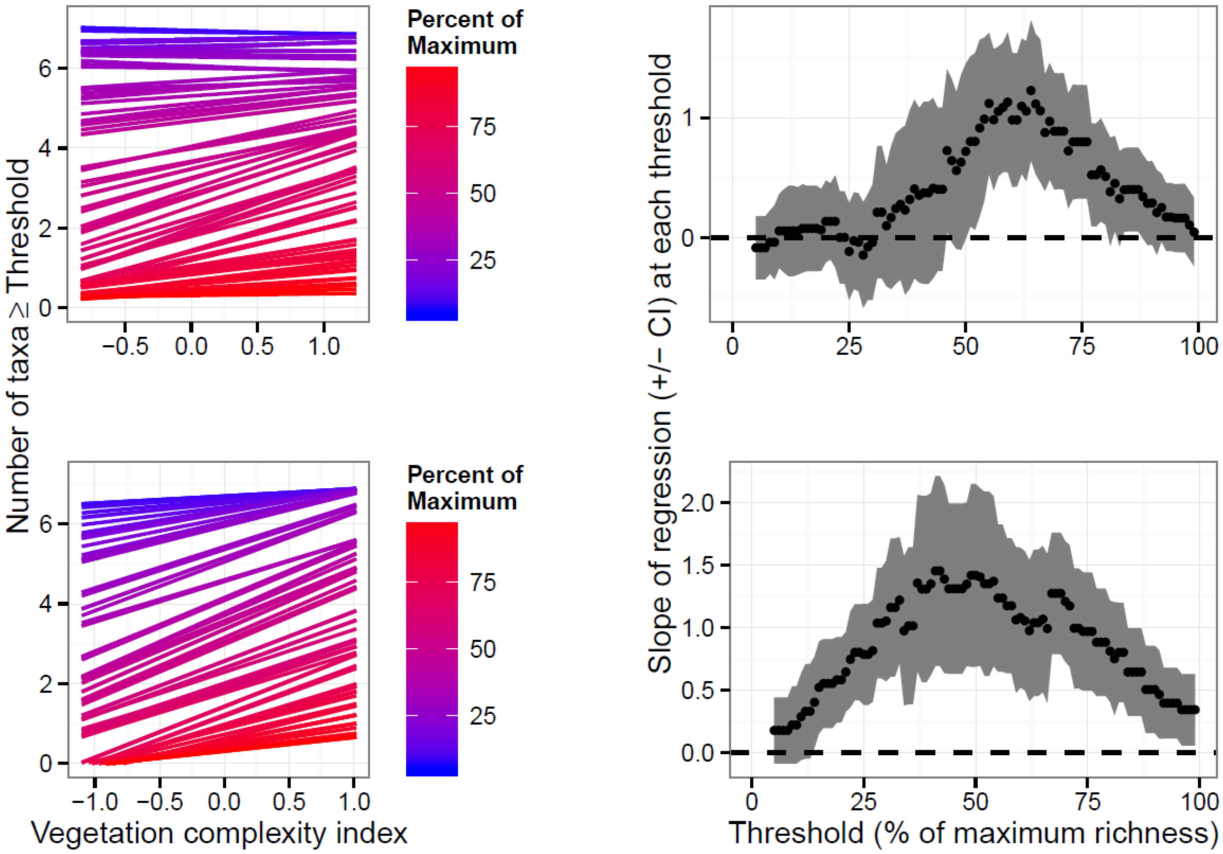
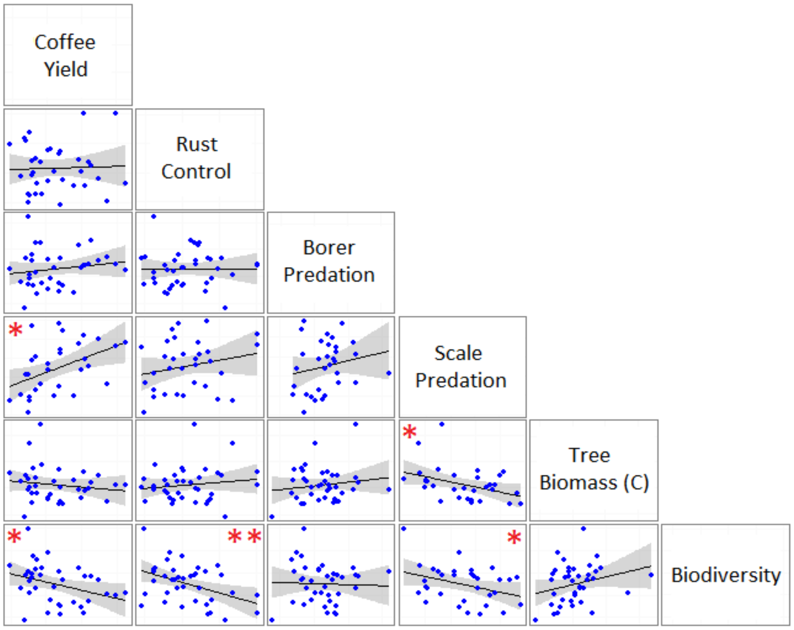


Figure 5.1. Multiple threshold plots (a,c) and slope significance plots (mean ± 95% CI) (b,d) for biodiversity in Mexico (a,b) and Puerto Rico (c,d). Each line in (a,c) represents one threshold (each threshold from 1-100% is represented), where the y-axis corresponds to the number of biodiversity clades that are more speciose than the given threshold (percent of highest species diversity at any site for that taxon) at each individual site (each having a given ecological complexity score (x-axis)). Slope significance plots show the slope (± 95% CI) of all 100 regressions from the multiple threshold plot with increasing threshold % on the x-axis. In b,d, values where the CI do not overlap the dashed line (slope=0) are considered significantly different from 0. The primary advantage of the slope significant plots is to observe over how large of a range and at what strength there is a significant benefit of ECI on ‘multi-biodiversity’.

A.


Coffee Yield
Rust Control
Borer Predation
Scale Predation
Tree Biomass (C)
Biodiversity
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B.

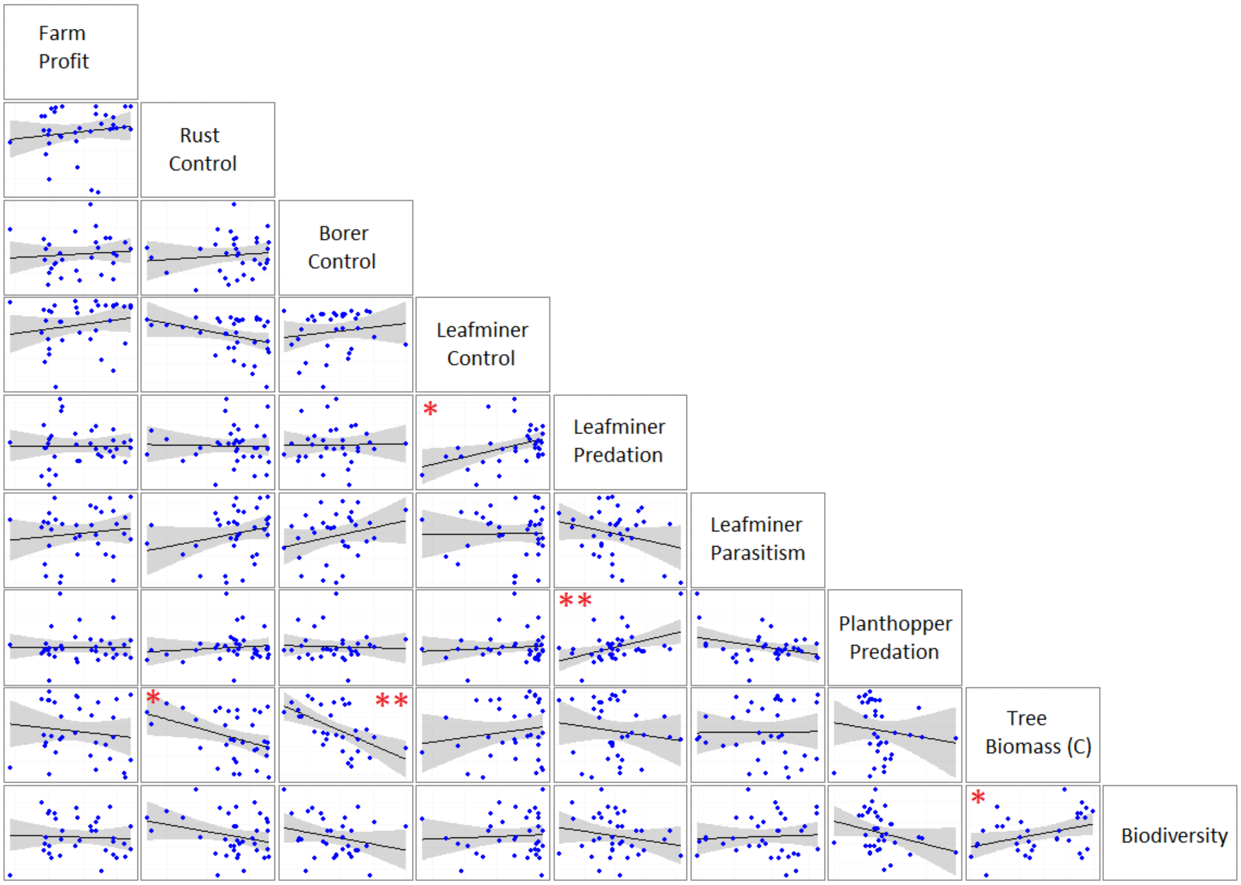
Farm Profit
Rust Control
Borer Control
Leafminer Control
Leafminer Predation
Leafminer Parasitism
Planthopper Predation
Tree Biomass (C)
Biodiversity
*
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Figure 5.2. Pairwise comparisons of ecosystem services in Mexico (a) and Puerto Rico (b) allowing for visualization of tradeoffs and synergies. *p=<0.05, **p=<0.01.

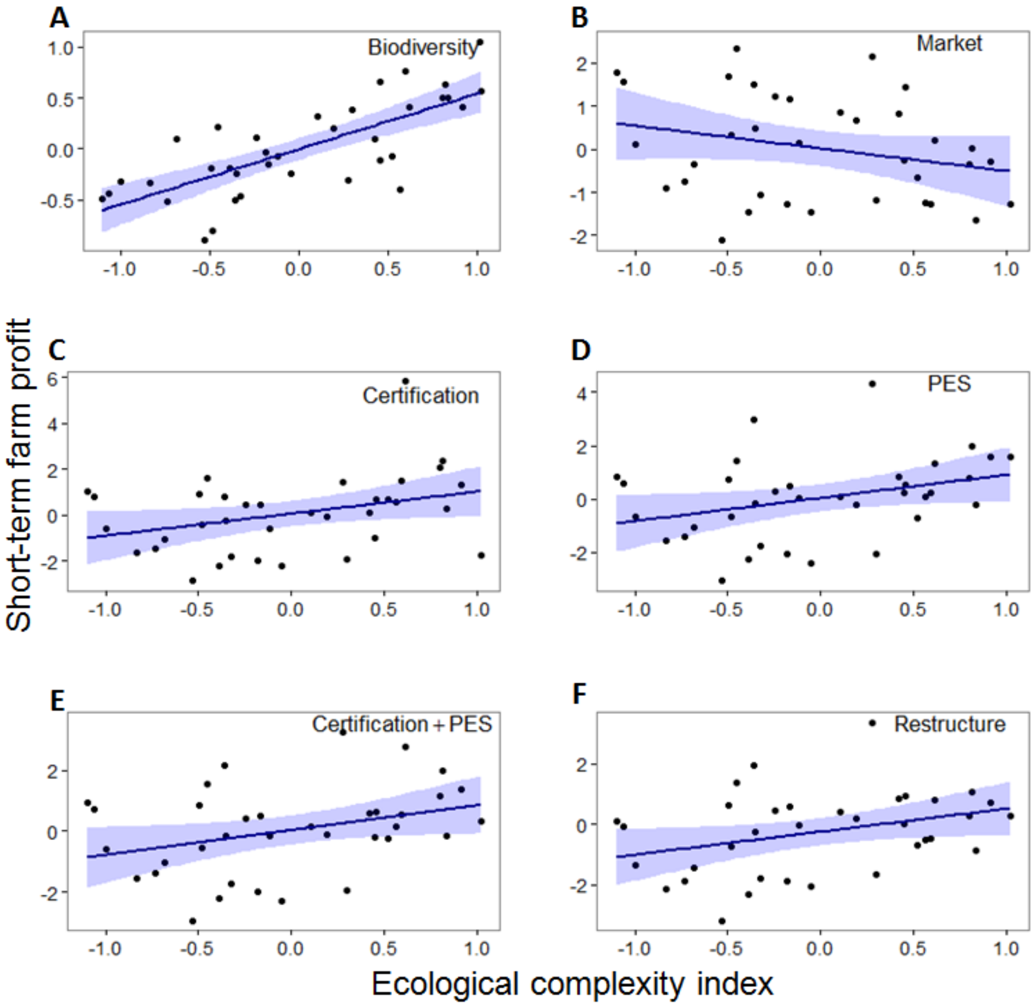


Figure 5.3. Linear models of (a) composite biodiversity values (unweighted) (R^2=0.539, p=<0.0001) and (b-f) weighted composite ecosystem service values with increased levels of ecological complexity for Puerto Rico. In (b), weighted with all coffee receiving same (uncertified market value) price (R^2=0.041, p=0.128). In (c), weighted with ecologically complex (ECI >0.5) farms receiving a premium (150% above market price) for coffee (R^2=0.093, p=0.042). In (d), farms received payments for ecosystem services (carbon) in proportion to the tree biomass on the farm, equating to \$52 t^{-1} of tree biomass (R^2=0.087, p=0.047). In (e), highly ecologically complex farms received a 50% premium for coffee, but also received payments for carbon storage, equating to \$30 t^{-1} of tree biomass (R^2=0.091, p=0.044). In (f), we show a restructuring of subsidies, where all low complexity farms lost subsidies valued at \$532 ha^{-1} and high complexity farms received subsidies (carbon PES) of the same value (R^2=0.089, p=0.046).

Supplementary material

Table S5.1. Summary statistics of local and landscape variables included in GLMM models.

			Mexico					Puerto Rico				
Variable Category	**Variable**	**Units**	**Mean**	**SE**	**Min**	**Max**	**GLMM[a]**	**Mean**	**SE**	**Min**	**Max**	**GLMM[a]**
Vegetation structure	Shade tree biomass	t ha-1	38.6	5.6	2.2	193.0	yes	19.5	3.8	0.0	76.6	no
	Shade	Percent cover	53.2	4.7	2.5	94.3	yes	36.2	4.9	0.0	83.6	yes
	Coffee shade	Percent cover	NA	NA	NA	NA	NA	49.2	3.1	7.7	78.8	yes
	Flowering trees	Number	106.8	7.0	24.0	193.0	yes	NA	NA	NA	NA	NA
	Groundcover extent	Percent cover	56.8	4.8	4.0	100.0	yes	22.4	2.5	0.9	50.0	yes
	Groundcover flowers	Number	55.0	18.7	0.0	452.0	yes	159.1	49.7	0.0	1370.0	yes
	Groundcover height	cm	16.3	1.1	6.1	32.5	no	11.4	0.8	3.7	18.6	yes
Vegetation spp. richness	Tree species richness[b]	Number	28.7	1.9	9.8	73.3	yes	4.6	0.3	0.0	10.0	yes
	Groundcover spp. richness	Number	14.9	0.8	6.0	27.0	yes	14.8	1.1	3.0	33.0	yes
Azteca sericeasur	Distance to Azteca nest	m	17.9	2.1	3.3	60.0	yes	NA	NA	NA	NA	NA
	Azteca nest density	Number	14.2	1.4	0.0	37.0	yes	NA	NA	NA	NA	NA
Landscape[c]	Forests	Percent cover	7.2	1.4	0.0	29.3	yes	68.3	2.0	36.4	86.7	yes
	High intensity coffee farms	Percent cover	27.8	5.7	0.0	1.0	yes	NA	NA	NA	NA	NA
	Distance to nearest forest	m	483.9	80.0	44.4	1926.7	yes	98.1	11.7	15.0	300.0	yes
	Coffee farms	Percent cover	92.6	1.4	70.7	1.0	no	9.0	1.4	0.4	31.5	yes
Other	Altitude	m asl	942.6	28.7	594.7	1273.4	yes	509.7	25.7	251.0	856.0	yes
	Agrochemicals[d]	0=no, 1=yes	0.6	0.1	0.0	1.0	yes	NA	NA	NA	NA	NA

[a] Signifies whether or not variable was included in GLMMs for biodiversity clades and ecosystem services (see Table 5.1). Some were not included due to absence or lack of importance in ecosystem (e.g. *Azteca sericeasur* in Puerto Rico), or high collinearity with other variables.

[b] Tree species richness in Mexico was calculated using a richness estimator (ICE) to approximate actual species richness had every individual been sampled in area. In Puerto Rico, species richness levels were too low to perform such an estimate. In Mexico, tree species richness is reported ha^{-1}, whereas in Puerto Rico it is reported for a 625 m^2 plot area.

[c] Landscape variables, with the exception of distance to nearest forest, were calculated as percent cover of each land use within a 500 m radius.

[d] Agrochemicals refer to synthetic fertilizers, insecticides, and herbicides. In Puerto Rico, only two farms did not use synthetic agrochemicals and therefore this variable was not included in the analysis.

Table S5.2. Weights for ecosystem services for Puerto Rico.

Ecosystem service[a]	**Weight**
Coffee yield[b]	1.00
Equalizer (accounting for net profit)[c]	0.74
Rust resistance[d]	0.30
Shade tree biomass[e]	0.05
Borer control[f]	0.26
Leafminer abundance[g]	0.20
Leafminer predation[g]	0.10
Leafminer parasitism[g]	0.10
Planthopper predation[h]	0.01

[a] Rust resistance and biocontrol variable (borer, leafminer, planthopper) values reflect the realistic potential damage that each of the pests could have on coffee yield in years or localities of high infestation based on research by the authors or based on primary literature.

[b] Basis to which all other services are compared (each service weighted for its effect on yield or income in relation to yield).

[c] Average ratio of net costs (including income from alternative crops but not coffee) divided by coffee value. This ratio was multiplied by the standardized same cost/income ratio of each individual farm.

[d] Reports vary as to the impact of rust on yield. During the most recent rust epidemic in Central America (2012-13), yield drops averaged 16% (Avelino et al. 2015). However, in some countries yield dropped an average of 54% the following year (El Salvador) (Avelino et al. 2015). In Colombia, yield dropped 31% from the most recent epidemic (Avelino et al. 2015). At Finca Irlanda and neighboring farms (where our study was conducted in Mexico), individual coffee bushes lost an average of 56% of all branches (Gonthier, unpublished data) during the epidemic. However, some branch death regularly occurs without rust infestation, so this rate is an overestimate of death from rust. Given these data, we chose a 30% drop as a conservative estimate of the potential impact that coffee yield could incur due to the fungus during times/localities of severe infestation. So far Puerto Rico has not succumbed to the same epidemic that recently hit Central and South America, although an eventual epidemic is not improbable.

[e] In Puerto Rico, few farmers earn direct income from tree biomass. Making charcoal is illegal. We set a conservative value (0.05) to account for the farmers that harvest non-market fruits or trees for construction or other uses. Trees that produced marketable fruits were included in net profit 'equalizer' calculations.

[f] This value is the highest infestation rate observed on any farm in Puerto Rico. We chose the highest value to represent the potential damage the borer could have on any farm.

[g] Studies report yield values dropping as much as 40% under bad infestations (Reis & Souza 1996). As we have three variables that relate to leafminer infestation, we divided the 40% into 20% for abundance, 10%

for predation, and 10% for parasitism. Although abundance could be a result of predation and parasitism, there is much evidence of environmental (e.g. shade cover) influences on leafminers, which we also observed as a strong determinant of leafminer abundance in the field. For this reason, we set leafminer abundance as the highest weight.

[h] We were unable to find any studies that assessed the planthopper's effect on coffee yield. We assigned a low value based on the planthopper's low infestation rates and their indirect effects on coffee yield (sucking phloem). However, planthoppers can be important vectors of plant diseases and thus it is possible that this insect could become more damaging in the future.

Table S5.3. Results from each final GLMM explaining local and landscape predictors of individual ecosystem services or clades.

A. Biodiversity

Mexico

Taxon	Model	Type of variable	Variable	Estimate	SE	z/t value	p-value
Ants	Poisson	Vegetation structure	No. of groundcover flowers (sqrt)	0.020	0.012	1.652	0.098
		Vegetation structure	Forest land	2.228	0.844	2.638	0.008
		Other	Distance to nearest Azteca nest (sqrt)	-0.103	0.046	-2.217	0.027
Spiders	Poisson	Other	Altitude	-0.196	0.048	-4.041	0.000
			Chemicals	0.352	0.169	2.083	0.037
Parasitoid wasps	Poisson	Vegetation structure	Tree biomass (log)	0.152	0.090	1.691	0.091
		Landscape	High intensity farmland	-0.550	0.204	-2.691	0.007
		Other	Altitude	0.001	0.000	2.551	0.011
			Chemicals	0.363	0.151	2.408	0.016
Bees	Poisson	Vegetation structure	Tree biomass (log)	-0.286	0.085	-3.378	0.001
		Other	Distance to nearest Azteca nest (log)	0.363	0.133	2.727	0.006
			No. of Azteca nests	0.027	0.011	2.510	0.012
Birds	Gaussian	Vegetation structure	No. of groundcover flowers (sqrt)	-0.501	0.275	-1.825	0.078
			Groundcover extent	5.609	1.667	3.364	0.002
			No. of flowering trees	-2.554	1.276	-2.002	0.054
		Plant spp. richness	Tree spp. richness	3.322	1.212	2.740	0.010
Groundcover	Poisson	Vegetation structure	Groundcover extent	0.009	0.002	5.572	0.000
Trees (log)	Gaussian	Vegetation structure	Shade	0.016	0.005	2.894	0.006

Puerto Rico

Taxon	Model	Type of variable	Variable	Estimate	SE	z/t value	p-value
Ants	Gaussian	Vegetation	Shade	-0.010	0.004	-2.804	0.011

		structure					
			No. of groundcover flowers (log)	-0.198	0.049	-4.040	0.001
			Shade from coffee	0.009	0.005	1.951	0.070
		Other	Anole abundance	0.021	0.010	1.971	0.066
Parasitoid wasps (sqrt)	Gaussian	Vegetation structure	Shade from coffee	0.026	0.015	1.807	0.080
			Groundcover extent	0.057	0.019	3.053	0.005
		Plant spp. richness	Tree spp. richness	0.410	0.155	2.643	0.013
Bees	Gaussian	Vegetation structure	Shade	-0.010	0.005	-2.211	0.035
		Landscape	Forest land	0.047	0.016	2.835	0.008
Anoles (sqrt)	Gaussian	Vegetation structure	Groundcover extent	0.009	0.003	3.043	0.005
		Landscape	Forest land	-0.009	0.004	-2.379	0.023
Birds	Poisson	Vegetation structure	Shade	0.008	0.003	3.296	0.001
		Landscape	Forest land	-0.014	0.008	-1.663	0.096
Groundcover plants	Poisson	Vegetation structure	Shade	-0.003	0.002	-1.754	0.079
			Groundcover extent	0.023	0.003	7.841	0.000
		Landscape	Distance to nearest forest	0.002	0.001	2.588	0.010
Trees	Gaussian	Vegetation structure	Shade	0.039	0.008	4.704	0.000
		Other	Altitude	0.004	0.002	2.037	0.054

B. Ecosystem services

Mexico

Service	Model	Type of variable	Variable	Estimate	SE	z/t value	p-value
Scale predation	Binomial	Vegetation structure	Tree biomass (log)	-0.652	0.218	-2.989	0.003
		Other	Distance to nearest Azteca nest (log)	0.354	0.134	2.653	0.008
			No. of Azteca nests	-0.020	0.012	-1.710	0.087
Coffee yield	Gaussian	Vegetation structure	Groundcover extent	-0.010	0.005	-2.071	0.047
Borer predation	Binomial	Vegetation structure	Shade	-0.370	0.171	-2.165	0.030
			Tree biomass (log)	0.604	0.330	1.828	0.068
Rust resistance	Binomial	Vegetation structure	Groundcover extent	0.239	0.047	-5.061	0.000
		Plant spp. richness	Groundcover spp. richness	-0.075	0.028	2.689	0.007
		Other	Distance to nearest Azteca nest (log)	0.544	0.183	-2.973	0.003
Herbivory resistance	Binomial	Vegetation structure	Shade	-0.010	0.005	1.984	0.047
			Groundcover extent	0.111	0.048	-2.312	0.021
		Plant spp. richness	Groundcover spp. richness	-0.063	0.021	3.025	0.002
		Landscape	Distance to nearest forest	0.001	0.000	-1.817	0.069

			Altitude	0.003	0.001	-2.674	0.008

Puerto Rico

Service	Model	Type of variable	Variable	Estimate	SE	z/t value	p-value
Coffee yield	Gaussian	Landscape	Distance to nearest forest	-7.366	3.863	-1.907	0.067
			Coffee farmland (sqrt)	6.472	2.340	2.766	0.010
		Other	Altitude	-6.913	1.800	-3.841	0.001
Farm profit	Gaussian	Landscape	Coffee farmland (sqrt)	9.789	2.598	3.768	0.001
		Other	Altitude	-4.618	1.969	-2.345	0.025
Borer control	Gaussian	Vegetation structure	Shade	0.099	0.035	2.807	0.009
		Other	Altitude	0.016	0.005	2.932	0.007
Rust resistance	Binomial	Vegetation structure	Shade	-0.009	0.001	6.458	0.000
			Shade from coffee	-0.006	0.001	4.075	0.000
Leafminer control	Gaussian	Vegetation structure	No. of groundcover flowers (log)	-0.282	0.087	3.239	0.003
Leafminer parasitism	Binomial	Plant spp. richness	Tree spp. richness	-0.118	0.060	-1.971	0.049
			Groundcover spp. richness	0.047	0.013	3.722	0.000
Leafminer predation	Gaussian	Vegetation structure	Groundcover height	-0.955	0.347	-2.752	0.007
			Shade	-0.043	0.025	-1.728	0.087
		Plant spp. richness	Tree spp. richness	0.802	0.384	2.089	0.039
		Landscape	Distance to nearest forest	0.021	0.011	1.978	0.051
			Forest land	0.159	0.064	2.499	0.014
		Other	Bird abundance	-0.331	0.136	-2.439	0.021
Planthopper predation	Gaussian	Plant spp. richness	Tree spp. richness	1.050	0.514	2.044	0.044
		Landscape	Distance to nearest forest	0.029	0.011	2.518	0.013
		Other	Bird abundance	-0.487	0.160	-3.052	0.003
			Anole spp. richness	-2.217	1.015	-2.184	0.031
Borer predation	Gaussian	Other	Altitude	0.000	0.000	-1.810	0.075
Herbivore predation	Gaussian	Plant spp. richness	Groundcover spp. richness	0.006	0.003	1.772	0.080
		Landscape	Distance to nearest forest	-0.001	0.000	-1.802	0.075
		Other	Altitude	0.000	0.000	-2.752	0.007

[a] Model type determined by distribution of the response variable

[b] Landscape variables, except nearest distance to forest, are calculated at a 500m radius. For a description of variables see ‘Methods’.

Table S5.4. Range of significance from multiple threshold analysis with biodiversity for variables that showed any significant effect on 'multi-biodiversity'.

	Mexico	Puerto Rico
Variable	Range of significance[a]	
Shade	-	65-83
Biomass	-	62-83
ECI[b]	49-81	13-99

[a] Range numbers refer to the threshold values (percent of maximum species richness for each taxon found in any individual site) where the slope of the regression between the variable listed and the number of sites where different taxon groups are above the threshold level is significantly different from zero (for ECI, see Fig. 5.1b,d).

[b] Ecological complexity index

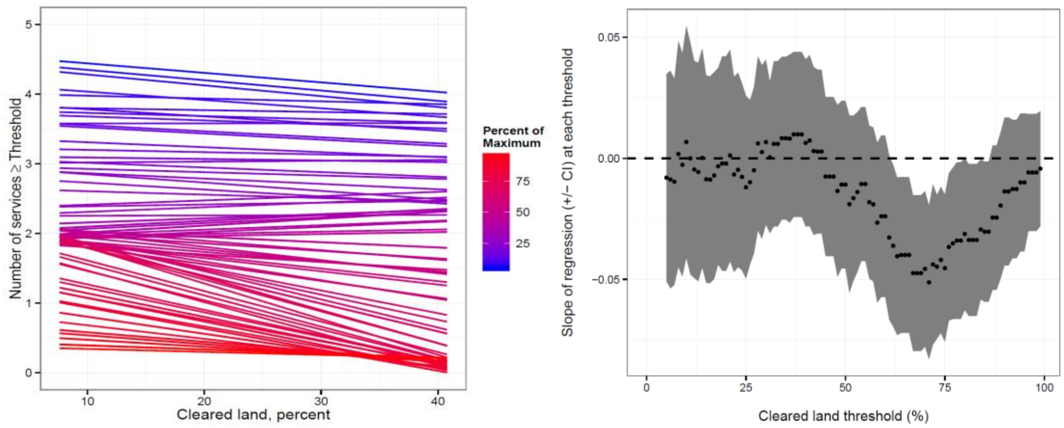
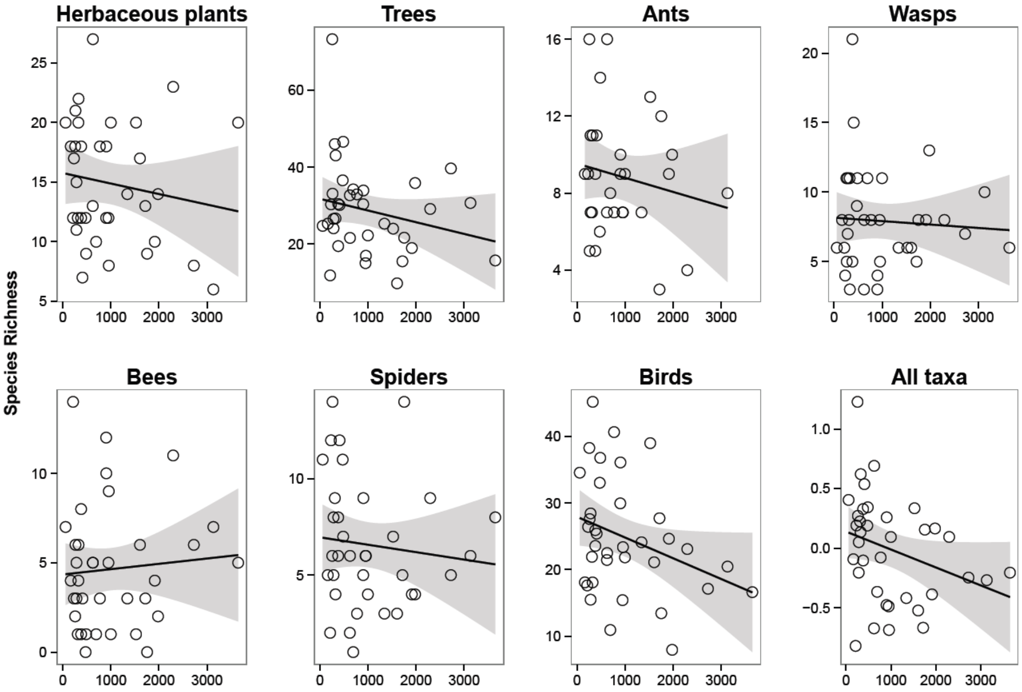


Figure S5.1. Multiple threshold (a) and slope significance (b) plots for Puerto Rico, showing the response of multiple ecosystem service provision to amount of cleared land (including grassland, non-coffee agriculture, and natural barrens) in the landscape (500 m radius).

A


Herbaceous plants
Trees
Ants
Wasps
Bees
Spiders
Birds
All taxa
Species Richness
0
1000
2000
3000

B

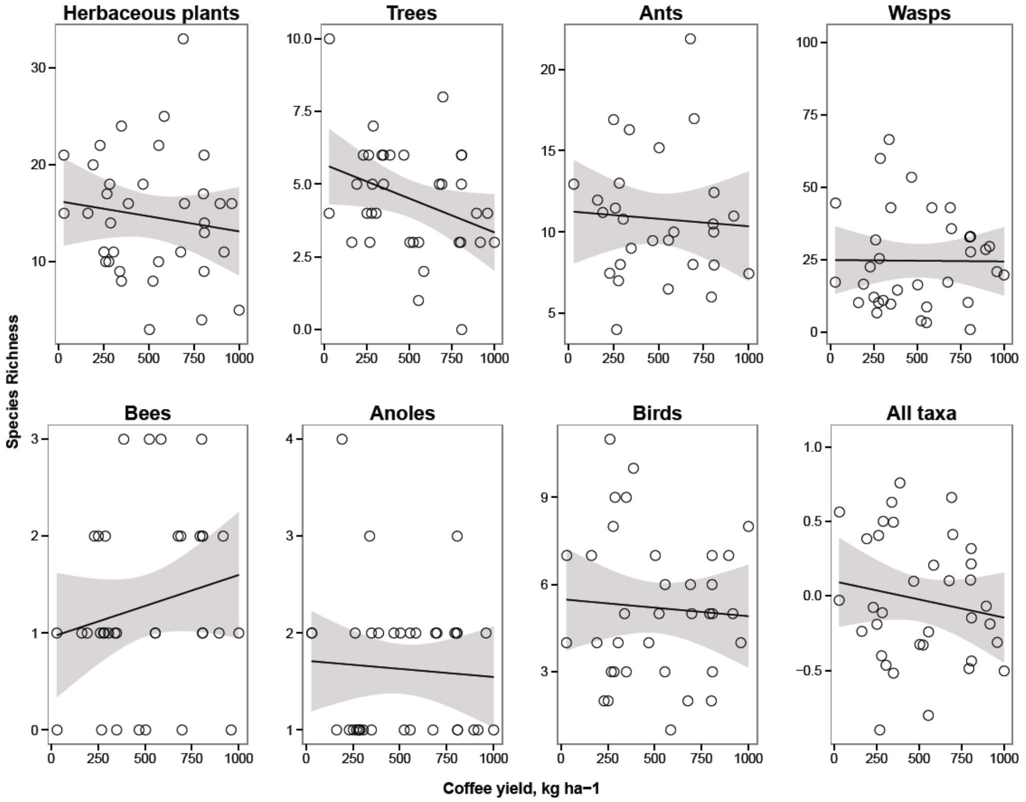

Herbaceous plants
Trees
Ants
Wasps
Bees
Anoles
Birds
All taxa
Species Richness
0
250
500
750
1000
Coffee yield, kg ha−1

Figure S5.2. Relationship between species richness of various clades and coffee yield (kg green coffee ha^{-1}) in Mexico (a) and Puerto Rico (b). All linear regressions non-significant at $p<0.05$; marginally significant linear relationships in (a): birds, $R^2=0.076$, $P=0.057$; all clades, R2=0.065, $P=0.073$; and (b): trees, $R^2=0.084$, $P=0.050$.

Appendix 5.1 Species lists of organisms from biodiversity surveys in Mexico and Puerto Rico. For spiders sampled in Mexico, see Hajian-Forooshani, et al. (2014). For ants sampled in Mexico and Puerto Rico, see Ennis, et al. (in prep).

Appendix 5.1a. Parasitoids sampled in Puerto Rico. For parasitoids of Mexico, see ‘Chapter 3’ or Pak et al. (2015).

	Individuals	**# Sites***	**Relative abundance (individuals)**	**Relative abundance (species)**
Agaonidae			0.74%	1.23%
Agaonidae sp.1	3	2		
Agaonidae sp.2	2	2		
Aphelinidae			2.38%	5.56%
Aphelinidae sp.1	4	4		
Aphelinidae sp.2	1	1		
Aphelinidae sp.3	2	2		
Aphelinidae sp.4	3	3		
Aphelinidae sp.5	1	1		
Aphelinidae sp.6	1	1		
Aphelinidae sp.7	2	2		
Aphelinidae sp.8	1	1		
Aphelinidae sp.9	1	1		
Bethylidae			1.04%	1.85%
Bethylidae sp.1	4	3		
Bethylidae sp.2	1	1		
Bethylidae sp.3	2	2		
Braconidae			4.17%	8.64%
Braconidae sp.1	1	1		
Braconidae sp.2	1	1		
Braconidae sp.3	7	4		
Braconidae sp.4	5	3		
Braconidae sp.5	2	2		
Braconidae sp. 6	2	2		
Braconidae sp.7	1	1		

Braconidae sp.8	1	1		
Braconidae sp.9	2	2		
Braconidae sp. 10	2	2		
Braconidae sp.11	1	1		
Braconidae sp. 12	1	1		
Braconidae sp.13	1	1		
Braconidae sp.14	1	1		
Ceraphronidae			1.64%	3.70%
Ceraphronidae sp.1	2	2		
Ceraphronidae sp.2	3	3		
Ceraphronidae sp.3	2	2		
Ceraphronidae sp.4	1	1		
Ceraphronidae sp.5	1	1		
Ceraphronidae sp.6	2	2		
Chalcididae			0.74%	1.85%
Chalcididae sp.1	2	2		
Chalcididae sp.2	1	1		
Chalcididae sp.3	2	2		
Chrysididae			0.30%	1.23%
Chrysididae sp.1	1	1		
Chrysididae sp.2	1	1		
Cynipidae			0.45%	1.23%
Cynipidae sp.1	1	1		
Cynipidae sp.2	2	1		
Diapriidae			3.87%	6.17%
Diapriidae sp.1	2	1		
Diapriidae sp.2	2	2		
Diapriidae sp.3	3	2		
Diapriidae sp.4	1	1		
Diapriidae sp.5	1	1		
Diapriidae sp.6	1	1		
Diapriidae sp.7	11	6		
Diapriidae sp.8	2	1		
Diapriidae sp.9	2	2		
Diapriidae sp.10	1	1		
Dryinidae			0.30%	0.62%
Dryinidae sp.1	2	1		
Encyrtidae			12.50%	16.05%
Encyrtidae sp.1	1	1		
Encyrtidae sp.2	2	2		
Encyrtidae sp.3	3	2		
Encyrtidae sp.4	6	5		
Encyrtidae sp.5	1	1		

Encyrtidae sp.6	1	1		
Encyrtidae sp.7	1	1		
Encyrtidae sp.8	7	5		
Encyrtidae sp.9	12	8		
Encyrtidae sp.10	3	3		
Encyrtidae sp.11	8	3		
Encyrtidae sp.12	1	1		
Encyrtidae sp.13	3	2		
Encyrtidae sp.14	9	4		
Encyrtidae sp.15	1	1		
Encyrtidae sp.16	4	3		
Encyrtidae sp.17	2	2		
Encyrtidae sp.18	1	1		
Encyrtidae sp.19	2	2		
Encyrtidae sp.20	1	1		
Encyrtidae sp.21	3	3		
Encyrtidae sp.22	1	1		
Encyrtidae sp.23	1	1		
Encyrtidae sp.24	2	2		
Encyrtidae sp.25	1	1		
Encyrtidae sp.26	7	4		
Eucharitidae			2.08%	2.47%
Eucharitidae sp.1	3	1		
Eucharitidae sp.2	6	6		
Eucharitidae sp.3	2	1		
Eucharitidae sp.4	3	1		
Eucoilidae			0.45%	1.23%
Eucoilidae sp.1	1	1		
Eucoilidae sp.2	2	2		
Eulophidae			23.21%	8.64%
Eulophidae sp.1	8	7		
Eulophidae sp.2	2	2		
Eulophidae sp.3	3	2		
Eulophidae sp.4	1	1		
Eulophidae sp.5	2	2		
Eulophidae sp.6	23	8		
Eulophidae sp.7	1	1		
Eulophidae sp.8	1	1		
Eulophidae sp.9	1	1		
Eulophidae sp.10	1	1		
Eulophidae sp.11	1	1		
Eulophidae sp.12	4	2		
Eulophidae sp.13	106	18		

Eulophidae sp.14	2	2		
Eupelmidae			7.44%	6.17%
Eupelmidae sp.1	3	3		
Eupelmidae sp.2	1	1		
Eupelmidae sp.3	15	11		
Eupelmidae sp.4	1	1		
Eupelmidae sp.5	1	1		
Eupelmidae sp.6	21	12		
Eupelmidae sp.7	1	1		
Eupelmidae sp.8	1	1		
Eupelmidae sp.9	3	1		
Eupelmidae sp. 10	3	3		
Evaniidae			1.34%	1.23%
Evaniidae sp.1	2	2		
Evaniidae sp.2	7	3		
Ichneumonidae			2.53%	1.85%
Ichneumonidae sp.1	1	1		
Ichneumonidae sp.2	14	6		
Ichneumonidae sp.3	2	1		
Mymaridae			6.25%	12.35%
Mymaridae sp.1	2	1		
Mymaridae sp.2	1	1		
Mymaridae sp.3	1	1		
Mymaridae sp.4	2	2		
Mymaridae sp.5	3	3		
Mymaridae sp.6	1	1		
Mymaridae sp.7	1	1		
Mymaridae sp.8	1	1		
Mymaridae sp.9	1	1		
Mymaridae sp.10	2	2		
Mymaridae sp.11	2	2		
Mymaridae sp.12	1	1		
Mymaridae sp.13	1	1		
Mymaridae sp.14	3	3		
Mymaridae sp.15	3	1		
Mymaridae sp.16	11	6		
Mymaridae sp.17	1	1		
Mymaridae sp.18	1	1		
Mymaridae sp.19	3	3		
Mymaridae sp.20	1	1		
Platygastridae			20.39%	5.56%
Platygastridae sp.1	11	6		
Platygastridae sp.2	67	11		

Platygastridae sp.3	4	1		
Platygastridae sp.4	7	5		
Platygastridae sp.5	1	1		
Platygastridae sp.6	40	13		
Platygastridae sp.7	1	1		
Platygastridae sp.8	2	2		
Platygastridae sp.9	4	4		
Pompilidae			1.93%	1.23%
Pompilidae sp.1	12	7		
Pompilidae sp.2	1	1		
Proctotrupidae			0.15%	0.62%
Proctotrupidae sp.1	1	1		
Scelionidae			3.42%	6.17%
Scelionidae sp.1	1	1		
Scelionidae sp.2	5	1		
Scelionidae sp.3	1	1		
Scelionidae sp.4	6	4		
Scelionidae sp.5	1	1		
Scelionidae sp.6	2	2		
Scelionidae sp.7	3	3		
Scelionidae sp.8	1	1		
Scelionidae sp. 9	1	1		
Scelionidae sp.10	2	2		
Signiphoridae			0.15%	0.62%
Signiphoridae sp.1	1	1		
Tanaostigmatidae			0.45%	1.85%
Tanaostigmatidae sp.1	1	1		
Tanaostigmatidae sp.2	1	1		
Tanaostigmatidae sp.3	1	1		
Vespidae			0.60%	1.85%
Vespidae sp.1	2	1		
Vespidae sp.2	1	1		
Vespidae sp.3	1	1		
Unknown	10	9	1.49%	NA

Total: 162 species, 672 individuals

* refers to the total number of sites (out of 36) where the parasitoid was found.

Appendix 5.1b. Parasitoids which emerged from leafminer (*Leucoptera coffeella*) pupae in Puerto Rico (measure of parasitism rate).

Family	Subfamily	Species	Individuals	# Sites*	Relative abundance
Braconidae	Cheloninae	*Phanerotoma rufescens*	1	1	0.19%

Braconidae	Cheloninae	*Phanerotoma* Sp.1	1	1	0.19%
Braconidae	Helconinae	*Mirax insularis*	38	16	7.25%
Encyrtidae	Unknown	Sp.1	1	1	0.19%
Eulophidae	Entedoninae	Sp.1	1	1	0.19%
Eulophidae	Eulophinae	*Colpoclypeus* Sp.1	1	1	0.19%
Eulophidae	Eulophinae	*Zagrammosoma multilineatum*	70	17	13.36%
Eulophidae	Etedoninae	*Chrysocharis* Sp. 1	298	30	56.87%
Eulophidae	Etedoninae	*Chrysocharis* Sp. 2	38	16	7.25%
Eulophidae	Etedoninae	*Chrysocharis* Sp. 3	54	5	10.30%
Eulophidae	Etedoninae	*Chrysocharis* Sp. 4	9	1	1.72%
Eulophidae	Etedoninae	*Chrysocharis* Sp. 5	10	5	1.91%
Eulophidae	Tetrastichinae	Sp.1	1	1	0.19%
Ichneumidae	Acaentinae	*Yezoceryx* Sp.1	1	1	0.19%
Total:		14	524		

* refers to the total number of sites (out of 36) where each parasitoid was found.

Appendix 5.1c. Bees sampled in Puerto Rico. For bees of Mexico, see Ennis, et al. (in prep).

Family	Subfamily	Individuals	# Sites*	Relative abundance (individuals)	Relative abundance (species)
Halictidae				44.12%	37.50%
Halictidae sp.1		41	17		
Halictidae sp.2		1	1		
Halictidae sp.3		3	3		
Melittidae				0.98%	12.50%
Melittidae sp.1		1	1		
Megachilidae	**Lithurginae**			51.96%	25%
	Lithurginae sp.1	51	19		
	Lithurginae sp.2	2	2		
Andrenidae	**Panurginae**			0.98%	12.50%
	Panurginae sp.1	1	1		
Colletidae	**Xeromelissinae**			1.90%	12.50%
	Xeromelissinae sp.1	2	2		

Total: 8 species, 102 individuals

*refers to the total number of sites (out of 36) where the bee species was found.

Appendix 5.1d. Anoles sampled in Puerto Rico.

Scientific name	# Sites*
Anolis cristatellus	36
Anolis cuvieri	2
Anolis evermanni	4
Anolis gundlachi	1
Anolis krugi	13
Anolis pulchellus	2
Anolis stratulus	4

Total species: 7

*refers to the total number of sites (out of 36) where each anole species was found.

Appendix 5.1e. Birds sampled in Puerto Rico. For birds in Mexico, see Gonthier, et al (in prep).

Scientific name	Common name	# Sites*
Anthracothorax dominicus	Antillean mango	1
Anthracothorax viridis	Green mango	11
Chlorostilbon maugaeus	Puerto Rican emerald	5
Coereba flaveola	Bananaquit	33
Columba livia	Rock dove	1
Columba squamosa	Scaly-naped pigeon	8
Columbina passerina	Common ground-dove	1
Estrilda melpoda	Orange-cheeked waxbill	7
Euphonia musica	Antillian euphonia	1
Falco sparverius	Kestral falcon	1
Icterus prosthemelas	Black-cowled oriole	1
Loxigilla portoricensis	Puerto Rican bullfinch	6
Margarops fuscatus	Pearly-eyed thrasher	5
Melanerpes portoricensis	Puerto Rican woodpeaker	7
Mimus polyglottos	Northern mockingbird	5
Molothrus bonariensis	Shiny cowbird	1
Myiarchus antillarum	Puerto Rican flycatcher	3
Nesospingus speculiferus	Puerto Rican tanager	1
Quiscalus niger	Greater Antillean grackle	6
Saurothera vieilloti	Puerto Rican lizard cuckoo	3
Spindalis zena	Stripe-headed tanager	3
Tiaris bicolor	Black-faced grassquit	25
Tiaris olivacea	Yellow- faced grassquit	1
Todus mexicanus	Puerto Rican tody	9
Turdus plumbeus	Red-legged thrush	3
Tyrannus caudifasciatus	Loggerhead kingbird	5
Tyrannus dominicensis	Gray kingbird	10
Vireo altiloquus	Black-whiskered vireo	12
Zenaida asiatica	White-winged dove	12

Zenaida aurita	Zenaida dove	1

Total: 30 species, 198 individuals

* refers to the total number of sites (out of 36) where the bird species was found.

Appendix 5.1f. Trees and shrubs (≥1 cm dbh) sampled in Mexico.

Family	Genus	Species	Common (Spanish)	Common (English)	# Sites*
Agavaceae	*Yucca*	*elephantoides*	izote		32
Anacardiaceae	*Mangifera*	*indica*	mango	mango	4
Anacardiaceae	*Tapirira*	sp. 1			2
Anacardiaceae	*Tapirira*	sp. 2			1
Annonaceae	*Annona*	sp. 2			3
Annonaceae	*Annona*	*cherimola*	cherimoya	cherimoya	4
Apocynaceae	*Aspidosperma*	*cruentum*	chiche		10
Araliaceae	unknown				1
Arecaceae	*Roystonea*	*regia*	palma real	royal palm	1
Asparagaceae	*Dracaena*		gigante		2
Asteraceae	*Critonia*	*morifolium*	broton		7
Asteraceae	*Vernonanthura*	*deppeana*	siquinay		15
Asteraceae	unknown				2
Asteraceae	unknown		trapoquilete		2
Bignoniaceae	*Spathodea*	*campanulata*	tulipa africana	African tulip	3
Bignoniaceae	*Tabebuia*	*rosea*	roble		4
Bignoniaceae	*Tabebuia*	sp. 2	primavera		25
Bixaceae	*Bixa*	*orellana*	achiote	lipstick tree	1
Boraginaceae	*Cordia*	*alliodora*		Spanish elm	1
Boraginaceae	*Cordia*	sp. 2	posól		6
Burseraceae	*Bursera*	*simaruba*	jiote	gumbo-limbo	6
Cannabaceae	*Trema*	*micrantha*	capulín		19
Caprifoliaceae	*Viburnum*				1
Casuarinaceae	*Casuarina*	*cunninghamiana*	pino australiano	Australian pine	1
Clethraceae	*Clethra*				3
Clusiaceae	*Calophyllum*	*brasiliense*			2
Cupressaceae	*Cupressus*		ciprés		1
Cyatheaceae	*Cyathea*			tree fern	2
Euphorbiaceae	*Alchornea*	*latifolia*	canaco		14
Euphorbiaceae	*Codiaeum*	*variegatum*	cola de gallo	croton	3
Euphorbiaceae	*Croton*				1
Euphorbiaceae	*Euphorbia*				3
Euphorbiaceae	*Jatropha*	*curcas*	jatropha	jatropha	1
Euphorbiaceae	*Ricinus*	*communis*	higuerilla	castor bean	3

Euphorbiaceae	*Sapium*				2
Fabaceae	*Albizia*		plumajillo		8
Fabaceae	*Calliandra*				1
Fabaceae	*Enterolobium*	*cyclocarpum*	guanacaste		1
Fabaceae	*Erythrina*				5
Fabaceae	*Hymenea*				1
Fabaceae	*Inga*	*fagifolia*	caspirol del monte		3
Fabaceae	*Inga*	*micheliana*	chalum		38
Fabaceae	*Inga*	*punctata*	caspirol		17
Fabaceae	*Inga*	*sapindoides*	paterna		36
Fabaceae	*Inga*	sp. 6	chalum colorado		1
Fabaceae	*Inga*	sp. 7	chaperna		6
Fabaceae	*Inga*	*vera*	guagua		33
Fabaceae	*Ormosia*				1
Fabaceae	*Pterocarpus*				1
Fabaceae	*Schizolobium*	*parahyba*	zope		11
Fabaceae	*Senna*	*reticulata*			1
Fabaceae	*Senna*	sp. 2			1
Fabaceae	*Swartzia*				1
Fabaceae	unknown				1
Fabaceae	unknown				1
Fabaceae	unknown				1
Fagaceae	*Quercus*	sp. 1	encino	oak	2
Fagaceae	*Quercus*	sp. 2	encino	oak	1
Lamiaceae	*Aegiphila*				2
Lamiaceae	*Tectona*	*grandis*	teca	teak	1
Lauraceae	*Nectandra*				5
Lauraceae	*Ocotea*	sp. 1			2
Lauraceae	*Ocotea*	sp. 2			1
Lauraceae	*Ocotea*	sp. 3			1
Lauraceae	*Ocotea*	sp. 4	tepemistle		17
Lauraceae	*Persea*	*americana*	aguacate	avocado	24
Lauraceae	unknown	sp. 1			1
Lauraceae	unknown	sp. 2			1
Lauraceae	unknown	sp. 3			1
Lauraceae	unknown	sp. 4			1
Malpighiaceae	*Byrsonima*	*crassifolia*	nance		3
Malpighiaceae	unknown				1
Malvaceae	*Ceiba*	*pentandra*	ceiba	kapok	4
Malvaceae	*Dombeya*	*wallichii*			1
Malvaceae	*Guazuma*	*ulmifolia*	guácima		1
Malvaceae	*Heliocarpus*	*donnellsmithii*	cajete		3
Malvaceae	*Hibiscus*				2

Malvaceae	*Pachira*	*aquatica*	castaño de Guinea	malabar chestnut	1
Malvaceae	*Trichospermum*				2
Melastomataceae	*Clidemia*	*octona*			1
Melastomataceae	*Conostegia*	*xalapensis*	cinco negritos		20
Melastomataceae	*Miconia*	*affinis*	siete negritos		12
Melastomataceae	*Miconia*	sp. 2			1
Melastomataceae	*Miconia*	sp. 3			1
Melastomataceae	unknown				1
Meliaceae	*Cedrela*	*odorata*	cedro	tropical cedar	16
Meliaceae	*Cedrela*	sp. 2			1
Meliaceae	*Cedrela*	sp. 3	cedrillo		2
Meliaceae	*Cedrela*	sp. 4			1
Meliaceae	*Guarea*				3
Meliaceae	*Melia*	*azedarach*	paraiso	chinaberry tree	2
Meliaceae	*Swietenia*				1
Meliaceae	unknown				1
Meliaceae	unknown				2
Moraceae	*Castilla*	*elastica*			2
Moraceae	*Ficus*	sp. 1			2
Moraceae	*Ficus*	sp. 2	matapalo	strangler fig	3
Moraceae	*Ficus*	sp. 3	matapalo	strangler fig	1
Moraceae	*Ficus*	sp. 4	matapalo	strangler fig	3
Moraceae	*Ficus*	sp. 5	matapalo	strangler fig	3
Moraceae	*Ficus*	sp. 6	matapalo	strangler fig	1
Moraceae	*Ficus*	sp. 7	matapalo	strangler fig	3
Moraceae	*Ficus*	sp. 8	matapalo	strangler fig	2
Moraceae	*Ficus*	sp. 9	matapalo	strangler fig	1
Moraceae	*Ficus*	sp. 10	matapalo	strangler fig	5
Moraceae	*Ficus*	sp. 11	matapalo	strangler fig	2
Moraceae	*Ficus*	sp. 12	matapalo	strangler fig	1
Moraceae	*Ficus*	sp. 13	matapalo	strangler fig	1
Moraceae	*Helicostylus*				1
Moraceae	unknown				1
Moraceae	unknown				1
Moraceae	unknown				1
Musaceae	*Musa*	*x paradisiaca*	banana	banana	2
Myristicaceae	*Virola*	*guatemalensis*	volador		4
Myrtaceae	*Eugenia*	*oerstediana*			5
Myrtaceae	*Eugenia*	sp. 2			1
Myrtaceae	*Psidium*	*guayava*	guayaba	guava	7
Myrtaceae	*Syzygium*	*jambos*	pomarrosa	rose apple	9
Oxalidaceae	*Averrhoa*	*carambola*	carambola	star fruit	1
Piperaceae	*Piper*				2

Poaceae	*Bambusa*		bambú	bamboo	1
Polygonaceae	*Triplaris*				2
Proteaceae	*Grevillea*	*robusta*			2
Rhamnaceae	*Frangula*	*capreifolia*	costé		6
Rosaceae	*Eriobotrya*	*japonica*	nispero	loquat	2
Rosaceae	*Prunus*				3
Rosaceae	unknown		moquillo		2
Rubiaceae	*Calycophyllum*		guayabo		16
Rubiaceae	unknown				1
Rutaceae	*Citrus*	*reticulata*	mandarina	mandarin	1
Rutaceae	*Citrus*	*sinensis*	naranja	orange	6
Rutaceae	*Citrus*	*limon*	limón	lemon	1
Rutaceae	*Zanthoxylum*				2
Salicaceae	*Casearia*				1
Sapindaceae	*Allophylus*	*camptostachys*			1
Sapindaceae	*Cupania*	*glabra*	cola de pavo		4
Sapindaceae	*Cupania*	sp. 2			1
Sapotaceae	*Pouteria*				1
Solanaceae	*Cestrum*				2
Solanaceae	*Solanum*		cuernavaca		4
Staphyleaceae	*Turpinia*				2
Thymeleaceae	*Daphnopsis*				1
Urticaceae	*Cecropia*	*obtusifolia*	guarumbo		20
Urticaceae	*Laportea*	sp. 1	chichicaste		1
Urticaceae	*Laportea*	sp. 2			1
Urticaceae	*Myriocarpa*				1
Verbenaceae	*Citharexylum*		cola de iguana		1
Verbenaceae	unknown				1

Total: 148 species

* refers to the total number of sites (out of 38) where the tree/shrub species was found.

Appendix 5.1g. Trees and shrubs (≥1 cm dbh) sampled in Puerto Rico.

Family	Scientific name	Common (Spanish)	Common (English)	# Sites*
Arecaceae	*Acrocomia media*	Corozo		1
Bignoniaceae	*Spathodea campanulata*	tulipa africana	African tulip	2
Boraginaceae	*Cordia alliodora*			1
Cannabaceae	*Trema micrantha*			1
Caricaceae	*Carica papaya*	papaya	papaya	2
Combretaceae	*Bucida buceras*	ucar		1
Fabaceae	*Andira inermis*	moca		7
Fabaceae	*Cajanus cajan*	gandul	pigeon pea	1
Fabaceae	*Gliricidia sepium*	madre de cacao		4

Fabaceae	*Inga fagifolia*	guama		2
Fabaceae	*Inga vera*	guaba		17
Fabaceae	*Samanea saman*	dormilon		1
Lauraceae	*Persea americana*	aguacate	avocado	2
Malpighiaceae	*Byrsonima spicata*	maricao		1
Malvaceae	*Thespesia grandiflora*	maga		2
Melastomataceae	*Clidemia eggersii*			1
Melastomataceae	*Miconia racemosa*			1
Meliaceae	*Cedrela odorata*	cedro	tropical cedar	2
Meliaceae	*Guarea guidonia*	guaraguao		6
Myrtaceae	*Pimenta racemosa*	malagueta	bayrum	1
Rutaceae	*Citrus aurantifolia*	lima	lime	1
Rutaceae	*Citrus reticulata*	mandarina	mandarin	9
Rutaceae	*Citrus sinensis*	china/naranja	orange	15
Rutaceae	*Citrus* sp.	chironja		2
Rutaceae	*Citrus x paradisi*	toronja	grapefruit	4
Sapindaceae	*Cupania* sp.			1
Solanaceae	*Solanum rugosum*			3
Urticaceae	*Cecropia obtusifolia*	guarumbo		1

Total species: 28

*refers to the total number of sites (out of 36) where the tree/shrub species was found.

Appendix 5.1h. Groundcover plants sampled in Puerto Rico.

Family	**Scientific name**
Acanthaceae	*Blechum pyrimidatum*
Acanthaceae	*Lepidagathis alopecuroidea*
Amaranthaceae	*Achyranthes aspera*
Amaranthaceae	*Amaranthus viridis*
Apocynaceae	*Asclepias nivea*
Araceae	*Dieffenbachia sanguine*
Araceae	*Philodendron consanguineum*
Araceae	*Syngonium podophyllum*
Asteraceae	*Ageratum conyzoides*
Asteraceae	*Bidens pilosa*
Asteraceae	*Conyza laevigata*
Asteraceae	*Crassocephalum crepidioides*
Asteraceae	*Cyanthillium cinereum*
Asteraceae	*Emilia fosbergii*
Asteraceae	*Melanthera nivea*
Asteraceae	*Mikania fragilis*
Asteraceae	*Pseudelephantopus spiralis*
Asteraceae	*Sonchus oleraceus*

Asteraceae	*Synedrella nodiflora*
Asteraceae	*Verbesina alata*
Asteraceae	*Youngia japonica*
Begoniaceae	*Begonia hirtella*
Bignoniaceae	*Dolichandra unguis-cati*
Brassicaceae	*Cardamine flexuosa*
Campanulaceae	*Lobelia cliffortiana*
Commelinaceae	*Callisia monandra*
Convulvaceae	*Ipomoea* sp.
Convulvaceae	*Merremia aegyptia*
Cucurbitaceae	*Cayaponia americana*
Cucurbitaceae	*Cayaponia racemosa*
Cucurbitaceae	*Melothria pendula*
Cyperaceae	*Cyperus odoratus*
Cyperaceae	*Cyperus sphacelatus*
Cyperaceae	*Kyllinga nemoralis*
Dioscoreaceae	*Smilax domingensis*
Euphorbiaceae	*Caperonia palustris*
Euphorbiaceae	*Euphorbia heterophylla*
Euphorbiaceae	*Euphorbia heterophylla*
Euphorbiaceae	*Euphorbia hirta*
Euphorbiaceae	*Euphorbia hypericifolia*
Fabaceae	*Chamaecrista nictitans*
Fabaceae	*Clitoria falcata*
Fabaceae	*Crotalaria pallida*
Fabaceae	*Macroptilium lathyroides*
Fabaceae	*Mimosa pudica*
Fabaceae	*Senna obtusifolia*
Fabaceae	*Vigna adenantha*
Fabaceae	*Vigna luteola*
Fabaceae	*Senna occidentalis*
Hypoxidaceae	*Hypoxis decumbens*
Lamiaceae	*Hyptis atrorubens*
Lamiaceae	*Hyptis capitata*
Lamiaceae	*Hyptis lantanifolia*
Lamiaceae	*Spermacoce ocymifolia*
Lamiaceae	*Spermacoce remota*
Lindsaeaceae	*Lonchitis hirsuta*
Lindsaeaceae	*Odontosoria aculeata*
Lythraceae	*Cuphea strigulosa*
Malvaceae	*Malvastrum coromandelianum*
Malvaceae	*Sida rhombifolia*
Marattiaceae	*Danaea* sp.

Melastomataceae	*Clidemia hirta*
Melastomataceae	*Miconia prasina*
Melastomataceae	*Miconia serrulata*
Melastomataceae	*Nepsera aquatica*
Ochnaceae	*Sauvagesia erecta*
Onagraceae	*Ludwigia erecta*
Oxalidaceae	*Oxalis barrelieri*
Oxalidaceae	*Oxalis corniculata*
Oxalidaceae	*Oxalis violacea*
Passifloraceae	*Passiflora rubra*
Phyllanthaceae	*Phyllanthus urinaria*
Phytolaccaceae	*Petiveria alliacea*
Phytolaccaceae	*Phytolacca* sp.
Piperaceae	*Piper hispidum*
Piperaceae	*Piper peltatum*
Plantaginaceae	*Mecardonia procumbens*
Poaceae	*Axonopus compressus*
Poaceae	*Chloris radiata*
Poaceae	*Chloris sagrana*
Poaceae	*Digitaria ciliaris*
Poaceae	*Digitaria horizontalis*
Poaceae	*Digitaria violascens*
Poaceae	*Echinochloa colona*
Poaceae	*Eleusine indica*
Poaceae	*Ichnanthus pallens*
Poaceae	*Paspalum fasciculatum*
Poaceae	*Setaria barbata*
Polygalaceae	*Polygala paniculata*
Pteridaceae	*Adiantum pyramidale*
Rubiaceae	*Mitracarpus frigidus*
Rubiaceae	*Oldenlandia lancifolia*
Rubiaceae	*Psychotria* sp.
Solanaceae	*Capsicum annuum var. glabriusculum*
Solanaceae	*Solanum americanum*
Thelypteridaceae	*Thelypteris kunthii*
Thelypteridaceae	*Thelypteris* sp.
Urticaceae	*Parietaria* sp.
Urticaceae	*Pilea nummulariifolia*
Urticaceae	*Pilea parietaria*
Vitaceae	*Cissus verticillata*

Total species: 101