**Biotic Drivers of Leaf Litter Decomposition in Shaded Coffee Agro-ecosystems in Chiapas, Mexico**

by

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## Dedication

To my parents, who have always been my biggest supporters.

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**Abstract**

Decomposition is a critical ecosystem process, essential for the cycling of nutrients in and through ecosystems. Abiotic factors, like temperature and precipitation, drive decomposition processes, but biotic factors can also play a key role via direct and indirect pathways. This dissertation interrogates biotic drivers of leaf litter decomposition in shaded coffee agro-ecosystems. Coffee agro-ecosystems provide a tractable model system for testing biotic drivers of decomposition, given the often-reduced diversity of agro-ecosystems. The global distribution of coffee and range of management styles also makes it important to understand the dynamics underlying ecosystem function.

In Chapter 2, I proposed that decomposer specialization may be acting to the advantage of litter types that are grown at a particular location. This ecological theory, known as home-field advantage, was tested using two commercially grown species of *Coffea* with a reciprocal transfer experiment that measured the decomposition of each species in its home environment, a con-generic away environment and a forested-away environment. Results revealed support for home-field advantage during a shorter six-week experiment, but this effect did not persist in a year-long litterbag study.

I assessed the role of two common shade trees, *Inga micheliana* and *Alchornea latifolia,* on the decomposition of *Coffea arabica* leaf litter in Chapter 3. Non-additive effects can occur when multiple species are decomposing in combination, though the direction and strength of such effects tends to be highly context dependent. I found that that *C. arabica* decay accelerated in mixture, and that being in mixture with *I. micheliana* provided the biggest boost in decay rate,

likely due to the relatively high nitrogen content in its litter. Being in mixture with *C. arabica* led to mixed results for decay, which may be a result of nitrogen being tied up in the secondary defense compound, caffeine. Micro-topography—being uphill or downhill—of a non-focal species did not have a significant effect on decay rates, suggesting that decomposers are not dispersing via rainwater runoff.

Coffee flowers synchronously, representing a potentially important contribution of flower petals to the detrital pool. In Chapter 4, I quantified the magnitude of a *C. arabica* bloom and its effects of the decomposer community and leaf litter decay rates. Results indicated that the bloom represents an ecologically relevant quantity of nutrients. While the decomposer did not respond on the time scale of one week, decay accelerated with the petals after one and two months.

Finally, in Chapter 5, I examined the indirect effects of a keystone ant species, *Azteca sericeasur,* on the decomposition of *Inga micheliana* leaf litter by determining the community of ants and decomposers in a radius around trees that had nests and trees that did not have nests. I found no decrease in ant species richness, but a different community of ants near nests. The decomposer community was not changed in the presence of *A. sericeasur* nests. A litterbag study found no differences in decay rates of litter at or away from nests, suggesting that decomposition function is maintained around *A. sericeasur* nests.

This research increases our understanding of biotic drivers of leaf litter decomposition in coffee agro-ecosystems and underscores the role of biota in decomposition processes.

## Chapter 1: Introduction

### 1.1 Biotic drivers of decomposition

Understanding the drivers of ecosystem function is of broad interest to the scientific community and essential for those involved in land management. Ecosystem function, which encompasses processes like nutrient cycling, decomposition and pollination, is crucial in regulating ecosystems. Ecosystem functions, including decomposition, are a product of biodiversity and necessary to sustain biodiversity (Hättenschwiler et al. 2005, Gessner et al. 2010, Handa et al. 2014). As the precursor to nutrient cycling, decomposition is a key ecosystem function. Untangling the drivers of decomposition requires a systems approach, given the vast number of factors that drive the decomposition process in ecosystems.

Temperature, precipitation and leaf chemistry can explain approximately 70% of variation in litter decomposition (Aerts 1997). The remaining 30% results from a combination of biotic and contextual factors. Biotic factors include all pathways that stem from living components of an ecosystem. This can include direct and indirect influences of microbes, primary producers, herbivores and upper trophic levels.

The physical and chemical properties of a decomposing plant substrate are key in determining the rate of decomposition, with higher nutrient concentrations and fewer structural components typically resulting in faster decomposition (Taylor et al. 1989, Silver and Miya 2000). However, the assemblage of litters present is also important in driving decomposition processes. Species decompose differently in mixture than in monocultures, or single species assemblies, with non-additive effects resulting in overall slower or faster decomposition,

depending on the mixture (Butenschoen et al. 2014, Cuchietti et al. 2014). Effects of mixture have been found on the scale of weeks to years. A number of potential mechanisms have been suggested to explain the variability in mixture effects including nutrient transfer between litter species and litter mixtures supporting heterogeneous decomposers communities (Gartner and Cardon 2004, Chapman et al. 2013).

Herbivores can influence decomposition directly, through inputs of frass, cadavers, and throughfall, and via indirect linkages, including the physical modification of plant material, induction of defenses, or selective foraging (Bardgett and Wardle 2010, Chomel et al. 2016, Hunter 2016). The organisms present—both the individual species and functional groups—can influence decomposition rates and interact with substrate, resulting in accelerated or decelerated decomposition rates dependent on time scale (Butenschoen et al. 2014). These interactions can occur with microbes, macro-arthropods and other plant species, highlighting the importance of studying multiple trophic levels in combination (Hättenschwiler & Gasser 2005, Cuchietti et al. 2014). Predators can indirectly influence inputs to the detrital pool by changing the abundance, community or behavior of herbivores (Schmitz et al. 1997, Gessner et al. 2010, Sitvarin et al. 2016).

Both direct and indirect biotic drivers of decomposition are likely to vary over space and time. Spatial variation can come from primary producers, microbes, and upper trophic levels, which are typically more mobile than primary producers or microbes. Temporal variation can take place hourly for microbial communities, daily, seasonally, or across years. Landscape variation and management will interact to generate further spatial and temporal variation. Decomposition is known to vary across space and time, though the basis for the expected relationships between decomposition and variability are conceptualized in terms of abiotic

variation. For example, decomposition is expected to correlate with seasonal temperatures (Aerts et al. 2012) – and seasonality can also alter herbivore population dynamics, with implications for the quality and form of plant tissue entering the detrital pool (Hunter 2001, Hunter 2016). In order to achieve a comprehensive understanding of biotic drivers of decomposition, a holistic approach is necessary, as is consideration of variability through space and time.

**1.2 Coffee agro-ecosystems**

Agro-ecosystems provide a tractable and compelling system in which to interrogate biological drivers of decomposition. Agro-ecosystems are among the most important managed ecosystem given their obvious necessity for feeding the world, the millions of farmers that rely on them for economic security and the increasing amount of land dedicated to agriculture (Tscharntke et al. 2012, Laurance et al. 2014). Agro-ecosystems are important to study and understand for these reasons, but the manipulated biodiversity and management practices in agro-ecosystems can also make them a more tractable study system, with reduced species diversity (Perfecto et al. 2014). Tropical agro-forestry systems are touted as a potential ecologically and socially sustainable alternative to intensified agriculture. However, in order to improve management of these systems, it is necessary to better understand the many ecosystem processes that contribute to productivity and sustainability of these agro-forestry systems. In this dissertation I focused on decomposition, which is a vital ecosystem function for understanding the fate of nutrients and is essential for managing fertilizer regimes and maximizing yield.

Coffee is grown globally throughout the tropics by an estimated 25 million smallholder farms and most of that production is exported, making it a valuable industry (Borella et al. 2015, International Coffee Organization 2019). The tropical lands on which coffee is most often grown

are frequently biodiverse, making them of high value ecologically (Perfecto et al. 1996). Further, coffee agro-ecosystems exist along a gradient of management from unshaded monocultures to biodiverse agro-forests that closely resemble natural forests (Moguel and Toledo 1999). The progenitor of cultivated coffee evolved in the understory, which, in combination with the variation in management, presents an opportunity to manage coffee in a sustainable manner and make it a useful study system in which to test ecological principles (Perfecto et al. 2014). A large body of research has found that ecosystem services and functions vary across this management gradient, but little work has investigated decomposition in these systems (Jha et al. 2014, Perfecto et al. 2014).

I focused my work on coffee agro-ecosystems of Chiapas, Mexico. Chiapas, the most southern state in Mexico, produces more than 40% of Mexico's coffee (USDA 2017), owing in part to its subtropical latitude and the Sierra Madre mountain range that provides altitudes suitable for *Coffea* cultivation. Farms range from a couple of hectares in size to 300 hectares and management styles span the gradient of intensification. The matrix of coffee in Chiapas and the dominance and management of coffee agro-ecosystems make it an interesting and viable study system for investigations of decomposition.

## 1.3 Framework and summary of chapters

Interactions between biota and below-ground processes are important in both managed and unmanaged ecosystems, but here I focus on their dynamics within shaded coffee agro-ecosystems. All plants, including crops in agro-ecosystems, are embedded in a network of interactions that includes upper trophic levels and belowground processes (Figure 1.1). In my system of focus, coffee plants interact with upper trophic levels, including birds, lizards and

arthropods that have the potential to become pests. Physical and chemical properties of the coffee plants influence belowground processes, including decomposition and nutrient cycling. Belowground processes are also connected to the upper trophic levels, indirectly, through the mechanisms discussed above, including their alteration of plant tissue, and direct inputs. These three fundamental levels are variable throughout time and across space. In coffee agro-forests, I have focused on shade trees that are planted within coffee farms as a primary driver of spatial heterogeneity. Mass flower blooms, which occur annually in the dry season, provide one source of temporal heterogeneity, though seasonal dynamics of arthropods and seasonal abiotic conditions themselves provide other examples.

This dissertation addresses key knowledge gaps by investigating multiple biotic drivers of leaf-litter decomposition in shaded coffee agro-ecosystems (Figure 1.2), specifically 1) home-field advantage and 2) contribution of canopy shade trees, 3) impacts of synchronous flowering and 4) cascading effects of a keystone ant species, *Azteca sericeasur*. Each of these biotic drivers is explored in a dissertation chapter, described in more detail below.

**Chapter 2: Leaf litter decomposition of *Coffea arabica* and *Coffea robusta* at home and away: short term home-field advantage.** In this chapter I evaluate a popular ecological theory, known as home-field advantage (HFA), which posits that litter will decompose more quickly in a "home" environment – where it was grown—compared to "away" environments (Vianco and Austin 2008, Ayres et al. 2009, Veen et al. 2015). This is among the first tests of HFA acting on plant material in agricultural systems. I used a reciprocal transfer experimental design with the two cultivated species of *Coffea,* where decomposition of *C. arabica* and *C. robusta* were quantified in their home environments, the con-generic away environment (e.g. *C. robusta* in environments where *C. arabica* is grown), and a forested-away environment, where

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coffee is not grown. I employed a tethered line methodology over six weeks and a year-long litterbag study to assess HFA on a short and long time scale.

**Chapter 3: Interactive effects of *Inga micheliana* and *Alchornea latifola* shade trees on mixed litter decomposition.** Leaf litter decay can be non-additive when multiple species are decomposing in mixture, where the resulting rate of decay is not equal to the sum or average of each species on its own (Hättenschwiler et al. 2005b, Lecerf et al. 2011). These interactions are important in understanding decomposition in coffee agro-forestry systems where coffee is grown beneath the canopy of a potentially diverse canopy of shade trees. Shade trees are often chosen for their ability to fix nitrogen and provide nutrients to plants, or provide fruit or timber, but they may also alter decomposition dynamics (Romero-Alvarado et al. 2002, Peeters et al. 2003, Tully and Lawrence 2012, Jha et al. 2014). This chapter evaluated the ways in which shade tree litter from *Inga micheliana,* a nitrogen fixing legume, and *Alchornea latifolia,* a non-leguminous species, impact the decomposition of *C. arabica* coffee litter. Tethered lines were used in the coffee farm and adjacent forest, with species in monoculture and all possible combinations. Pairwise comparisons with species above and below focal samples were used to test the role of micro-topography in mediating mixture effects.

**Chapter 4: Synchronous flowering of *Coffea arabica* accelerates leaf litter decomposition.** This chapter assesses the impacts of temporal variation in litter inputs that results from a synchronous pulse of flower petals into the detrital pool after a mass bloom of *C. arabica.* Floral tissue generally has high nutrient concentrations, making a temporally synchronous pulse of petals potentially important for the decomposer community and decomposition processes (Martinez et al. 2003, Whigham et al. 2013). I quantified the magnitude of the bloom and the nutrients that bloom represented to the ecosystem. Then, I assessed the

short-term impact of the bloom to the leaf litter invertebrate decomposer community and to *C. arabica* leaf litter decomposition in a two-month litterbag experiment.

**Chapter 5: Evaluating community effects of *Azteca sericeasur* on *Inga micheliana* leaf litter decomposition.** In this chapter I examine the role of a keystone arboreal ant species, *Azteca sericeasur* on the leaf litter decomposition of a common shade tree, *Inga micheliana.* Previous research has highlighted the ability of *A. sericeasur* to modify the ant and broader arthropod community within a radius of its nest (Ennis 2010, Vandermeer et al. 2010, Vannette et al. 2017). I used a combination of community sampling, with baits and pitfall traps, and litterbags to test the indirect impacts of *A. sericeasur* nests on litter decomposition, as mediated by their impacts on the arthropod community.

In a concluding chapter, I contextualize the results of each chapter, placing my work in a broader context and highlighting priorities for future work.

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**1.5 Figures**
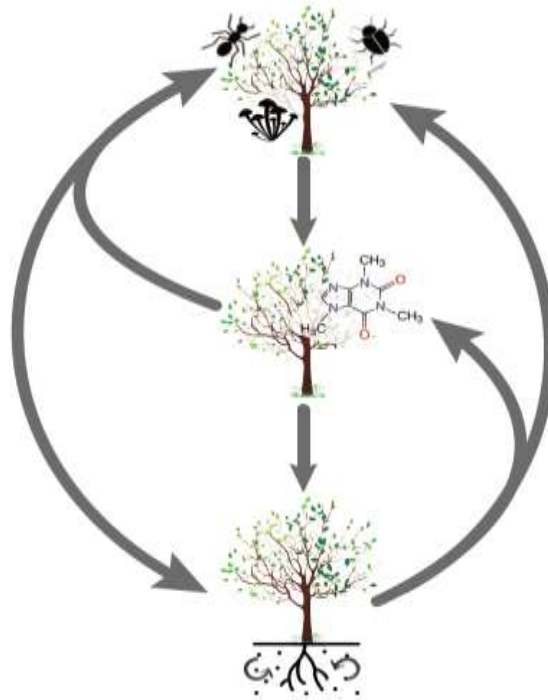


**Figure 1.1.** Conceptual framework. A coffee plant, the primary producer of focus, is at the center of the framework and is pictured with a caffeine molecule as a reminder of chemical interactions, as well as physical properties. Upper trophic levels, including herbivores and fungi are depicted above the coffee plant and nutrient cycling and below ground processes are below. These three levels are all linked, directly and indirectly. While this work focuses on coffee systems, this framework could be generalized across ecological systems and processes.
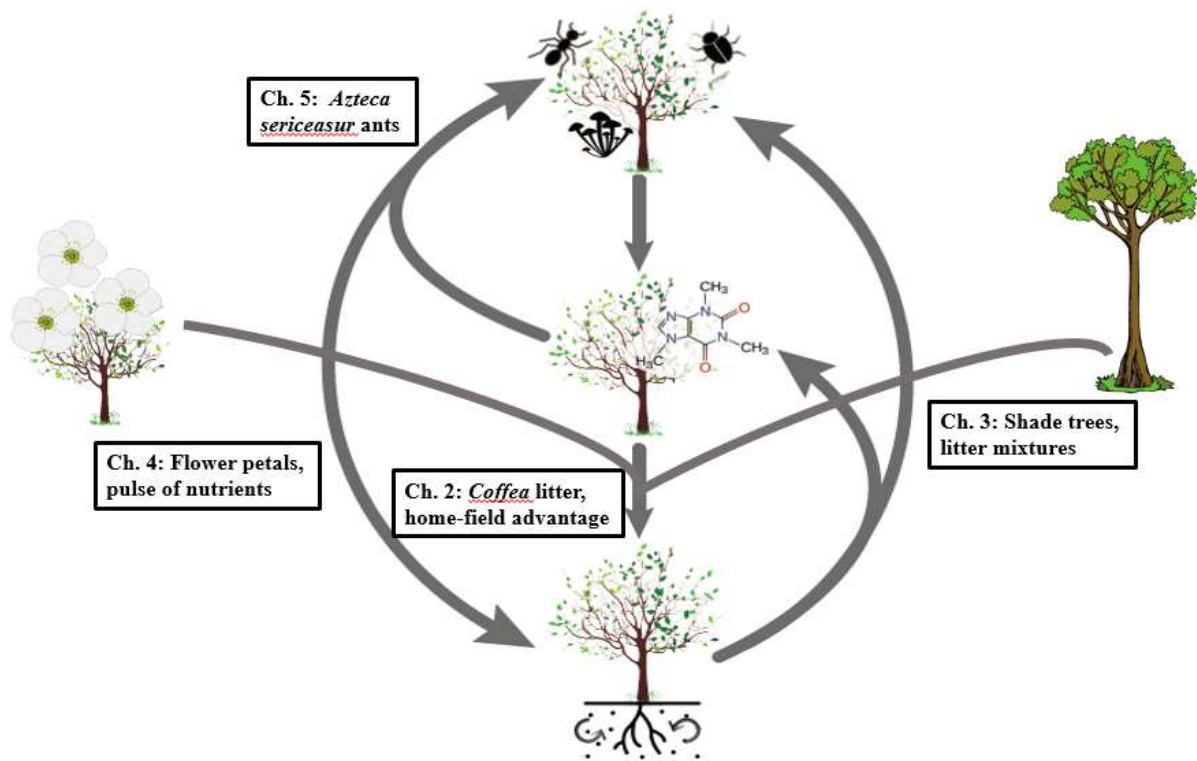
**Figure 1.2.** Summary of dissertation chapters. The four data chapters build from the conceptual framework. Chapter 2 explores the role of home-field advantage in coffee decomposition. Chapter 3 investigates the role of shade trees in driving leaf litter decomposition, and as a source of spatial variation. Chapter 4 focuses on flowers as a source of temporal variation in the detrital pool. The final data chapter links upper trophic levels to leaf litter decomposition, testing the role of a keystone ant species.

**Chapter 2: Leaf Litter Decomposition of *Coffea arabica* and *Coffea robusta* at Home and Away: Short Term Home-field Advantage**

## 2.1 Abstract

Home-field advantage (HFA), when applied to decomposition, predicts that a substrate will decompose more quickly in a home environment compared to away environments, presumably due to specialized decomposer communities. Few empirical tests of HFA have been done in agricultural environments, where manipulated species composition and reduced biodiversity could increase the effects of HFA. We used both a six week tethered line experiment and a yearlong litterbag study as complementary methodologies to assess the decomposition of *Coffea arabica* and *Coffea robusta* leaf litter in three environments: (a) where *C. arabica* is grown, (b) where *C. robusta* is grown and (c) an adjacent forest, where coffee is not cultivated. Using the decay constant (*k)* and carbon to nitrogen ratios, we tested for evidence of a HFA in decomposition, compared to congeneric-away and forested-away environments. We found evidence of HFA with the shorter-term tethered line experiment, where *C. arabica* decayed twice as quickly in its home environment and 50% faster in the congeneric away environment than it did in the forested-away environment. We found no evidence of HFA in the longer litterbag study, with no difference in decay based on species or environment. The carbon to nitrogen ratios for tethered line samples differed over time and by environments, driven by differences between the coffee environments and the forest. Our results provide some of the first evidence of HFA in an agricultural system, with effects even in a congeneric-away environment. While we found no evidence of HFA in the longer, yearlong

litterbag study, a short term HFA could still provide an ecologically important pulse of nutrients if this pulse is synchronized with plant demand.

## 2.2 Introduction

Home field advantage (HFA) is a ubiquitous concept in sports; it posits that a familiar arena and the support of local fans will give the home team an advantage over the visiting team. Ecologists have adopted this framework and applied it to comparison of decomposition dynamics in the environment in which they grew—or where conspecifics are growing—versus environments without conspecifics. This phenomenon has been studied across spatial scales– from individual trees in a watershed (Jackrel & Wootton 2014) to across-biome comparisons (Heneghan et al. 1999)—and temporal scales—with evidence of HFA acting at the scale of weeks (Jackrel and Wootton 2014) and persisting for years (Gholz et al. 2000).

HFA is most often evaluated with reciprocal transfer experiments, wherein the litter from each of two environments is observed in its "home" environment and in the "away" environment of the second focal species. Such studies have demonstrated that HFA is a common, though not universal, phenomenon (Vianco and Austin 2008, Ayres et al. 2009, Veen et al. 2015). Multiple mechanisms may drive HFA, including plant-herbivore interactions, microbial symbiosis, phyllosphere legacies, and specialization of decomposer communities (Austin et al. 2014). While HFA is not the most important determinant of decomposition rates – approximately 70% of decomposition can be explained by climate and initial litter quality – a meta-analysis of reciprocal decomposition studies, including those specifically looking at HFA, found an 8% average increase in decomposition rates due to HFA across litter types in forests (Ayres et al. 2009). Other studies have reported increases in decomposition as high as 53% when manure was placed in "home-field"

pastures (Rashid et al. 2013). Ecosystem characteristics (e.g. total biodiversity and abiotic factors) can play a role in determining the importance of HFA (Heneghan et al. 1999, Gießelmann et al. 2011).

Leaf chemistry, including secondary metabolites, may also play an important role in mediating HFA. Wallenstein et al. (2013) found that the "home" environment accelerated microbially-derived transformations to a greater extent for the more slowly decomposing lodgepole pine than for aspen litter, suggesting that HFA may have a greater effect on more recalcitrant species. Secondary metabolites, which may be produced by the plant or associated endophytes, have the potential to impact decomposition through several pathways, operating from the fine scale of organismal inhibition to the broad scale of shaping microbial communities (Chomel et al. 2016). Secondary metabolites, which can act as chemical defenses against herbivory, can also deter detritivores (Asplund et al. 2013). Coq et al. (2009) found that condensed tannins were negatively correlated with decomposer fauna abundance, while fauna abundance correlated positively with mass loss, indicating that secondary metabolites could have a negative indirect effect on decomposition. Decomposer suppression through secondary metabolites (or other mechanisms, including the presence of endophytes [Lemons et al. 2005]) also slows the process of mineralization (Hättenschwiler et al. 2010).

To date, most research on HFA has occurred in natural ecosystems, with very little investigation of HFA in agricultural systems. Agricultural systems are typified by intensive management, which frequently can include moving biomass in and out of systems (i.e. imports in the form of cover crops and fertilizers, exports of cleared or pruned vegetation). Further, crops are often planted outside their naturally occurring ranges, which can lead to mismatches between the arthropod decomposer communities and the crop detritus. Since HFA is expected to increase with

environmental dissimilarity, it may be more important in agricultural settings where the crop is non-native. The only study of HFA in agricultural systems, to our knowledge, focused on decomposition of manure (Rashid et al. 2013). The study found an increase in nitrogen recovery of 14-53%, depending on the application rates, which corresponded with the decomposition of the manure (Rashid et al. 2013). While the literature on HFA in crop systems is lacking, it is reasonable to assume management decisions, like crop rotation and input management, could influence the outcome and relative role of HFA in agricultural settings. Micro-arthropods can distinguish between quality differences in detritus that result from farm management choices, as demonstrated through feeding preference tests of isopods in cork-oak agroforestry systems (Reis et al. 2018). Additionally, Barel et al. (2018) found that material characteristics as well as rotational history affected decomposition of cover crop residues underscoring additional pathways by which farm management could influence decomposition.

Here we test for HFA in leaf litter decomposition in a coffee agroforestry system. Coffee agroforestry systems provide a compelling system in which to study HFA. They combine elements of both intensive agricultural systems and forested systems, and, as in all agricultural systems, a variety of farm management decisions could influence the magnitude of HFA. For example, a range of management decisions can alter the ways in which plant material enters the detrital pool: clearing can reduce herbaceous cover; canopy cover is managed; coffee plants are pruned and fertilized. Finally, two species of coffee (*Coffea arabica* and *Coffea robusta*), differing in physical and chemical properties, including secondary metabolites like caffeine, are commonly cultivated in proximity, including in our study site.

In the study reported here, we compared the decomposition of *C. arabica* and *C. robusta* with a reciprocal transfer experiment where leaves were placed in their home environment and in

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two away environments: 1) the environment of the other species (hereafter congeneric-away) and 2) a forested environment, where coffee is not cultivated (hereafter forested-away). We used both tethered lines and litterbags to assess HFA because, in combination, these methods allow us assess decomposition at short and longer timescale, and because each method has different bias, with tethered lines overestimating decomposition and litterbags underestimating decomposition (Robertson and Paul 2000, Karberg et al. 2002). We hypothesized that:

a) Home-field advantage will allow both species to decompose more quickly in the home environment than the congeneric-away environment, but *C. arabica* will decompose quicker than *C. robusta,* irrespective of HFA.

b) Decomposition will be slower for both species in the forested-away environment than in the congeneric-away environment, due to relative similarity between the agricultural environments.

## 2.3 Methods

*Study system and study site*

Two species of coffee are cultivated for commercial sale. *Coffea robusta* makes up about 30% of global production and is typically relegated to lower altitudes and lower quality lands (Bunn et al. 2015). *Coffea arabica* is valued more highly than *C. robusta* and requires cooler temperatures, and thus higher elevations. While the two species are similar in many respects, *C. arabica* is smaller in stature, with smaller and thinner leaves. The leaf chemistry of *C. arabica* leaves differs from that of *C. robusta* in two important ways: 1) there is less lignin and other structural compounds, and 2) there are lower levels of the secondary defense compound caffeine. *Coffea arabica* has a higher carbon to nitrogen ratio compared to *C. robusta* (Vega et al. 2020).

Caffeine, the primary defensive compound in coffee, is a nitrogenous alkaloid, known to deter generalist herbivores (Nathanson 1984, Hollingsworth 2002). *Coffea arabica* leaves are approximately 1% caffeine by dry weight, where *C. robusta* leaves are closer to 2% (Ashihara and Suzuki 2004). This difference in caffeine has potentially important corollaries for nitrogen use and demand since caffeine is approximately 29% N by molecular weight (Vega et al. 2020). The difference in chemistries between *Coffea* species could push decomposition rates in either direction. It could be that higher-caffeine leaves could be preferred by decomposers due to the nitrogen present (caffeine being nitrogen-based), leading to faster decomposition of *C. robusta* compared to the lower caffeine *C. arabica* leaves. Alternatively, defensive compounds that are toxic to herbivores, as caffeine can be, may also negatively affect decomposers, resulting in avoidance of higher caffeine leaves and slower decomposition rates of *C. robusta*. Interspecific differences in nutrient quantity may confound or exacerbate the effects of the defensive compounds, irrespective of HFA.

This study was conducted at *Finca Irlanda*, a 300 hectare organic shaded coffee farm in the Soconusco region of Chiapas, Mexico. The farm ranges from 900-1200 m.a.s.l. and experiences mean annual rainfall of approximately 4500 mm (Li et al. 2016). The region has a distinct rainy season from May through October and a dry season from November through April.

The certified organic status of *Finca Irlanda* informs farm management decisions. Herbaceous vegetation in the understory is controlled by periodic manual cutting with machetes. The canopy layer includes a diverse range of species, but is dominated by species in the *Inga* genus (Perfecto and Vandermeer 2002). Canopy trees are pruned periodically and the clippings are generally left in the field. The altitudinal variation at *Finca Irlanda* permits both *C. arabica* and *C. robusta* to be grown; most of the farm is dedicated to *C. arabica* production, with lower

elevations dedicated to *C. robusta* and some cacao. The distribution of the two species within the farm has been approximately static for ≥10 years. The adjacent forest reserve has steep topography, which is part of reason why it is not in cultivation. The area is approximately 15 ha and contains some large trees (>25 m) and patches of secondary forest (Moorhead et al. 2010, Briggs et al. 2013).

*Sampling methods*

We used two methods to assess decomposition: tethered lines and litterbags. Each method is associated with distinct, opposing methodological issues (Vitousek et al. 1994, Robertson and Paul 2000, Karberg et al. 2002, Kurz-Besson et al. 2005). Tethered lines are entirely exposed, so that a piece of leaf material is counted as "lost" or "decomposed" once it is separated from the part of the leaf tied to the fishing line. This approach can therefore greatly overestimate decomposition. On the other hand, estimates of decomposition from litterbags face the opposite issue. Pieces of leaf tissue are retained until they are smaller than the bag mesh size. Additionally, only a partial community of decomposers (species smaller than the litterbag mesh openings) has access to the decomposing material. Thus, relative rates of decay cannot be meaningfully compared between methods, but both are informative in comparing across treatments using the same method.

*Tethered line design*

We collected and dried recently senesced *C. arabica* and *C. robusta* leaves. Using four bunches of leaves—two bunches of each species—we created tethered lines. Each line consisted of a 2 meter-long piece of fishing line, with four leaf bunches attached to the line and separated by 40 centimeters from each other by their petioles. Six lines, arranged like spokes of a wheel,

combined to make one experimental unit (Figure 2.1). Bunches were weighed so that the starting dry mass was known.

We selected 13 sites: five in plots where *C. arabica* is grown, five in plots where *C. robusta* is grown and three sites in a forested area where coffee is not grown. This design allowed us to assess the decomposition rate of both species in areas where they are typically grown (home environment), in areas where the other species is grown (congeneric-away environment), and in a forested area where neither species of coffee is cultivated (forested-away environment). The forested area was included to provide a non-agricultural point of comparison. Selected sites were relatively flat and away from areas of high human activity. We assessed canopy cover at each site using the iPhone application "CanopyApp" (version 1.0.2, University of New Hampshire).

At each site, one wheel was placed on the existing leaf litter. All wheels were set out within a week of each other in June 2016, during the rainy season. Each week of 6 consecutive weeks, one line was collected from every wheel. Collected lines were dried in at 50 deg C to a constant weight and weighed. We used mass loss as a proxy for decomposition and saved samples for carbon and nitrogen analysis.

*Litterbag design*

We repeated the same reciprocal design from the tethered lines with litterbags. We used 5 mm fiberglass mesh for the litterbags to allow micro-arthropods to access the litter. There were a total of 225 litterbags; one third of the litterbags contained *C. arabica* leaves, and another third contained *C. robusta* leaves. The final third of the litterbags had a plastic fabric mimicking the starting density of leaves, to monitor sediment accumulation in the litterbags. As with the tethered line design, we collected and dried recently senesced leaves. We screened leaves for significant

blemishes (discoloring, tears in the leaves, heavy herbivory) before homogenizing acceptable leaves into one batch and sewing approximately 50 g of leaves in each of the litterbags.

We selected fifteen sites, with 5 in each of the following environments: *C. arabica* plots, *C. robusta* plots and forested plots. Litterbags were placed on the litter surface in the field in July 2017, and a set of bags was collected after the following intervals: 1, 3, 6, 9 and 12 months. Upon re-collection, bags were dried to a constant weight in a 50 deg C oven and re-weighed.

*C:N analysis*

We ground dried samples from the tethered line experiment using a Krups brand coffee grinder at its finest setting. We analyzed a subset of samples from each week of collection (thus, 6 time points). From the total ground sample, a representative sub-sample was analyzed for total C and total N using a LECO Trumac CN combustion analyzer (LECO Corporation, St. Joseph, MI). We used the total C and total N data to calculate the carbon to nitrogen ratio (C:N).

*Statistical methods*

For the tethered line experiment, the mass loss was averaged for each species, across both bunches in each line. In a few cases where bunches were lost, only one data point was available for a line. We used the exponential decay equation ($N_t = N_0 * e^{-kt}$) to calculate the decay constant, *k,* as is standard in decomposition literature (Olson 1963). While many equations have been used to look at the rate of decay, the simple exponential equation is among the most widely used and appropriate for our shorter time frames (Wider and Lang 1982, though see Cornwell and Weeden 2014). A higher *k* is indicative of faster decomposition.

We made linear mixed models using the "lmer" function in the "*lme4*" package in R (De Boeck et al. 2011) to further assess the effects of species and environment on the decay constant. With $k$ as the dependent variable, we used species, environment, and the interaction between species and environment as potential predictors. If home-field advantage (HFA) was acting, we would expect an interaction between species and environment. Time is not included in the model because it is incorporated in $k$. We included wheel as a random variable because wheels were sampled at each sampling point, and thus, decay would be expected to correlate between samples at that site. Wheel was incorporated as a random intercept because, since theoretically no decay would have taken place at day 0, $k$ has a theoretical intercept of 0. The same analysis was repeated for tethered line and litterbag data sets. In the litterbag analysis days was used as the time variable and for the tethered line data weeks was used. This was done to avoid partial weeks in the litterbag study and to make the values comparable to the published literature.

The assumptions of independence and equal variance were met. However, assumption of normality was not met, even after log-transforming the data. The results of the log-transformed analyses were qualitatively the same as with the untransformed data. Violations of normality primarily affect the residuals, which are not our focus here, and transformations without justification beyond a lack of normality has come under increasing scrutiny (Chanyoung et al. 2014, Mena et al. 2017). Given this and our sample size (Schmidt and Finan 2018), we used un-transformed data for these analyses, despite the violation of the normality assumption.

We used post-hoc tests to generate contrasts that allowed us to make pairwise comparisons between the three environments. We calculated estimated marginal means, or least square means, using the "emmeans" function from the "*emmeans*" package in R (Lenth et al. 2018). With three environments, the linear mixed model output only provides 2 of the potential 3 environment

comparisons with any given reference category. The model could be re-paramaterized using different reference categories, but using contrasts provides comparisons between all levels of a factor, without the algebra of re-calculating intercepts. For both tethered line and litterbag models we used "emmeans" to calculate pairwise contrasts between environments. For the tethered line data we also calculated pairwise contrasts of environments, by species. This was not done for the litterbag data because it was not warranted based on the model results.

We built a linear mixed model to test for difference in the carbon to nitrogen (C:N) ratio in the tethered line samples. As with the decay constant analysis, we used the "lmer" function from the "*lme4*" package in R (De Boeck et al. 2011). The first run of the model included time, species and environment and all of the two and three way interactions between the three main effects. We included wheel, nested with time, as a random variable to account for similarity between the repeated samples from each wheel. We used model selection to create a second model with time and environment, both of which were significant in the full model. Again, we used the "emmeans" function from the "*emmeans*" package in R to calculate the estimated marginal means for each of the three pairwise combinations of environments (Lenth et al. 2018).

## 2.4 Results

*Tethered lines*

Over six weeks, the decay constant, *k,* was lower in both the congeneric and forested away environments, compared to the *C. arabica* environment (Fig 2.2A). The decay constant for *C. robusta* varied less, but was also lower in the forested away environment (Fig 2.2A). In areas where *C. arabica* is grown, the decay constant, *k,* was higher for *C. arabica* leaves than *C. robusta* leaves ($k_{CA}$=20.509 ± 2.01, $k_{CR}$=12.698 ± 1.12, *p<0.005*, Table 2.1). In areas where *C. robusta* is

grown, $k$ was still higher for *C. arabica* ($k_A$=15.880 ± 1.64, $k_R$=13.935 ± 1.49), though the difference between the species decay constants was smaller.

In the forest, the rate of litter decay did not differ between species ($k_A$=8.673 ± 1.99, $k_R$=8.32 ± 1.23). Based on the pairwise comparisons, litter decay for both species in the forest was significantly slower than in the *C. arabica* environment ($p$= 0.0271) and slower than in the *C. robusta* environment ($p$= 0.0946), though the forest and *C. robusta* environments were not significantly different. The decay of *C. arabica* in coffee environments is driving the difference between the coffee environments (*C. arabica* and *C. robusta*) compared to forest environment (Table 2.1C).

*Carbon to nitrogen ratio*

C:N ratios decreased over time, as would be expected with decay ($p < 0.005$, Table 2.2, see supplementary table A2.2 for full model results). Litters decomposing in the forest environment had significantly higher C:N ratio compared to both *Coffea* spp. environments (*C. arabica* – forest, $p$=0.0117, forest-*C. robusta,* $p$=0.0162). However, the C:N ratio did not differ significantly between species ($p$=0.307) or between environments ($p$=0.9821).

At the end of the 6 week tethered line experiment, C:N ratios were higher for *C. robusta* litter in the *C. arabica* environment than they were for *C. arabica* litter (C:N$_{CR}$=18.0 ± 0.49, C:N$_{CA}$=15.2 ± 0.64), but did not differ between litter species in the *C. robusta* or forested environments (Figure 2.2C).

*Litterbags*

Over the one year study period of the litterbag experiment, decomposition rates did not differ between species or between environments (Fig 2.2B, Table 2.3). There was no significant interaction between species and environment (Table 2.3).

## 2.5 Discussion

Our study finds support for home-field advantage in litter decomposition over the span of weeks with the tethered line methodology, but these HFA effects did not persist for months in litterbags – nor was there any detectable HFA acting on shorter time scales with the litterbag methodology. Our experimental design provided two away environments for each species - one agricultural or congeneric-away and one non-agricultural forested-away environment. We found evidence for short-term HFA (up to one and a half months) acting between the home and congeneric-away environments, as demonstrated by the significant effect of the species x environment interaction on the decay constant for the tethered line experiment. Both *C. robusta* and *C. arabica* decomposed more quickly in their home environments compared to the forested away environment and *C. arabica* also decomposed more quickly in the congeneric away environment compared to the forested away environment, which supports our second hypothesis. This is among the first reports of home-field advantage in agricultural systems and could have important implications for nutrient cycling in tropical agricultural settings, even if HFA could not be detected over a longer time frame.

The slower decomposition that we found in the forested-away environment could be partially due to the abiotic conditions of a forest—e.g. increased canopy cover leads to less light and lower temperatures which may outweigh a possible increase in humidity. Similarly, the species

initial differences in leaf nutrients and secondary chemistry (which we did not measure, but has been established in previous studies) likely contribute to the faster decomposition of *C. arabica* relative to *C. robusta* that we saw across environments. However, we found an interaction between species and environment for the tethered line, when looking at *k,* and a higher *k* for both species in their home environments, which is indicative of HFA.

The difference between environments in the tethered line study is driven primarily by differences in the *C. arabica* leaves between home and congeneric-away environments and the forest, as indicated by the pairwise contrasts, when environments are separated by species. While both species are decomposing more quickly in coffee environments, the magnitude of change between rates of decay in agricultural and forest environments is greater for *C. arabica.*There was no difference in the decay rate of C:N ratio for *C. robusta* between coffee environments. *Coffea arabica* has smaller, thinner leaves, with less caffeine, than *C. robusta,* so the higher decay constants are not altogether surprising, particularly with the tethered line methodology where there is greater exposure to abiotic factors. However, if caffeine is an impediment to decomposers, we should expect *C. robusta* to benefit most from a specialized home community of decomposers. It may also be that less biodiversity and more disturbances in the agricultural environments prevent the expected development of specialized decomposer communities (Jangrid et al. 2008). While we cannot definitively disentangle the role of decomposer communities, or the potential effects of the physical and chemical attributes of the two *Coffea* spp., our results suggest that the decomposer community in the forest may be highly specialized or less able (or less inclined, given the other litter types that may be available) to break down any quantity of caffeine.

In the tethered line experiment, variation in C:N ratios supported the findings from the decay constant in that there was a significant difference between the two coffee environments and

the forest environment. However, C:N ratios did not differ between home and congeneric-away environments for either species. Ratios of carbon and nitrogen are traditionally used as a proxy for litter quality and an indicator of the decomposition stage of litter. Our C:N ratio results reflect the decomposition stage of the litter, though we know there are also initial species differences (Vega et al. 2020). Thus, the high $k$ for *C. arabica* in the *C. arabica* environment is reflected in a low C:N ratio for *C. arabica* in a *C. arabica* environment. The C:N ratio could be lower for our treatments with highest decay rates if more stable or inaccessible forms of N are left behind over time as relative labile C is lost. Most studies of HFA have used $k$ as a response variable, not C:N ratio. C:N ratios describe the quality of undecomposed litter, not the quantity of already decomposed materials (Bonanomi et al. 2013).

Our results suggest that HFA occurs on the scale of weeks, but does not play a significant role over a longer period of time. In the yearlong litterbag study we found no differences in the rate of decay between the home environment and congeneric-away or forested-away environments for either species. Other year-long tropical litterbag studies have also failed to find evidence of HFA (Bachega et al. 2016); it might be harder to detect HFA on longer time scales in our study system, given the rapid rate of decay in tropical systems. However, we did not find evidence of HFA, even at the one-month collection of litterbags (see supplementary table A2.4).

Our ability to detect HFA at the four-week time point in the tethered line experiment, but not the one-month collection of litterbag samples may be due to the inherent biases in the respective methodologies. We know of few studies that use both tethered line and litterbag examples (for exceptions see Woods and Raison 1983, Lawrence and Wise 2004). In contrast to our results, one such study in a sub-alpine forest reported similar decay rates between tethered lines and litterbags (Woods and Raison 1983). At first glance, it is perplexing to have a higher

proportion mass loss (and, thus, higher $k$) in a six-week experiment, compared to a year-long experiment, but it is congruent with the respective methodological biases. While microbes are more likely to be highly specialized than larger decomposers, perhaps specialized decomposers with larger body sizes were excluded from the litterbags. However, our study used 5 mm mesh, which allows access to most micro- and meso- fauna. We know of one decomposer larger than 5 mm, a common millipede species, at our field site, but small soil biota, which would have access to the litter in our bags, have been implicated as the drivers of HFA in grassland systems (Li et al. 2020), not larger decomposers. The two methods used offer different exposures to the largest decomposers, but also lead to disparities in abiotic conditions. The litter on the tethered lines is far more exposed to abiotic conditions compared to the litter in the litterbags, which may experience a different micro-climate than litter adjacent to the bags. The micro-climate in the litterbags is unlikely to have had a directional effect (that is, a reverse HFA effect), but could also have impeded our ability to detect HFA if HFA is happening in the early stages of decomposition and those early stages are elongated due to the litterbag design.

Our study did not seek to identify the mechanism behind the HFA operating in this system, and many potential mechanisms could be responsible for the observed patterns. Differences in vegetation quality and soil quality, and disparity in environments are often cited as determinants in predicting the strength of HFA (Veen et al. 2015, Palozzi and Lindo 2018), but here we see evidence of HFA despite using two species of the same genus and similar, adjacent environments. This suggests high levels of decomposer specialization may be responsible, which is congruent with other research (Austin et al. 2014, Lin et al. 2019), though we did not explicitly examine the soil biota. Our study also lacks data on soil chemistry. We assume that soil parameters did not differ, except in differences that might result from different plants, because the environments were

adjacent to one another, but future studies should incorporate chemical parameters into their analysis as well. While we do find evidence of HFA between home and congeneric-away environments, in some cases, decay rates were more similar in congeneric-away environments than in the forested-away environment, which highlights the role that environmental disparity and, potentially, microbial communities plays in driving HFA.

HFA could be important in agro-ecosystems, even though it appears to operate only on short time scales in this coffee agro-ecosystem. Given the rapid pace of decomposition in the tropics, differences in decomposition rates in the initial weeks could have a relatively large impact on plants if the pulse is synchronized with plant demand (Lodge et al. 1994). Moreover, work with agricultural cover crops finds that even a short-term pulse of nutrient availability can increase yields in temperate agricultural systems (Blesh 2018). Our results also suggest that farm-level management decisions could play a role in determining the magnitude of HFA. Increasing homogeneity in agro-ecosystems could lead to accelerated decomposition and potentially increased nutrient availability or tighter cycling if the nutrients are bioavailable and stay in the agro-ecosystem. However, there are many other, often negative, consequences of homogenization that could reduce yields and decrease the reliency of agro-ecosystems (Jha et al. 2014). These negative consequences of homogenization are unlikely to be outweighed by the accelerated decomposition possible with stronger HFA.

HFA is known to be situationally important in a variety of ecosystems and contexts. Many of the mechanisms proposed to explain HFA are not mutually exclusive; further work is needed to determine which suite of mechanisms might be acting and in which contexts those mechanisms and HFA are most prevalent. Additional research into HFA in agricultural systems is warranted to ascertain how exactly management decisions could drive HFA and if and how HFA is meaningful

in terms of nutrient availability to crops in an agricultural context. This study provides one of the first accounts of HFA in agricultural landscapes and highlights the potential role of farm-level management decisions in altering nutrient cycling dynamics.

## 2.6 Acknowledgements

## 2.7 References

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## 2.8 Tables and figures

**Table 2.1** Linear mixed model output for tethered line samples (A), pairwise estimated marginal means contrasts for the pairwise combinations of locations (B), and contrasts for pairwise combinations of locations, separated by species (C). Pairwise contrasts for all main effects and interactions are provided in supplementary table A2.1. *Coffea arabica* was the reference species and reference environment for the model (i.e. species estimates for *C. robusta* describe the difference between *C. arabica* and *C. robusta*).

| *Predictors* | *Estimates* | *Std. Error* | *df* | *t-value* | *P value* |
|---|---|---|---|---|---|
| **A. Linear mixed model output** | | | | | |
| (Intercept) | 20.946 | 1.787 | 18.927 | 11.724 | <0.005 |
| **Species** | **-7.711** | **2.051** | **196.567** | **-3.759** | **0.000225** |
| Environment | | | | | |
| *C. robusta* | -5.091 | 2.624 | 30.343 | -1.94 | 0.061731 |
| **forest** | **-12.273** | **3.067** | **12.642** | **-4.002** | **0.001589** |
| Species x environment | | | | | |
| *C. robusta* x *C. robusta* | 5.498 | 3.026 | 197.91 | 1.817 | 0.07077 |
| ***C. robusta* x control** | **7.363** | **3.433** | **195.540** | **2.145** | **0.033226** |
| **B. Pairwise contrasts for environments** | | | | | |
| ***C. arabica* – forest** | **8.59** | **2.54** | **7.08** | **3.389** | **0.0271** |
| *C. arabica* – *C. robusta* | 2.34 | 2.07 | 12.76 | 1.132 | 0.512 |
| Forest- *C. robusta* | -6.25 | 2.56 | 7.65 | -2.441 | 0.0946 |
| **C. Pairwise contrasts for environment, by species** | | | | | |
| Species: *C. arabica* | | | | | |
| ***C. arabica* – forest** | **12.273** | **3.08** | **15.5** | **3.985** | **0.003** |
| *C. arabica* – *C. robusta* | 5.091 | 2.68 | 36.5 | 1.902 | 0.1526 |
| **Forest- *C. robusta*** | **-7.182** | **3.19** | **18.4** | **-2.25** | **0.089** |
| Species: *C. robusta* | | | | | |
| *C. arabica* – forest | 4.910 | 3.04 | 14.7 | 1.613 | 0.2715 |
| *C. arabica* – *C. robusta* | -0.407 | 2.45 | 24.5 | -0.166 | 0.9849 |
| Forest- *C. robusta* | -5.317 | 3.03 | 15.1 | -1.754 | 0.2183 |

**Table 2.2** Linear mixed model output for carbon to nitrogen ratios from tethered line samples (A), and pairwise estimated marginal means contrasts for the pairwise combinations of locations (B). The full model, before variable selection, is provided in supplementary table A2.2. *Coffea arabica* was the reference species and reference environment for the model (i.e. species estimates for *C. robusta* describe the difference between *C. arabica* and *C. robusta*).

| Predictors | Estimates | Std. Error | df | t-value | P value |
|---|---|---|---|---|---|
| **A. Linear mixed model output** | | | | | |
| (Intercept) | 20.0102 | 0.40084 | 85.2111 | 49.920 | <0.005 |
| Time | -0.08585 | 0.01301 | 64.0475 | -6.599 | <0.005 |
| Environment | | | | | |
| *C. robusta* | 0.07957 | 0.41541 | 19.9047 | 0.192 | 0.85004 |
| **forest** | **1.55847** | **0.42893** | **14.7035** | **3.633** | **0.00252** |
| **B. Pairwise contrasts for environments** | | | | | |
| ***C. arabica* – forest** | **-1.5585** | **0.456** | **13.2** | **-3.418** | **0.0117** |
| *C. arabica* – *C. robusta* | -0.0796 | 0.439 | 17.9 | -0.181 | 0.9821 |
| **Forest- *C. robusta*** | **1.4789** | **0.469** | **15.8** | **3.153** | **0.0162** |

**Table 2.3** Linear mixed model output for litterbag samples (A) and pairwise estimated marginal means contrasts for the pairwise combinations of locations (B). Pairwise contrasts for all main effects and interactions are provided in supplementary table A2.3. *Coffea arabica* was the reference species and reference environment for the model (i.e. species estimates for *C. robusta* describe the difference between *C. arabica* and *C. robusta*).

| Predictors | Estimates | Std. Error | df | t-value | P value |
|---|---|---|---|---|---|
| **A. Linear mixed model output** | | | | | |
| (Intercept) | 4.12115 | 0.91886 | 134 | 4.485 | <0.005 |
| Species | 0.09197 | 1.29947 | 134 | 0.071 | 0.944 |
| Environment | | | | | |
| *C. robusta* | 0.27724 | 1.31294 | 134 | 0.211 | 0.833 |
| forest | 0.49054 | 1.35994 | 134 | 0.361 | 0.719 |
| Species x environment | | | | | |
| *C. robusta* x *C. robusta* | -0.23990 | 1.85677 | 134 | -0.129 | 0.897 |
| *C. robusta* x control | -0.656 | 1.92325 | 134 | -0.330 | 0.742 |
| **B. Pairwise contrasts for environments** | | | | | |
| *C. arabica* – forest | -0.1728 | 0.963 | 12.4 | -0.179 | 0.9824 |
| *C. arabica* – *C. robusta* | -0.1573 | 0.929 | 10.9 | -0.169 | 0.9843 |
| Forest- *C. robusta* | 0.0155 | 0.972 | 12.8 | 0.016 | 0.9999 |
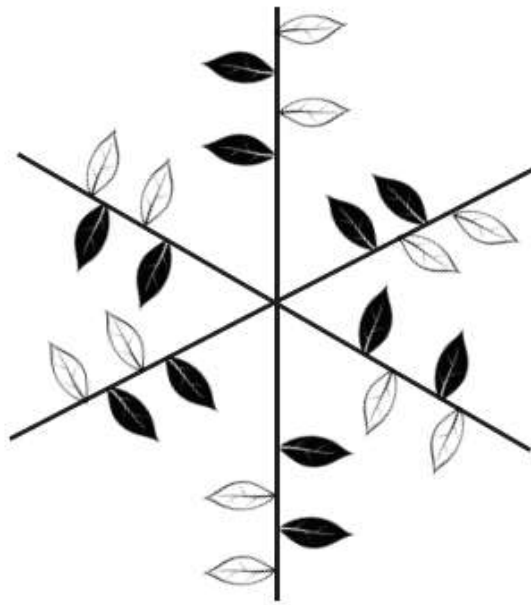
**Figure 2.1** Overhead schematic of tethered line design. Six lines with two alternating bunches of four *C. arabica* leaves (black) and four *C. robusta* leaves (white) were arranged into a wheel.
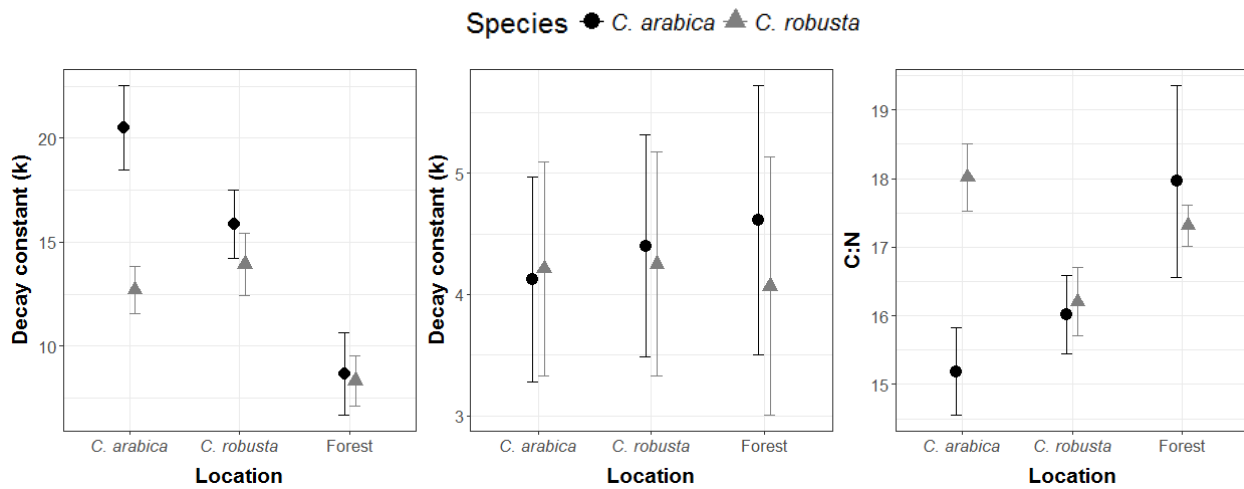


**Figure 2.2** Decay constant for tethered lines (A) and litterbags (B) and C:N ratio for tethered lines (C). Error bars represent standard error.

# Chapter 3: Interactive Effects of *Inga micheliana* and *Alchornea latifola* Shade Trees on Mixed Litter Decomposition

## 3.1 Abstract

The decay of litter from multiple plant species decomposing in mixture can be non-additive, where the resultant decay rate is not equal to the sum or average of the decay of each species in monoculture. These interactions are important to understanding and predicting nutrient cycling and decomposition generally and essential in agroforestry contexts where biodiversity is managed to optimize ecosystem function.

We quantified the rate of litter decay for leaves of three focal species: *Coffea arabica* and two shade tree species common in coffee agro-forestry systems, *Inga micheliana* and *Alchornea latifolia.* With locations in the forest and where *C. arabica* is grown, we were able to compare rates of decay in monoculture to rates of decay with all possible litter mixture combinations across locations. We expected that all species would decay more quickly in mixture but that *I. micheliana* would have the greatest accelerating effects, because it is a nitrogen-fixing species. Though *C. arabica* has a relatively low carbon to nitrogen ratio, we expected that nitrogen in caffeine, *C. arabica's* main defensive compound, might be a deterrent to decomposers. Lastly, we used pairwise comparisons with species located above and below focal species to assess the role of run-off and micro-topography in facilitating decomposer dispersal.

Of the three species tested, only *C. arabica* decayed significantly faster in mixture in both 2017 and 2018 studies. As predicted, decomposing in combination with the nitrogen-fixing *I. micheliana* accelerated decay more than decomposing in combination with *A. latifolia.*

Decomposing in combination with *C. arabica* had mixed and opposing results for *I. micheliana* and *A. latifolia* between settings and study years. There was little evidence to suggest that micro-topography was important in facilitating mixture effects.

In sum, our results highlight the context-dependency of mixture effects on leaf litter decomposition. We find evidence of a common nitrogen-fixing shade tree accelerating the decay of *C. arabica* leaf litter highlighting another potential benefit of maintaining canopy trees on coffee farms.

### 3.2 Introduction

Tree species composition can influence decomposition and nutrient cycling through a number of pathways, including interactive mixture effects, also known as non-additive effects. With non-additive effects, decomposition rates of litter occurring in a multispecies mixture cannot be predicted by its decomposition when occurring alone (Hättenschwiler et al. 2005). Such effects are widespread and can result in faster or slower decomposition (Hättenschwiler et al. 2005, Lecerf et al. 2011), yet how they might influence nutrient availability in soils in agroforests is poorly understood. The direction of non-additive effects is uneven across studies, and these effects can lead to overall faster or slower decomposition (Gartner and Cardon 2004, Ball et al. 2008, Hoorens et al. 2010). In a 32 species study, mixing litter led to large non-additive effects, but there were no patterns in the directionality or magnitude of the effects based on litter quality or functional group (Wardle et al. 1997). The effects of mixed litter may also change over time, with some studies finding greatest effects of mixture in shorter time frames and others finding non-additive effects that persist for years (Gartner and Cardon 2004, Chapman et al. 2013).

Mixture effects might occur through the following three overlapping and non-exclusive factors, explained below: a) identity and composition of the decomposing substrate, b) identity and composition of decomposers, and c) spatial dynamics. First, the quality of litter substrate (i.e. nutrient content, or recalcitrance) could be important in determining the strength and direction of additive effects (Wardle et al. 2002). Litter constituent species richness and/or identity could also be important if litters have complementary stoichiometry (Scherer-Lorenzen 2008).

Second, the identity and richness of decomposers—both invertebrate and microbial— could also drive ecosystem function outcomes for mixed substrates. If decomposers are switching between species or attracted to mixtures due to physical/habitat or nutritional heterogeneity, there may be more decomposers and a wider range of decomposers present in mixture (Handa et al. 2014, McDaniel et al. 2016). Decomposer diversity inextricably is linked to litter diversity, because microbial diversity often increases with plant litter diversity (Chapman and Newman 2010, for an exception see Griffin et al. 2019) and some microbial decomposers are endophytic, already present in the litter when it enters the detrital pool. Litter mixtures have been found to have 70% higher microbial activity after 10 months and 20% higher activity after 27 months, compared to litter monocultures (Chapman et al. 2013). While microbial decomposers are important, past research also underscores the role of invertebrate decomposers. Smith and Bradford (2003), comparing decomposition rates in litterbags with varying mesh size, found the strongest relationship between litter quality and decomposition with the coarsest mesh size at 30 days, suggesting that larger decomposers respond quickly and strongly to litter quality. In another study, the presence of millipedes and earthworms determined the direction and magnitude of mixture effects, with millipedes affecting the decomposition rate of more slowly

decomposing ("low quality", high C:N ratio) species and earthworms having a great effect on quickly decomposing ("high quality", low C:N ratio) species (Hättenschwiler and Gasser 2005). The decomposer community regulates changes in litter chemistry during decomposition (Wickings et al. 2012), further underscoring their importance in determining the rate and outcome of decomposition processes.

The third non-exclusive way in which mixtures might alter decomposition is through spatial dynamics. Decomposer dispersal is often invoked as a presumed mechanism to explain non-additive effects, but few empirical tests have been done. Larger decomposers are likely to be more mobile across the landscape, but microbial decomposers may disperse through vegetative dispersal (Rantalaninen et al. 2006), with larger organisms (Jacobsen et al. 2017), or via rain or wind (Fitt et al. 1989, Madden 1997). Considering spatial dynamics and decomposer dispersal offers a potential path to resolving some of the contradictory results from existing research on the decomposition of litter mixtures.

Agroforestry systems offer a compelling and tractable model system to investigate litter mixture effects. Agro-forestry systems, where crops are cultivated with trees, often have reduced biodiversity, when compared to natural forests. Furthermore, decomposition and nutrient cycling are of great interest to farmers who are invested in optimizing nutrient use efficiency. In coffee agro-forestry systems, leguminous shade trees are frequently planted because of a perceived benefit from nitrogen fixation that could increase nitrogen availability to crops, though the evidence for these perceived benefits is relatively scarce (Romero-Alvarado et al. 2002, Grossman et al. 2003, for an exception see Haggar et al. 2011, Sauvadet et al. 2019). The choice of which trees to plant in coffee agro-forestry is often framed in terms of nutrient inputs from fixation, without regard for the effects of tree species effects on decomposition, whether through

direct inputs or mixture effects. Importantly, decomposition is the primary way that nutrients from biological nutrient fixation become available to the coffee plants (Monroe and Isaac 2014).

One important crop frequently grown in agroforestry systems is coffee (*Coffea* spp.), which ranks among the most valuable global commodities and supports millions of farmers (Jha et al. 2014). Coffee is cultivated in a range of management styles from unshaded monocultures to diverse agro-forestry systems (often called "traditional" agroforestry systems) (Moguel and Toledo 1999). Across the Neotropics, coffee farmers commonly employ leguminous trees in the genus *Inga* as the primary canopy tree, in part for the perceived benefits for soil fertility and therefore yield. Some research has found increased nutrient availability from fixation in coffee agroforestry systems, including nitrogen and carbon, when shade trees are present (Snoeck et al. 2000). Yet direct comparisons of traditional and *Inga* dominated agro-forestry systems have found no evidence of differences in yield (Romero-Alvarado et al. 2002, Peeters et al. 2003). Furthermore, some studies have found a negative correlation between shade trees and weed density (Soto-Pinto et al. 2002), but this expectation of farmers has not been met under *Inga* canopies (Romero-Alvarado et al. 2002). Despite mixed evidence, farmer interviews in Mexico and Costa Rica found that *Inga* spp. were among the most commonly referenced shade trees by coffee farmers (Grossman 2003, Albertin and Nair 2004). The ubiquity of *Inga* spp. in coffee agroforests likely stems also in part from government policy. For example, in Mexico, the Instituto Mexicano del Café (INMECAFE), a now defunct extension agency, heavily promoted the use of *Inga* spp. over traditional (mixed) agroforestry systems (Peeters et al. 2003).

The inconsistent evidence for the contribution of *Inga* to nutrient availability in coffee agro-forestry systems stems from the context-dependent nature of nitrogen fixation more broadly. Nitrogen is cycled more conservatively in shaded coffee plantations than in unshaded

plantations, with more available nitrogen and less leaching in shaded systems (Babbar and Zak 1994, Tully et al. 2012), but root nodules of leguminous trees and biological nitrogen fixation are highly variable in agro-forestry systems (Winbourne et al. 2018). Spatial variation in *Inga* symbionts and variable fixation by nodules may also add to inconsistent contributions of nitrogen (Grossman et al. 2005). *Inga oerstediana,* among the common *Inga* sp. in Mexico, was slow to nodulate in a greenhouse study, with little evidence of fixation at 150 days, meaning it is unlikely that younger stands are making a significant contribution to nitrogen availability, especially for neighboring coffee plants (Grossman et al. 2006). In another study, *Inga edulis* provided an estimated 100 kg N/ha/yr in a mature stand, but only 60% of individual plants fixed N, with no clear pattern distinguishing those contributing N from non-contributors (Leblanc et al. 2007).

The lack of a clear pattern of fixation and variable nutrient contributions suggests that promotion of leguminous shade trees – including *Inga* spp.– on the basis of their potential to increase soil fertility via the addition of fixed N may be premature; however, the presence of leguminous shade trees is still likely to affect nitrogen cycling through direct litter inputs (i.e. conserving and recycling N within the system and maintaining it in relatively bioavailable pools) and potentially through mixture effects that speed up decomposition and nutrient cycling (Sharma et al. 1997, Youkhana and Idol 2009, Tully and Lawrence 2012). Litterfall from the leguminous shade tree *Erythrina poeppigiana* added the same amount of nutrients as the highest fertilizer recommendation when *E. poeppigiana* was pruned at least twice per year (Beer 1988).

We conducted a study that considered the effects of two shade tree species – *Inga micheliana* (Facaceae)*,* a leguminous nitrogen fixer, and *Alchornea latifolia* (Euphorbiaceae)*,* a species that does not fix nitrogen –on *Coffea arabica* leaf litter decomposition. Trees in the *Inga*

genus are the most common shade tree at our study site, accounting for more than half of all the shade trees (Philpott and Bichier 2012), and the most common genera in coffee agroforests throughout the region (Grossman et al. 2006). The second focal shade tree species, *Alchornea latifolia,* is the most common non-*Inga* shade tree at our study site. Both species are managed by pruning to control canopy cover and tree architecture.

*Coffea* spp., including *C. arabica,* are well-known for their secondary defensive compounds, namely caffeine. Caffeine is an alkaloid, and thus contains nitrogen, which may attract decomposers if they can access the nitrogen and if nitrogen is limiting. The accessibility of nitrogen in caffeine to decomposers may drive the decay rate of *C. arabica* and other species decomposing with it. Conversely, caffeine can deter generalist herbivores (Nathanson 1984, Hollingsworth et al. 2002) and it may have the same deterring effect on decomposers (Arora and Ohlan 1997, Rudgers and Clay 2008, Chomel et al. 2016). *Coffea arabica* leaves are approximately 1% caffeine by dry weight (Ashihara and Suzuki 2004) and contain 3-3.5% foliar nitrogen, depending on the fertilization regime (Gonthier et al. 2011).

We conducted two experiments. In the first, we compared the decomposition rate of leaf litter from each species – the primary crop, *C. arabica*, and both shade trees – individually and with factorial combination of one and two other species both in an operational coffee farm and neighboring forested reserve. In the second experiment, we used a pairwise design where each species was uphill and downhill from each of the other species to examine the role of topography and rainwater run-off in decomposer dispersal. With these two experimental set-ups, we tested the following hypotheses:

1. Consistent with synergistic mixture effects, we expect all species will decompose more quickly in combination with other species, due to any of the potential mechanisms that could result in complementarity.

2. Given the relatively low carbon to nitrogen ratio of *Inga* litter, we hypothesize that the decay rate of *C. arabica* decomposing in mixture with *I. micheliana* would be higher than its decay rate when decomposing with *A. latifolia.*

3. If the nitrogen in *C. arabica* is bound up in caffeine, and caffeine is a deterrent for decomposers, the mixture effects from decomposing with *C. arabica* litter would smaller than expected based on the carbon to nitrogen ratio alone.

4. If micro-topography controls the dispersal of decomposers, then being down slope from a quickly-decomposing species, like *I. micheliana*, will lead to faster decomposition than being uphill of the same species.

## 3.3 Methods

*Study site*

This research was conducted at *Finca Irlanda*, a 300 ha organic, shaded coffee farm in the Soconusco region of Chiapas, Mexico. The site is located approximately 40 km north of Tapachula, Chiapas, Mexico at 900-1100 m a.s.l. It experiences distinct seasons with a rainy season that lasts from May through October and yields approximately 4200-6000 mm of rainfall annually (Burdine et al. 2014).

*Finca Irlanda* is certified by the Smithsonian Migratory Bird Center as a bird friendly coffee farm, a certification that requires a minimum density and diversity of shade trees. There are more than 100 shade tree species on the farm, though trees in the *Inga* genus make up

approximately 70% of the total shade trees (Philpott and Bichier 2012). *Finca Irlanda* encompasses areas where *C. arabica* is cultivated and areas with *Coffea robusta* and *Cacao* spp. The farm also includes a forest fragment, where coffee is not cultivated. The topography within the forest fragment is too steep for profitable agriculture and much of the forest in this fragment has never been cut (J. Vandermeer, personal communication).

*Experimental design*

Tethered lines are used in decomposition studies as a way to asses litter decomposition, particularly over shorter periods of time (Vitousek et al. 1994, Karberg et al. 2008, Kurz-Besson et al. 2005). Tethered lines are completely exposed, and thus abiotic conditions may further affect decomposition of litter on tethered lines. Tethered lines tend to overestimate decomposition because once a piece of the leaf is detached from the piece tethered to the line, it is considered to be decomposed. However, all decomposers have access to tethered lines because the lines sit on the leaf litter surface, providing an advantage over litterbags for the purposes of the research questions addressed here, which exclude all decomposers larger than the mesh used to make the litterbag.

*Tethered line wheels*

Recently senesced *Inga micheliana (*IM)*, Alchornea latifolia* (AL) and *Coffea arabica* (CA) leaves were collected from the research plot and air dried. All three focal species senesce throughout the year, without a mass senescence event. Damaged leaves were excluded. We assembled wheels composed of six identical tethered lines each. In total, 7 wheel types were

made: three with a single species (with IM, AL or CA alone); three with two species (IM-AL, CA-IM and CA-AL) and one with all three species (IM-AL-CA).

To create the lines, leaves were attached to fishing line (PowerPro®). *C. arabica* and *A. latifolia* leaves were sewn by their petiole in bunches of two leaves. *I. micheliana* leaflets were used to avoid conflating differences between the decomposition of compound versus simple leaves and were sewn onto the line approximately one inch from the base of the leaf. Six lines, with alternating species bunches spaced approximately 35 cm apart, were arranged to make one wheel (Figure 3.1A). Two bunches of each species on the line were used, and the mass loss was averaged between con-specific bunches. Thus, single species lines had 2 bunches, two species lines had 4 bunches and three species lines had 6 bunches. Each bunch was weighed before placement in the field. Lines were set in the field in June 2017 and the experiment concluded in July 2017.

Ten sites were chosen in relatively flat areas, five in areas of the farm where *C. arabica* is cultivated and five in an adjacent forest reserve. Each of the coffee sites were a minimum of 100 m from each other and in total, the 5 sites spanned a 15-ha area. Three wheels were placed in each of the *C. arabica* sites (CA-IM, CA-AL and CA-AL-IM) for a total of 15 wheels. It was not possible to capture the decomposition rate of *I. micheliana,* and *A. latifolia* in the absence of *C. arabica* in these sites, given that *C. arabica* is the dominant species in the leaf litter in this area of the farm. All 7 wheel types were placed at each of the 5 sites in the forest reserve for a total of 35 wheels. Coffee is not grown in the forest, and both *A. latifolia* and *I. micheliana* are rare.

For six weeks, one line was collected per week from each wheel at each site. Debris was removed from the samples upon collection; bunches were dried at 50 degrees C and re-weighed. Mass loss was used as a proxy for decomposition.

*Paired slope tethered lines*

We conducted a second tethered line experiment in 2018. Recently senesced leaves from *I. micheliana, A. latifolia* and *C. arabica* were collected and dried to a constant weight. Single species lines were created with a single bunch of leaves at the end of 0.5 m of fishing line. All pairwise combinations were created, with species at the top and bottom of each line, approximately 35 cm apart to mirror the design of the 2017 study. There were 9 treatments per site (IM, CA, AL, IM-CA, CA-IM, IM-AL, AL-IM, AL-CA, CA-AL). Three lines were tied to a triangular frame, such that each of the three lines were arranged parallel to one another (Figure 3.1B). All nine treatments were placed in eight forest sites, at least 50 m apart, for a total of 72 triangles, each with 3 tethered lines that were harvested for 3 consecutive weeks.

We used the same forest reserve that was used in the 2017 study. However, rather than choosing flat areas, we chose sloped sites such that each line ran along a slope of at least 20 degrees (36% grade). This allowed us to test if being downhill (or downstream of rainwater run-off) had an effect on the outcome on the decay rate of leaves that were in mixtures. One line was collected each week from each frame at every site, for a total of 4 weeks in the field for the last lines collected. Debris was removed from the samples, and samples were dried to a constant weigh and re-weighed to determine mass loss. Evidence of comminution was noted for each bunch of leaves by noting the presence or absence of visible hyphae, along with skeletonized leaves or herbivory of the edge of the leaves. The experiment ran throughout June 2018.

*Carbon to nitrogen (C:N) ratios*

Leaf samples from the 2017 tethered wheel experiment were ground using a Krups brand coffee grinder at its finest setting and analyzed for total C and total N using a LECO Trumac CN combustion analyzer (LECO Corporation, St. Joseph, Michigan). Samples of the initial homogenized leaf litter were used; therefore the results from this analysis represent the initial C:N ratio. We calculated the carbon to nitrogen ratio using total C and total N data.

*Statistical methods*

We used the mass loss calculated from the leaf bunches to calculate the decay constant, *k*, from the exponential decay equation ($N_t = N_0 * e^{-k*t}$) (Olson 1963). While many equations have been fit to decay data, the exponential decay equation remains the most widely used in the literature, particularly for decomposition experiments of this duration (Aerts 1997, Bärlocher 2005).

For both the tethered line and slope line data sets, we built a linear mixed model using the "lmer" function in the *"lmerTest"* package in R (Kuznetsova et al. 2015). For the tethered line data, we used a treatment variable that included information on the leaf litter species, the location (forest or *C. arabica* farm plot) and the species that were present on the line. This resulted in a total of 19 treatments, and we used an intercept of zero to the model to parameterize a model without needing to designate a reference category. We included two random variables, one to account for the wheel and another for the interaction between wheel and line. Wheels, which were replicated as sites in both the forest and coffee farm, were sampled six times throughout the experiment and lines were nested within the wheels.

We built an equivalent model for the slope line data. Here the treatment identifier included information on the leaf litter species, its relative position (uphill or downhill) and the other species present on the line—for a total of 15 unique identifiers. Again, we used a zero intercept and included random variables for site and the interaction of site and line. Each site had 3 lines that were sampled at 3 time points, necessitating an interaction term to account for correlation within sites.

After building models for both the tethered line and slope experimental set-ups we tested our hypotheses enumerated above.

Our first hypothesis predicted that mixtures would decay more quickly than species in monoculture. To test this, we compared the decay of all three species in monoculture – both in the 2017 tethered line wheels and the 2018 slope lines – to the decay of species in all combinations. Thus, the decay of *C. arabica* in monoculture was compared to the decay of *C. arabica* when with *I. micheliana,* with *A. latifolia,* and with both species, in the case of the 2017 tethered line experiment. Equivalent comparisons were made for *I. micheliana* and *A. latifolia*. Four comparisons were made for the 2018 slope lines, with the decay in monoculture compared to the decay above and below each of the other two species. The composite hypotheses were tested using the "contrast" function in the "*lmerTest"* package in R (Kuznetsova et al. 2015). We generated arrays (3 rows by 19 columns for the 2017 tethered lines, and 4 rows by 15 columns for the 2018 slope lines). Testing the hypotheses with one contrast prevents multiple comparisons and eliminates the need for p-value corrections.

Our second hypothesis predicted there would be a greater benefit to decomposing in mixture with *I. micheliana* compared to decomposing in mixture with *A. latifolia,* because *I. micheliana* is a nitrogen-fixing legume with a lower carbon to nitrogen ratio. We tested the

impact of decomposing in combination with *I. micheliana* and *A. latifolia* on *C. arabica* decay. We compared the decay of *C. arabica* with *I. micheliana* and the decay of *C. arabica* with *A. latifolia* in both the forest and farm in the 2017 tethered line study. We compared *C. arabica* above and below both *I. micheliana* and *A. latifolia* in the 2018 slope study. These were all single comparisons using the "contrast" function in the "*lmerTest*" package in R. Additionally, for the 2017 tethered line study, we compared the predicted versus observed decay of *C. arabica* with *I. micheliana* and *A. latifolia*. Predicted decay was calculated as the decay of *C. arabica* in monoculture and observed decay was calculated as the decay of *C. arabica* in single species mixtures. The decay of *C. arabica* with *I. micheliana* was also compared to the decay of *C. arabica* with *A. latifolia*.

Third, we hypothesized that decaying in combination with *C. arabica* would accelerate decay more than decomposing with *A. latifolia*, but less than decomposing with *I. micheliana* because, while *C. arabica* has a lower carbon to nitrogen ratio than *I. micheliana*, a portion of the total nitrogen is tied up in caffeine. To test this, we compared the decay of *I. micheliana* with *C. arabica* and with *A. latifolia* and the decay of *A. latifolia* with *C. arabica* and with *I. micheliana*. These same comparisons were made in the 2018 slope line study, testing comparisons with the focal leaves in the uphill and downhill position. All comparisons were made with the pairwise "contrast" function from the "*lmerTest*" package in R. We also compared the observed and predicted decay of *A. latifolia* with *I. micheliana* to that of *A. latifolia* with *C. arabica* and the decay of *I. micheliana* with *C. arabica* to *I. micheliana* with *A. latifolia*.

Our final hypothesis predicted that, if micro-topography controled the dispersal of decomposers, being downhill of a different species will accelerate decay more than being uphill

of that same species. We tested this with the 2018 slope study and made 6 comparisons with the "contrast" function in the *"lmerTest"* package, testing each of our three focal species above and below the other two species.

We perfomed an ANOVA (using the *"aov"* function) and Tukey post-doc test to determine if carbon to nitrogen ratios differed significantly between litter species.

## 3.4 Results

Average initial carbon to nitrogen ratios for *A. latifolia* were $27.29 \pm 1.18$, $22.06 \pm 0.99$ for *I. micheliana* and $17.76 \pm 0.27$ for *C. arabica* ($F[2,32]=50.27$, $p<0.005$)

*Hypothesis 1*

The decay rate for all three species differed by treatments (Figure 3.2, Figure 3.3, supplementary Table B3.1 and B3.2). When decomposing in monoculture in the 2017 tethered line study, *C. arabica* had a lower decay rate ($k=9.99 \pm 0.89$) than when decaying with *I. micheliana* ($k=15.99 \pm 1.57$) or with *I. micheliana* and *A. latifolia* ($k=12.85 \pm 1.01$). In the 2018 slope line study, *C. arabica* similarly had a higher decay rate when decomposing above ($k=10.63 \pm 0.87$) or below ($k=10.29 \pm 1.17$) *I. micheliana* and below *A. latifolia* ($k=8.42 \pm 0.91$) than it did when decomposing in monoculture ($k=7.49 \pm 0.48$). In both 2017 and 2018, *C. arabica* decayed more quickly in mixture than in monoculture (2017, *F[3,918.8]=8.034,p< 0.005*; 2018, *F[3, 314]=7.389, p<0.005*).

*Inga micheliana* had a lower rate of decay when decomposing alone ($k=8.58 \pm 1.54$) than when decomposing with *C. arabica* ($k=12.52 \pm 1.65$). This difference drove an overall significant increase in decay for *I. micheliana* in mixture in the 2017 tethered line study

(*F[3,919.2]=6.295, p=0.003149),* but decay did not differ between treatments for *I. micheliana* in the 2018 slope line study (*F[4, 314.9]=0.8854, p=0.4729). Alchornea latifolia* decay did not differ significantly between leaves in monoculture and mixture in the 2017 tethered line study (*F[3,920.5]=0.6718, p=0.5694).* In the 2018 slope study, *A. latifolia* decayed more quickly in monoculture ($k=7.78 \pm 0.51$) than in mixture, driven largely by lower decay rates when above ($k=4.28 \pm 0.82$) and below ($k=4.36 \pm 0.44$) *C. arabica* (*F[4,314.9]=6.266, p<0.005).*


*Hypothesis 2*

      *Coffea arabica* decayed more quickly when in mixture with *I. micheliana* than with *A. latifolia* in the forest ($k_{IM}=15.97 \pm 1.57$, $k_{AL}=9.59 \pm 0.78$, *F[1,918.7]=18.004, p<0.005)* and farm locations ($k_{IM}=13.55 \pm 0.91$, $k_{AL}=9.46 \pm 0.67$, *F[1,927.2]=6.596, p=0.01037)* in the 2017 tethered line study. When comparing monoculture decay rates and observed mixture decay rates, 87% of *C. arabica* leaf bunches decayed more quickly in combination with *I. micheliana* compared to in monoculture, while only 43% of leaf bunches decayed quicker with *A. latifolia* than would be expected based on monoculture rates (Figure 3.4). Further, 87% of *C. arabica* leaves in the forest and 93% of *C. arabica* leaves in the farm decayed more quickly with *I. micheliana* than with *A. latifolia* (Figure 3.5). In the 2018 slope study, *C. arabica* also decayed more quickly with *I. micheliana* than with *A. latifolia.* This was true when *C. arabica* was above ($k_{IM}=8.42 \pm 0.89$, $k_{AL}=6.20 \pm 0.49$, *F[3,314.02]=24.507, p<0.005)* and below ($k_{IM}=10.29 \pm 1.17$, $k_{AL}=8.42 \pm 0.91$, *F[1,314.02]=4.386, p=0.037)* the other species.

*Hypothesis 3*

The effect of decomposing in mixture with *C. arabica* was tested in both the 2017 and 2018 studies. In the 2017 study, *I. micheliana* decomposed more quickly in mixture with *C. arabica* than in mixture with *A. latifolia* ($k_{CA}$=12.52 ± 1.65, $k_{AL}$=9.60 ± 1.44, $F[1,918.8]$=4.671, $p$=0.03093). In total, 69% of *I. micheliana* samples decayed more quickly with *C. arabica* than with *A. latifolia* (Figure 3.6A). There was no significant difference in the decay of *A. latifolia* in mixture with *I. micheliana* or *C. arabica* ($k_{IM}$=10.34 ± 0.95, $k_{CA}$=9.82 ± 0.94, $F[1,920.1]$=0.149, $p$=0.6998), with 54% of *A. latifolia* samples decaying more quickly with *I. micheliana* than with *C. arabica* (Figure 3.6B). In the 2018 slope study, *A. latifolia* decayed more slowly below *C. arabica* than below *I. micheliana* ($k_{CA}$=4.36 ± 0.44, $k_{IM}$=7.27 ± 0.55, $F[1,314.7]$=9.417, $p$=0.00234), and more slowly above *C. arabica* than above *I. micheliana* ($k_{CA}$=4.28 ± 0.82, $k_{IM}$=6.36 ± 0.52, $F[1,314.7]$=5.448, $p$=0.0202). There were no significant differences in the decay of *I. micheliana* in mixture with *C. arabica* compared to rates in mixture with *A. latifolia*, for either topographical orientation ($F_{above}[1,314.9]$=2.45, $p_{above}$=0.1185; $F_{below}[1,314.7]$=0.322, $p_{below}$=0.5709)

*Hypothesis 4*

All combinations of the three species above and below the other species were tested in the 2018 slope study (Figure 3.3, supplementary table B3.2). The only case in the 2018 study in which the decay rate of a species was significantly different based on the location (above or below) of another species was for *C. arabica* with *A. latifola,* where *C. arabica* leaves below *A. latifolia* decayed more quickly than when they were above *A. latifolia* ($k_{above}$=6.20 ± 0.49, $k_{below}$=8.42 ± 0.91, $F[1,314]$=6.118, $p$=0.0139, see supplementary table B3.3 for full results).

**3.5 Discussion**

Our results highlight species-specific responses to litter mixtures and the complicated, context-specific interactions that can take place when multiple litter species are decomposing in combination. Our findings further provide evidence that use of *I. micheliana,* a nitrogen-fixing legume, can accelerate decomposition processes in shaded coffee farms, potentially providing a benefit to nutrient cycling if nutrients are retained in the agro-ecosystem and bioavailable. The carbon and nitrogen data confirmed our expectation in that the ratio was highest for *A. latifolia* litter. Being a nitrogen-fixing legume, we expected *I. micheliana* litter to have a lower carbon to nitrogen ratio. The lowest initial ratio, and thus highest proportion of nitrogen, was found in *C. arabica* litter, though it's unclear how much of the nitrogen is tied up in caffeine.

Contrary to our expectation, all species did not decay more quickly in mixture than they did alone. Only *C. arabica* consistently decayed more quickly in mixture across both experiments (when averaging across treatments; *C. arabica* with *A. latifolia* did not decompose more quickly). There are many reasons for farmers to include shade trees in their coffee farms, including benefits to biodiversity, alternate sources of income and reduced spread of disease (Jha et al. 2014). These results further suggest that increased decay rates could be added to this list of benefits associated with shade trees. Because this study does not determine the fate of decaying nutrients, further research would be needed to determine if faster decay is resulting in faster nutrient cycling or tighter nutrient cycling, where less is lost from the system. Further, past research indicates that nutrients may be released from shade tree biomass at variable rates, depending on the diversity of the canopy. Tully and Lawrence (2012) found that release of nitrogen was maximized with a single species canopy, but phosphorus was released more quickly in mixture. Additionally, the management intensity on farms can mediate the impact of shade

trees on nutrient availability, with a greater impact reported on organically managed farms which are more dependent on decomposition processes for nutrient availability (Sauvadet et al. 2019). This highlights the importance of tracking the fate of individual nutrients and considering the full suite of management choices on farms.

Evidence for the effects of mixture on litter decay rates of the other two focal species was inconsistent across species and studies. The only significant difference in mixture for *I. micheliana* was driven by an increase in decomposition in the 2017 tethered line study when *I. micheliana* was in mixture with *C. arabica.* Past research has found that the fastest decaying species will not necessarily have higher decay in mixture (Kominoski et al. 2007). In our study, monoculture decay rates did not differ significantly between species in the 2017 study and the average *I. micheliana* decay rate was slower than the average for *A. latifolia,* which has a higher carbon to nitrogen ratio. Still, the highest quality species would be expected to benefit less from mixture, so the lack of accelerated decay for *I. micheliana* in mixture is not altogether surprising.

The lack of a change in decay rates in the 2017 tethered line study for *A. latifolia* (the species with the highest carbon to nitrogen ratio) in mixture is unexpected. Furthermore, *A. latifolia* decayed more quickly in monoculture in the 2018 slope study than it did in combination with either of the other two species. This was driven by a deceleration of decay when *A. latifolia* was in mixture with *C. arabica.* However, since *C. arabica* decayed as quickly as or more quickly than *A. latifolia,* the presence of *C. arabica* likely is not slowing the decay when in mixture. One possibility is that decomposers preferred *C. arabica* to *A. latifolia,* and therefore the length of the 2018 study—just four weeks—was not long enough to see any of the benefit for the adjacent *A. latifolia* litter.

In this study, we assessed the decay of each focal species, as well as the impact of each species on the others. We hypothesized that decaying in combination with *I. micheliana* would accelerate decay more than decaying with *A. latifolia* and that the impact of decaying with *C. arabica* would be smaller than in mixture with *I. micheliana,* but larger than in mixture with *A. latifolia.* Our results, across years, studies, contexts and topographical orientations supported our hypothesis that being in mixture with *I. micheliana* would accelerate decay more than being in mixture with *A. latifolia.* This was true for *C. arabica* leaves in the forest and the farm in the 2017 study and when found above and below the other species in the 2018 slope line study. Because we compared the effects of *I. micheliana* and *A. latifolia* on the decay of *C. arabica,* it is an important caveat that this effect may not hold true for all species. However, this is an important conclusion for land managers of *C. arabica* farms where the detrital pool is dominated by *C. arabica* leaf litter.

The impacts of being in mixture with *C. arabica* were inconsistent for the other two species. The 2017 and 2018 studies both found significant effects for *C. arabica* litter in mixture, but in different contexts. In the 2017 study, *I. micheliana* litter decayed more quickly with *C. arabica* litter than with *A. latifolia* litter in the forest, but this was not the case in the 2018 study. In the 2018 study, *A. latifolia* litter decayed more quickly with *C. arabica* litter than with *I. micheliana* litter, but there had been no significant differences in the 2017 study. We had expected that the lower carbon to nitrogen ratio in *C. arabica* could be complicated by some of their nitrogen being tied up in the alkaloid secondary defensive compound, caffeine (Chomel et al. 2016). It was not possible to assess the impact of *C. arabica* litter on the decomposition of shade tree species in the coffee farm because – by definition—all locations on a coffee farm have *C. arabica* litter. Other studies have found evidence of home-field advantage on the rate of litter

decomposition on the span of weeks in *C. arabica* farms, meaning that *C. arabica* leaves decompose more quickly in areas where *C. arabica* is cultivated, compared to adjacent forests (Schmitt and Perfecto, in review). If there are specialized decomposers in *C. arabica* farms, well suited to breaking down caffeine, there might be more consistent results of decomposing with *C. arabica* in those farms. The complication of caffeine, and secondary compounds more generally, is ecologically important to understanding mixtures, but is less important for applications of this work – as *C. arabica* litter will be omnipresent and dominant on farms. Still, understanding how caffeine and other alkaloids shape decomposer communities is important in predicting the outcome of any litter mixture on coffee farms.

Finally, we evaluated the influence of topography in potentially driving decomposer dispersal. We found little support that topography, at least on this micro scale, was important in facilitating the transfer of decomposers between leaf species. Only one of the six combinations of focal species litter above or below another tested had a significant difference in decay rates. While we detected one significant effect of spatial location on decomposition, where *C. arabica* decayed more quickly below *A. latifolia* than it did above it, we know of no reason why dispersal would be important for this combination but not others and conclude that this apparent effect may not be biologically relevant. While dispersal may be important in determining the rate and end products of decomposition, it more likely occurs via fungal hyphae, or more mobile larger decomposers that, in consuming litter material, may move between litter species and transfer microbes in the process (Nemergut et al. 2013), and these dispersal mechanisms may not depend on micro-topography.

Predicting the outcome of mixtures on leaf litter decay remains a challenge for ecologists, as the identity of the species and context appear to be important in determining the magnitude

and direction of mixture effects. As expected, the non-nitrogen fixing focal species tested here did not have the same magnitude of effect on the rate of decay, but decay is just one of many ecosystem functions to consider. Further research is necessary to disentangle the mechanisms driving mixture effects and maximize ecosystem services and benefits from canopy trees in coffee farms and across agro-forestry systems. Here we find evidence of a commonly used nitrogen-fixing shade tree accelerating the decay of leaf litter, in particular *C. arabica* leaf litter. While many decomposition studies of mixture effects use litterbags, where litter species are enclosed in a bag, we used tethered lines, where litter was adjacent, but not touching. The directional effects that emerged—even from 35 cm away—underscore the strength of mixture effects in agro-ecosystems. Our findings suggest yet another potential benefit of maintaining canopy trees on coffee farms.

## 3.6 Acknowledgements

## 3.7 References

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**3.8 Tables and figures**
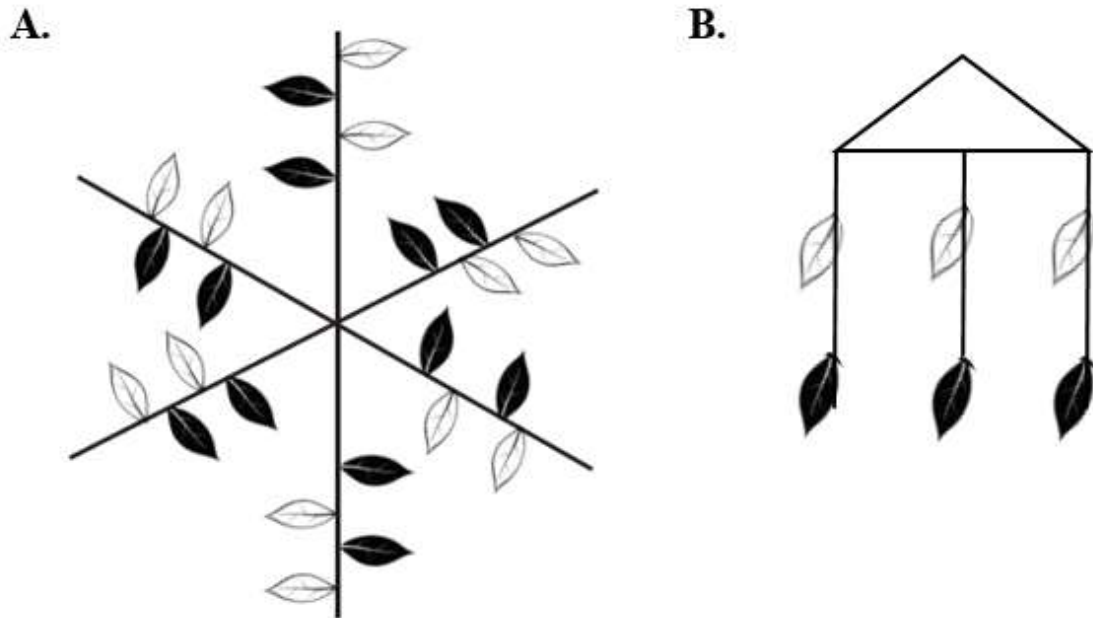


**Figure 3.1** Overhead view of experimental set-up for 2017 tethered line "wheels" (A), illustrating a two species wheel as an example, and the 2018 slope study (B). The monoculture lines in the 2018 slope study had just one bunch of leaves. The colors of leaves represent different species. Each leaf represents a bunch of two leaves; bunches were spaced approximately 35 cm apart.
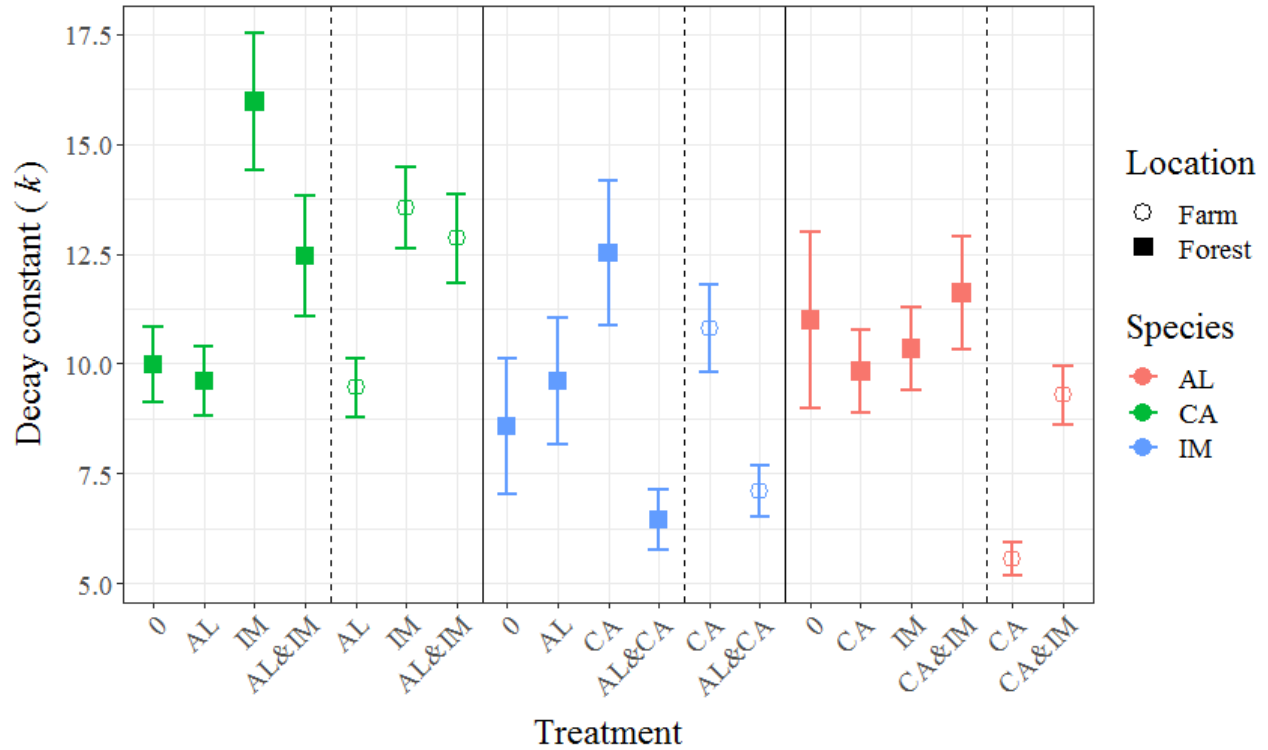
**Figure 3.2** Average decay constants ($k$) for treatments in the 2017 tethered line study. Error bars represent standard error. The colors indicate the species of leaf and the points indicate the location, either in the forest (filled square) or in the *C. arabica* farm (open circle). The treatment refers to the species of leaf that the focal leaf was in mixture with. A "0" indicates that the focal leaf was in monoculture.
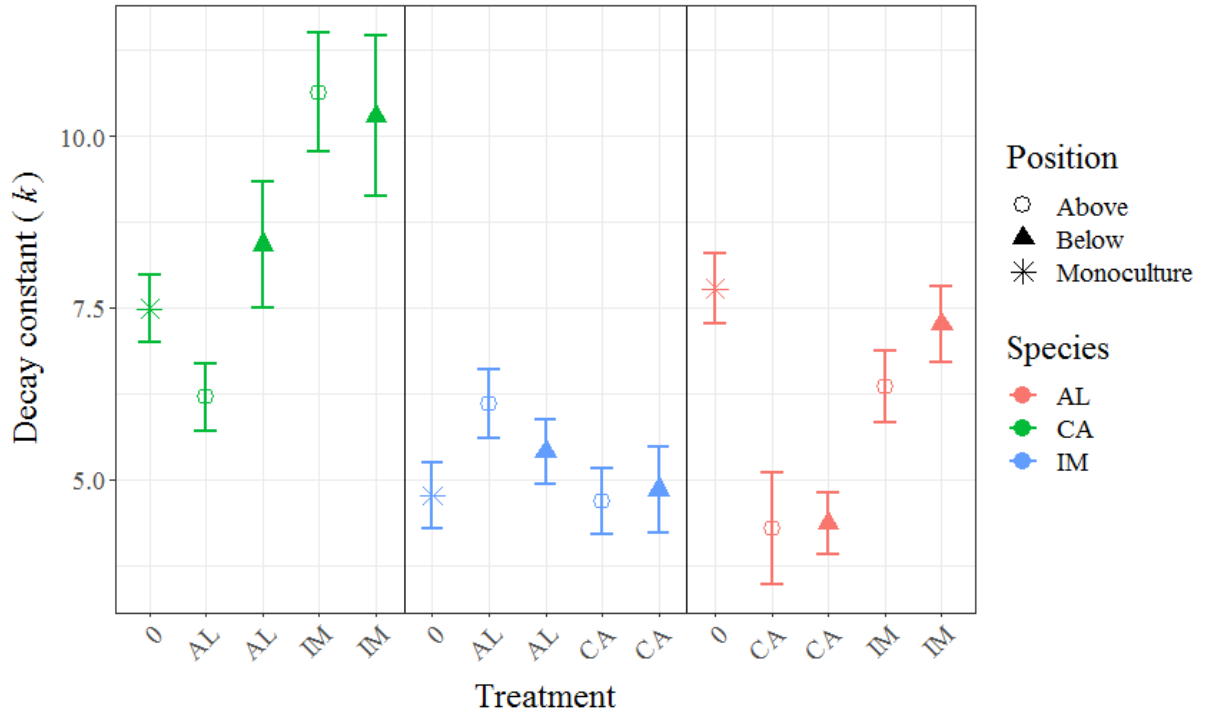
**Figure 3.3** Average decay constants ($k$) for treatments in the 2018 slope line study. Error bars represent standard error. The colors indicate the species of leaf and the points indicate the position of the focal species on the line, either in monoculture (star), above the mixture species (open circle) or below the mixture species (filled triangle). The treatment refers to the species of leaf that the focal leaf was in mixture with. A "0" indicates that the focal leaf was in monoculture.

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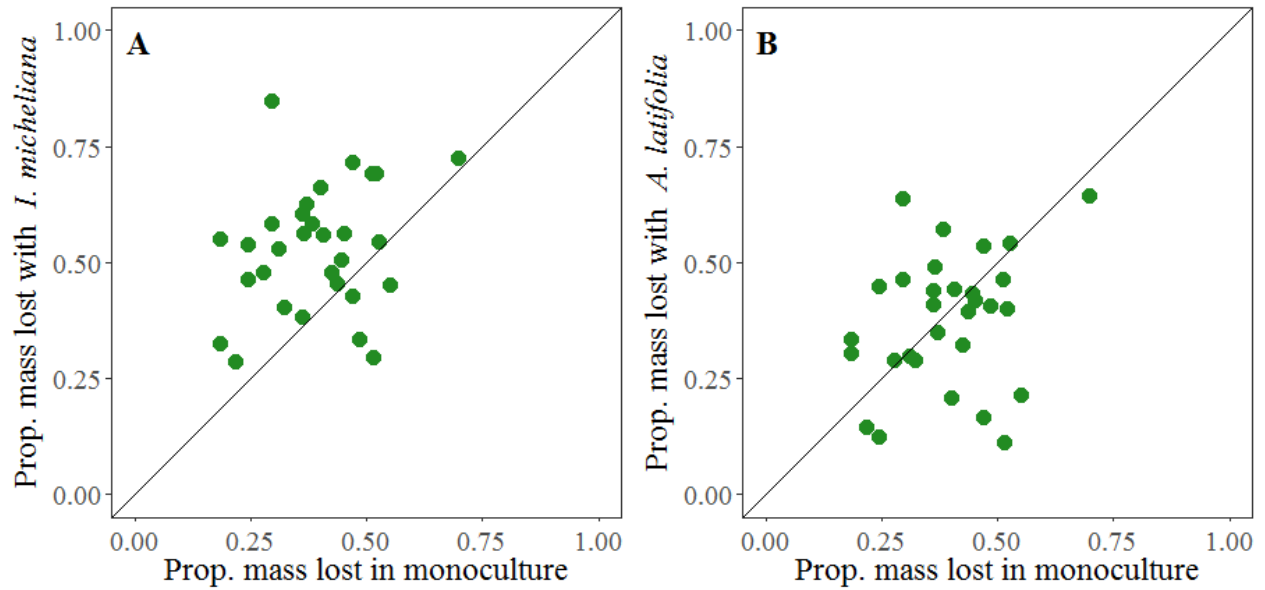
**Figure 3.4** Proportion mass lost for *C. arabica* leaves decomposing in monoculture compared to proportion mass lost with *I. micheliana* (A) and with *A. latifolia* (B) in the 2017 tethered line study. Each data point represents a sample from a given site in a given week. With *I. micheliana*, 26 of 30 samples decayed more quickly than *C. arabica* in monoculture. With *A. latifolia*, 13 of 30 samples decayed more quickly than in monoculture. The line represents 1:1 correspondence where the proportion lost in monoculture is the same as the proportion lost with the other species.
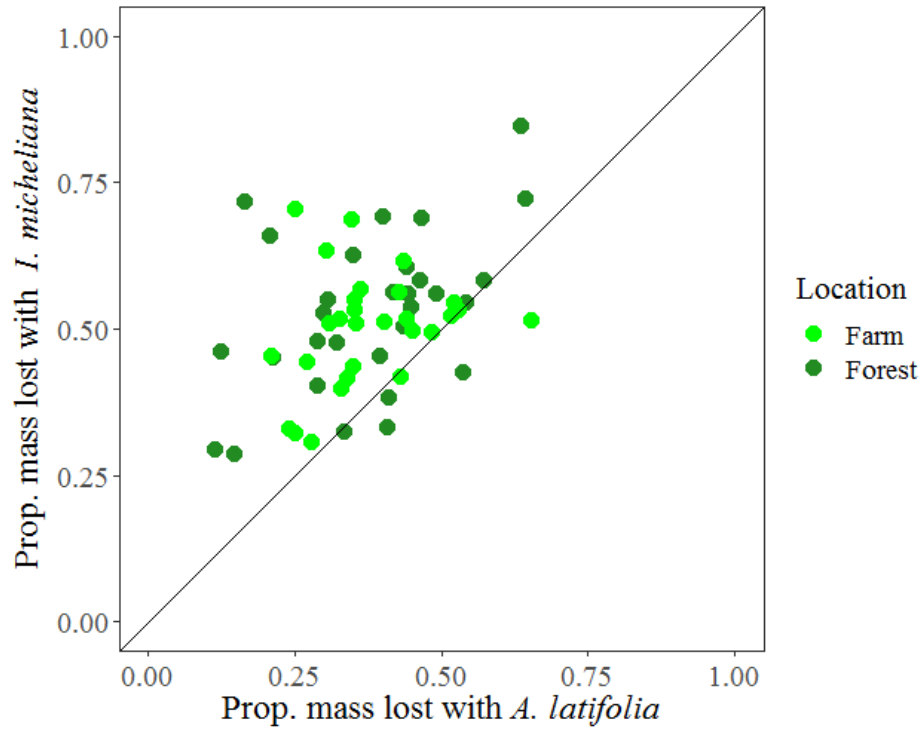
**Figure 3.5** Proportion mass lost for *C. arabica* leaves decomposing with *A. latifolia* compared to *C. arabica* leaves decomposing with *I. micheliana* in the forest and coffee farm in the 2017 tethered line study. Each data point represents a sample from a given site in a given week. For *C. arabica* in the forest, 26 of 30 samples decayed more quickly with *I. micheliana* than with *A. latifolia*. For *C. arabica* in the coffee farm, 26 of 28 samples decayed more quickly with *I. micheliana*. A 1:1 line illustrates where the proportion lost with both species is equal.
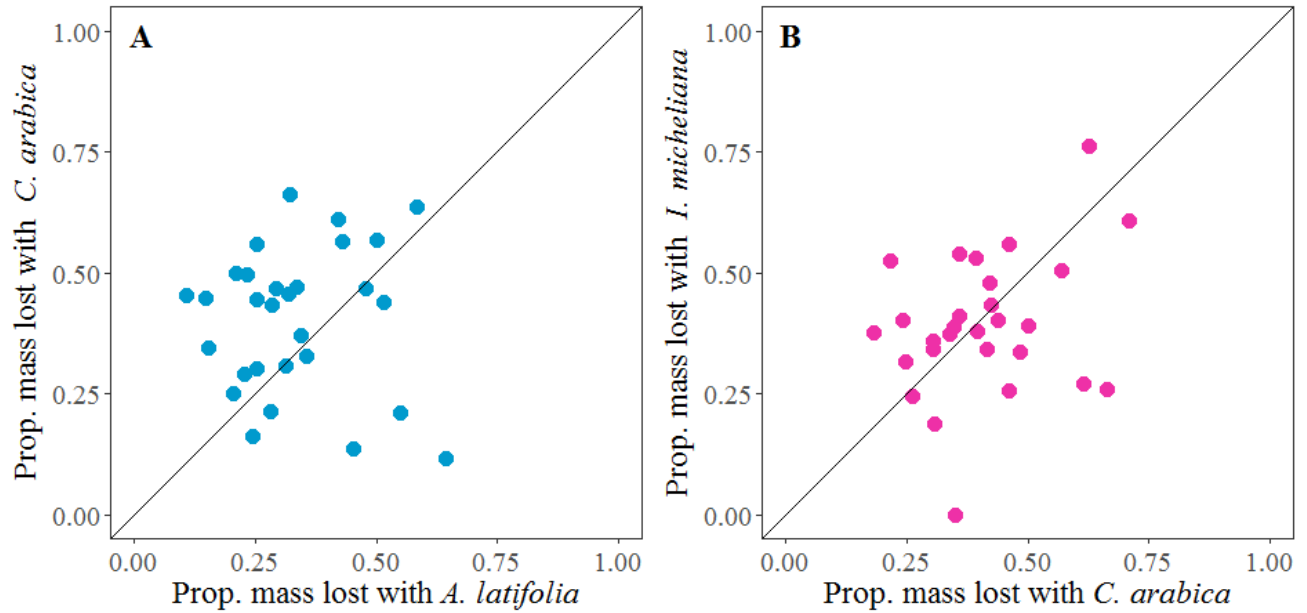
**Figure 3.6** Proportion mass lost for *I. micheliana* leaves decomposing with *A. latifolia* compared to with *C. arabica* in the forest (A) and *A. latifolia* decomposing with *C. arabica* compared to with *I. micheliana* in the forest (B) in the 2017 tethered line study. Each data point represents a sample from a given site in a given week. For *I. micheliana*, 20 of 29 samples decayed more quickly with *C. arabica* than with *A. latifolia*. For *A. latifolia*, 15 of 28 samples decayed more quickly with *I. micheliana*. The 1:1 line represents samples that lost equivalent proportions under the different treatments.

## Chapter 4: Synchronous Flowering of *Coffea arabica* Accelerates Leaf Litter Decomposition

### 4.1 Abstract

Coffee (*Coffea arabica)* flowers synchronously and flowers are only open for a few days before senescing. Flower petals often decompose easily, containing higher concentrations of nutrients relative to other plant tissues. Thus, a pulse of petals into the detrital pool could be beneficial for the decomposer community and accelerate decomposition processes.

Our research assessed the magnitude of the pulse of petals within a shaded coffee farm, and the impact of petals on the litter arthropod community and on the rate of leaf litter decomposition. Three plots of 12 coffee plants were monitored throughout the flowering period to estimate the magnitude of the bloom. Pitfall traps were used to assess the litter arthropod community before and after flowering. Finally, litterbags with *C. arabica* leaves alone and *C. arabica* leaves with flower petals were used to compare the effect of petals on decomposition rates.

The average number of flowers open per plant at the peak of the bloom was 792 flowers. When scaling to obtain an estimate per hectare in a year, our results indicate flower petals could contribute 26.27 kg of nitrogen, 2.03 kg of phosphorus and 26.7 kg of potassium. The leaf litter community did not change during our sampling, suggesting that any community effects may be acting on a longer time scale or smaller spatial scale. Leaf litter decomposed nearly three times as quickly in litterbags that included flower petals, relative to litterbags with only *C. arabica* leaf litter in the first month and twice as fast in the second month. The rate of decomposition with

petals exceeded the rate of decomposition without petals and was highest after one month, though the benefit continued after two months. Our results demonstrate that the presence of flower petals can accelerate short-term decomposition processes.

**4.2 Introduction**

Nutrient limitations constrain plant growth in all managed and unmanaged ecosystems. Annual and seasonal oscillations in availability can provide essential contributions to nutrient pools where natural cycles are not overshadowed by anthropogenic inputs (Pastor and Durkee Walker 2006, Galloway et al. 2008). Some seasonal or intra-annual inputs are dramatic and well-studied, such as oceanic upwellings or periodical insect outbreaks (Falkowski et al. 1998, Yang 2004). In other cases, low volume but synchronous inputs could have the potential to temporarily alleviate nutrient limitations, but have received little attention from researchers. Coffee flowering provides one such example, where blooms across fields occur synchronously with thousands of flowers open for just one or two days. Typical nutrient budgets are unlikely to include such inputs, given the brief residence times of the flowers in the ecosystem (Glover and Beer 1986, Dossa et al. 2008, Tully and Lawrence 2011). We propose that, despite their transient nature, mass coffee blooms could be an important source of nutrients in coffee agroecosystems, with the potential to affect both ecosystem function and the decomposer community.

Input pulses have the potential to alter ecosystem function through their contribution to the detrital pool. Increased quantity or quality of inputs to the detrital pool can influence decomposition rates and the pace of nutrient cycling. A pulse of relatively labile material, meaning material with easily available carbon and other nutrients, can act to "prime" decomposition of soil organic matter, temporarily accelerating decomposition processes

(Kuzyakov et al. 2000). Altered activity and relative abundances of decomposer microbes may explain this priming effect (Kuzyakov et al. 2000, De Graaff et al. 2010).

Nutrient pulses can also have cascading effects across trophic levels, with consequences for ecosystem function. Some of the most well-known and well-studied examples in ecology come from aquatic systems, including upwellings and turnover that can stimulate organismal growth across trophic levels (Tilman et al. 1982, Falkowski et al. 1998), but there are examples from plant ecology as well. For example, the nitrogen contribution of seed masting in nitrogen-limited boreal forests has been linked to tree regeneration (Zackrisson et al. 1999). Events like seed masting or inter-annual spikes in abundance of arthropods can drive consumer community dynamics, too (Ostfield and Keesing 2000). A pot experiment with a grass found that larger individuals benefit disproportionately from pulses, highlighting the importance of pre-pulse conditions (Lamb et al. 2012). These examples emphasize the potential of nutrient pulses to influence both primary producers and upper trophic levels in a range of contexts.

Flowering events have rarely been considered within the lens of periodic pulses, though many species, especially domesticated crop species (Jung & Müller 2009), flower synchronously and profusely (e.g. almonds, blueberries, canola and sunflower). Bamboo, which reproduces with a single mass flowering event at the end of a life span that can range 3-120 years, has been well studied and provides an exception (Janzen 1976). However, studies of the ecosystem consequences of bamboo flowering have focused on the input of the bamboo plant into the detrital pool, rather than the flowers themselves (Austin & Marchesini 2012), and there are key differences between bamboo, a monocotyledon, and other plant species that may make it harder to draw parallels to other plant systems.

Even annual flowering events, especially when conspecifics are abundant and the bloom is short-lived, have the potential to provide an important pulse of resources. This is particularly true given that floral tissue is likely to have an outsized influence on decomposition dynamics, relative to its biomass (Whigham et al. 2013). Flowers generally have higher concentrations of nutrients compared to leaves and other plant tissue (Belkhodja et al. 1998, Martinez et al. 2003). Additionally, petals have fewer structural compounds, e.g. lignin, which can constrain decomposer access to nutrients and increase the longevity of a tissue in an ecosystem. Further, detritivores have been shown to select flowers over leaves in preference experiments and grow faster when fed a diet of floral tissue (Smallengagne et al. 2007, Whigham et al. 2013). Detritivores, including protozoa, nematodes, Collembola, mites, millipedes, isopods, earthworms and others, can influence decomposition in several ways including by regulating bacterial and fungal populations and by fragmenting and consuming litter (Hättenschwiler et al. 2005, Wickings et al. 2011). Collembola (commonly known as springtails) can be especially important meso-fauna due to their ability to directly consume and alter organic material and regulate the fungal communities (Rusek 1998). Further, Collembola can increase microbial biomass, accelerating decomposition rates, linking their abundance to microbial biomass, and making them an important indicator group within the detritivore community (Hanlon and Anderson 1979, Seadtedt 1984, A'Bear et al. 2012, Yang et al. 2012).

In agricultural systems, naturally-occurring nutrient levels are often augmented by the addition of inorganic or organic fertilizers (Potter et al. 2010). This is especially true in tropical agriculture, where soils are known for being highly weathered and, subsequently, nutrient poor (Sanchez et al. 2013). Organic fertilizers, which are derived from plant and animal matter, are less available to plants than inorganic fertilizers, but avoid the externalities (notably, water

pollution) and costs of inorganic fertilizers and have long been used in agriculture to increase nutrient cycling (Drinkwater & Snapp 2007, Kremen & Miles 2012). Use of plant matter as nutrient supplements (e.g. cover crops, mulch and compost) is a widespread and ancient practice. The mass coffee bloom represents an endogenous source of plant matter, so the ecosystem level nutrient budget is unchanged. Thus, flower petals would be unlikely to replace the need for fertilizer, but the nutrients provided could supplement nutrient budgets and alter the seasonal timing of fertilizer needs.

Here we describe the magnitude of the nutrient flux of a coffee mass bloom and its consequences for decomposition and detritivore community composition in a shaded organic coffee farm in southern Mexico. We focused on Collembola as part of the detritivore community because of their important role in decomposition (Yang et al. 2012) and their abundance in our study system (Schmitt et al., in press). Our study addressed these three main objectives with the following hypotheses:

H1: Coffee flower petals will have higher nutrient concentrations than coffee leaf tissue and represent an important pool of nutrients on a farm scale. We used the nutrient concentrations in petal tissues to scale the density of the bloom on a plant basis to estimates of farm-level nutrient inputs for nitrogen (N), phosphorus (P) and potassium (K).

H2:  Senesced flower petals will positively influence coffee leaf litter decomposition rates. We predicted that leaf litter decomposition would be accelerated with the addition of relatively labile flower petals.

H3: Finally, we assessed the impact of the bloom on the leaf-litter invertebrate community.

We expected that the decomposers, namely Collembola, would increase in abundance where more flowers were present, if the petal biomass alleviates nutrient limitations.

## 4.3 Methods

*Study system and site*

Coffee is grown in the tropics and usually in areas that experience distinct dry and rainy seasons. Coffee blooms occur in the dry season (Drinnan and Menzel 1995). At our field site, coffee will have a mass bloom 2-4 times in a season, over the span of 1-2 months. We conducted this study in 2018, when flowering occurred from late January through early March, though in other years the bloom as occurred as late as mid-March through the end of April (Philpott et al. 2006). Blooms are cued by a period of dryness, followed by 7-10 mm of precipitation (Crisosto et al. 1992, DaMatta et al. 2007, Schroth et al. 2009). Flowers are open for approximately 48 hours before senescence (Cannell 1983), though they are most attractive to pollinators in the first 24 hours (Free 1993). *Coffea arabica,* the most common commercially grown species of coffee and the focus of this study, is self-compatible, but fruit set increases with outcrossing (Klein et al. 2003). Many varietals of *C. arabica* are grown commercially and are present at our study site. The most common varietals at our site include Catimor, Java and Carchimor, with lesser quantities of Arabe, Bourbon, Caturra, Costa Rica, Colombiano, Marceleza and Tupic varietals.

Field work was carried out at *Finca Irlanda*, a 300 ha, shaded, organic coffee farm in the Soconusco region of Chiapas, Mexico. *Finca Irlanda* is located approximately 950-1150 meters above sea level and receives 4500 mm of rainfall each year (Philpott and Bichier 2012). Precipitation is concentrated in the rainy season, which extends from May through October (Lin 2010). Few external inputs are added, with the exception of compost and "compost tea" (stewed

compost) which is made on-site from chicken manure, calcium carbonate and worm vermiculture of coffee parchment (Gonthier et al. 2013). One kilogram of compost is applied to each plant in March and September, and 200 milliliters of compost tea are applied to each plant in February and August.

*Density of the bloom and nutrient content of tissues*

Three plots with twelve coffee bushes in each were set-up mid-February 2018 and monitored nine times between February 21, 2018 and March 10, 2018. Upon set-up, the height and total number of branches were recorded, as well as the spatial arrangement of the plants. Plots ranged in size from 20 square meters to 30 square meters; plot size varied because coffee plants were not planted with perfectly even spacing. The number of branches with flowers and the total number of flowers on each plant were recorded at each observation. Plots were located 200 -1000 m apart. The sites were chosen where planted rows were intact and plants were of a similar size.

Recently senesced flower petals were collected from the ground and dried at 50 deg C to a constant weight. Samples were homogenized, ground and nutrient concentrations (N, P, K, S, Mg, Ca, Na, B, Zn, Mn, Fe, Cu, Al) were analyzed using Inductively Coupled Argon Plasma (ICAP) mass spectrometry, run on a Thermo iCap 6500 at A&L Great Lakes Laboratory (Fort Wayne, Indiana). Nutrient data were used, with the mass of flowers, to scale the nutrient pulse to a farm-relevant scale (kg/ha/yr). We focused on nitrogen, phosphorus and potassium, the three nutrients that most commonly limit plant growth. The same analysis was run on recently senesced *C. arabica* leaf samples collected from the same farm, to provide a relative comparison of flower and petal nutrient content.

*Leaf litter decomposition*

Litterbags made of 2 mm fiberglass mesh were placed at 15 sites around the farm. Mesh size was chosen to allow micro- and meso-fauna access to the litter, which includes Collembola (Bradford et al. 2002). Sites were a minimum of 75 m apart. Four litterbags were placed at each site on a 0.5 m diameter ring. Half the litterbags were filled with *C. arabica* leaf litter and half with a combination of *C. arabica* leaf litter and *C. arabica* flowers. Approximately 20 g of leaves were placed in each bag with approximately 6 g of petals in half the bags. This ratio is higher than would occur throughout the farm, but it could represent litter immediately beneath a plant or under a clump of petals. All plant tissue was collected when recently senesced, and dried to a constant weight before being sewn into the bags. The treatments were affixed to opposite sides of the ring to prevent direct contact. Precise masses were recorded for each bag and used to calculate mass lost upon collection. Metal identification tags with a unique number were used to track individual bags. Litterbags were put in the field in March 2018.

One bag of each treatment (leaves and leaves with petals) was collected from each site after one month, in April 2018, and after two months, in May 2018. Collected bags were dried and the leaf tissue was re-weighed. Petal and leaf tissue were distinguished upon collection at one month. No petal tissue remained in any bag after two months. Mass loss (the difference in mass between time points) was used as a proxy for decomposition.

*Community effects*

Twelve pitfall sites were set up throughout the farm, a minimum of 75 m from one other. Sites were approximately 4 m square and contained five pitfall traps (Figure 4.1). One trap was collected before the bloom (time A), after which the remaining four traps were manipulated

through the physical removal and exclusion of flowers or addition of 15 g of dried flower petals. To attract decomposers, petals were added around the edge of the pitfall trap in a ring approximately 3 inches in diameter. One addition and one exclusion trap were sampled after three days (time B) and the remaining two (one addition and one exclusion trap) were sampled 7 days after the manipulation (time C). These times were chosen with the aim of assessing short-term response of the litter arthropod and would denote recruitment rather than reproduction.

Plastic 16 oz. Deli containers were used as a traps. Containers were buried, flush with the soil surface, and allowed to sit for a minimum of 24 hours before lids were removed. Each was covered with a plate, propped up with wooden dowels, to allow organisms to walk beneath the plate and fall into the trap, but to prevent falling detritus or larger organisms from accessing the trap. Traps were filled with water with a drop of dish soap to break surface tension and facilitate the capture of organisms in the traps. They were collected 24 hours after opening and organisms were transferred to alcohol and identified to order, and then morpho-species.

*Statistical methods*

Nutrient content was measured in five homogenized floral samples and scaled to hectare-level estimates using the following equation (eq. 1):

$$\% \; nutrient * \frac{0.1848 \; g}{flower} * \frac{792 \; flowers}{plant} * \frac{2400 \; plants}{hectare} * \frac{3 \; mass \; blooms}{year} = X \; g \; / \; ha \; / \; year$$

The values in eq. 1 are estimated from the field site in this study. Because *Finca Irlanda* is relatively un-intensified, some of the parameter estimates (especially plants per hectare) are on the low end of the range seen in coffee farms. Thus, this calculation, which resulted in an estimate of grams of nutrient per hectare per year, provides a conservative estimate of the nutrient flux affected by the mass coffee bloom. We did not scale the leaf nutrient data to

g/ha/yr because leaves senescence continually throughout the year and, to our knowledge, there are no reliable estimates of leaves lost per year.

To test for differences across time or treatments in proportion of mass lost in the litterbags – a proxy for decomposition – we used a two-way ANOVA, implemented with the "aov" function in the "*dplyr*" package from R (Wickham et al. 2015). Assumptions of normality and homogeneity of variances were met. Looking at the proportion of mass lost allows us to assess the decay linearly.

The decay constant *k*, as derived from the exponential decay equation ($N_t = N_0 * e^{-kt}$), was also used to compare rates of decomposition. The exponential decay equation is commonly used in decomposition analyses (Olsen 1963, Aerts 1997, Bärlocher 2005). We used a linear mixed-effects model using the "lmer" function with "*lmerTest*" package in R (R Development Core Team, 2009, Kuznestova et al. 2017) to test for differences between treatments and across time points. Site was included as a random effect. The decay constant, *k,* was log-transformed to meet assumptions of normality and heterogeneity of variance.

Change in the litter arthropod community, as a function of time and treatment, was visualized using non-metric dimensional scaling plots (NMDS) and assessed with analysis of similarity (ANOSIM). The NMDS plots were made using the "metaMDS" function from "*vegan*" in R (Oksanen et al. 2007). ANOSIM was calculated using the "anosim" function from *"vegan"* in R (Oksanen et al. 2007). We calculated distances based on Bray-Curtis, since our data was count data, and ran 1000 permutations. We used 2 dimensions when calculating the Bray-Curtis distances, as our stress values were relatively low. *R* is the output of the ANOSIM analysis and, like $R^2$, indicates the amount of the variation that can be explained by the explanatory variable being tested. NMDS and ANOSIM were repeated with the full community

pitfall data at the order level and morpho-species level. At the order level, groups included Diptera, Arachnida, Colleoptera, Orthopteran, Hemipteran, Collembola, Hymenoptera and other. Ants were excluded from ANOSIM analysis because, due to the eusocial nature of ants, their counts in pitfall traps conflate activity level and abundance.

To test for differences in the abundance of Collembola across treatments and times, we used a two-way ANOVA, run with the "aov" function in the "*dplyr*" function (Wickham et al. 2015). To meet the assumption of normality, one outlier was removed and data were log-transformed. A Tukey HSD post-hoc test was used to look at pair-wise comparisons of time points.

## 4.4 Results

*Density of the bloom and nutrient content of tissues*

Two of the three plots we monitored experienced mass blooms during the observation period (Figure 4.2). Within those two plots, plants produced an average of 792 flowers at peak bloom. The maximum number of flowers recorded on a single plant on a single day was 1540 flowers, but there was considerable variability between plants in a given plot. In the two plots that had a mass bloom during the monitoring period, the peak number of flowers per plant ranged from 292 to 1540, with an average peak of $818.9 \pm 77.9$ flowers per plant.

Nutrient concentrations per sample and on a per hectare basis are given in Table 4.1. The petals averaged 2.49% nitrogen, 0.22% phosphorus and 2.53% potassium per sample. When scaling to an estimate per hectare and per season, our results indicate flower petals could contribute approximately 26.27 kg of nitrogen, 2.03 kg of phosphorus and 26.7 kg of potassium.

The same nutrient analyses were conducted on leaves from the same farm site. The leaves averaged 2.51 % nitrogen, 0.13% phosphorus and 1.83% potassium per sample.

*Leaf litter decomposition*

Leaves decomposed faster in the presence than in the absence of flowers ($F=736.067$, $p < 0.00$) and a greater proportion of mass was lost in two months compared to one month ($F=247.788$, $p < 0.005$). There was a significant interaction between time and treatment on proportion mass lost in the litterbags ($F[1, 52]=5.391$, $p=0.0242$); the difference in decay rates with and without petals is greater in month 2 compared to month 1 (Figure 4.3), leading to this ordinal interaction. Assumptions for homogeneity of variances (Levene's test, F=0.8505, p=0.4726) and normality (Shapiro-Wilk normality test, W=0.96583, p=0.113) were met.

Comparing the decay constant ($k$) confirms that the rate of decomposition is higher after one month and with petals (Figure 4.3). The linear mixed-effects model indicated that treatment ($\beta= 0.917$, df=39, $p < 0.0005$) and month ($\beta= -0.250$, df=39, $p< 0.0005$) were both significant predictors of $k$, and there was an interaction between treatment and month ($\beta= -0.163$, df=39, $p=0.0051$).

*Community effects*

Flower availability had no impact on the abundance of Collembola ($F=0.077$, $p= 0.7827$), though there were fewer Collembola over time ($F[1,52]= 4.692$, $p= 0.0134$). There was also no significant interaction between time and treatment ($F=1.232$, $p= 0.2721$). A post-hoc Tukey test showed that collembolan abundances were lower across sites before the manipulation as compared to the samples taken 3 days after manipulation ($p=0.0114$) or 7 days after

manipulation ($p=$ 0.0376). There were no significant differences in collembolan abundance between the two sampling times after manipulation ($p=0.8075$).

The NMDS plot illustrated the lack of separation of arthropod communities based on treatments or time points, regardless of the level of taxonomic resolution (for order-level resolution, see Figure 4.4). Analysis of similarity (ANOSIM), at the level of orders, indicated significant separation between treatments ($R=0.066$, $p=0.02$), but there was no separation between treatments at the level of morpho-species ($R= -0.001$, $p=0.64$). An R statistic of 0 indicates no separation and a negative R value indicates greater dissimilarity among replicates than between samples.

## 4.5 Discussion

Our study provides the first estimates of the nutrient flux associated with mass coffee flowering, and its effects on leaf litter arthropod communities. We report limited changes to the litter arthropod community but evidence of accelerated leaf litter decomposition as a result of petal inputs.

*Nutrient fluxes and decomposition*

We estimate that approximately 26 kg N/ha/yr, 2 kg P/ha/yr and 27 kg K/ha/yr are cycled from flower petals to the detrital pool during the flowering season at our field site. Senesced leaves from *C. arabica* have 2.51% N, 0.13% P and 1.83% K, whereas the petals had 2.49% N, 0.22% P and 2.53% K. While the percentage of nitrogen is nearly identical between tissues, the phosphorus and, in particular, potassium is substantially higher in the petals than in the leaves. Further, given the structural tissue in leaves, each of the nutrients is less accessible for microbes

and micro-invertebrates. This finding is consistent with other research showing high nutrient levels in floral tissue compared to leaves (Belkhodja et al. 1998, Martinez et al. 2003).

While coffee flowers have relatively high concentrations of nutrients, the impact of these nutrients in the agroecosystem will be dependent on the timing of the inputs, mineralization dynamics and the fate of mineralized N. Our study does not allow us to speak to the form of the nutrients or their retention in the system. However, it is clear that nutrient form greatly affects the accessibility of nutrients to plants and other organisms and the form of the nutrients will influence their likelihood of leaching out of a system. For example, phosphorus is often the limiting nutrient for plant growth in tropical systems, in part because much of the total phosphorus in the soils is bound up in iron oxides (Turner et al. 2018). Nitrogen, on the other hand, can be problematically mobile, particularly when present as nitrate (Fowler et al. 2013). Therefore, it is crucial to determine if the nitrogen from flower petals is retained in the system and if phosphorus is bioavailable. The mobility of nutrients can stem from both management decisions and inherent ecosystem properties. In a study of Costa Rican coffee farms, nitrogen leaching was negatively related to shade tree biomass, a management decision, whereas phosphorus leaching was correlated with soil iron pools, an inherent property (Tully et al. 2012). Overall, abiotic and management conditions will interact to drive the fate of floral-derived nutrients in coffee agro-ecosystems.

The timing, duration, and intensity of the blooms also mediate the potential effects of the bloom on nutrient cycling. The timing of the bloom is dependent on each year's climate, with blooms having the potential to occur over a two month period. In years when mass blooms occur in short succession and are highly synchronous at a local scale, we suspect that their nutrient impact is likely to be greater than in years when blooms are less synchronous or are spaced

across a longer period of time. The response of decomposer communities to pulses will depend on their life history strategy (Treseder et al. 2011), as well as the size of the pulse.

The timing of the bloom also has implications for nutrient availability for developing coffee fruit and, thus, for coffee yields. We scaled our nutrient estimates to an annual basis, but the blooms occurred on the scale of weeks and all petal tissue had decomposed within months. Flowering necessarily precedes the start of fruit development, a stage during which coffee plants have elevated nitrogen demands (Bruno et al. 2011). One study found that up to 20% of total plant nitrogen was found in flowers during the bloom (Malavolta et al. 2002). Thus, if flowers contain a significant portion of a plant's nitrogen and the demand for nitrogen is greatest immediately following a bloom during early fruit formation, the nutrient input from petals into the soil nutrient pool may reduce the need for external inputs at a time when crop nutrient demand is high. The nutrient demands of coffee plants in early fruit formation have been established in previous research, and it is likely the decomposition of petal tissue contributes to these nutrient needs. Further research is needed to determine the fate of decomposed nutrients from petal tissue.

The rate of leaf litter decomposition in litter bags, as measured by the proportion of leaf biomass lost and the decay constant $k$, increased over time and when petals were present in the litterbags with the leaves. No recognizable petal tissue was found in the litterbags at the end of two months, suggesting that the petal tissue decomposes quickly, but the effect of petals may alter decomposition even after the petals have gone. The presence of petals at the start of decomposition could have priority effects on microbial communities, altering the community and abundance of bacterial and fungal species (Strickland et al. 2009). In changing the composition

and trajectory of decomposer communities, the impact of petals could continue after the petal tissue has been entirely broken down.

The observed increase in decomposition rates with the addition of petals to the detrital pool indicates that petals represent a biologically accessible source of nutrients.

*Litter community effects*

We found little evidence of changes within the leaf litter invertebrate community on the whole, or Collembola, in response to the addition or exclusion of petals. The abundance of Collembola did decrease over our sampling times, but there were no differences between treatments. Our experiment was carried out during the bloom and at sites that measured approximately 4 meters squared. The farm, as a whole, was relatively saturated with flowers during this time, minimizing the potential impact of our smaller scale manipulations. We suggest that future experiments be executed in in the dry season, outside of the bloom, when abiotic conditions are similar but the environment is less saturated with petal tissue, or during the bloom season outside of a coffee farm. We do not expect the decrease in Collembola to be biologically important, but still, the direction of change is counter-intuitive given the increase in labile detritus across the farm during the mass blooms. An overall increase in Collembola would be expected as the result of migration of Collembola from soil to the leaf litter or from Collembola reproduction.

We did find some evidence of a shift in leaf litter invertebrate community composition at the level of orders, but the *R* value was less than 0.07, indicating the shift was relatively unimportant in explaining the community. Further, there was no indication of separation between treatments at the level of morpho-species in the ordination plots. Thus, while there was a

statistical effect at the level of orders, we do not expect the shift was biologically important. The

duration of our study also likely limited our ability to detect shifts in the community. The time

frame of our pitfall study, with the latest sample 7 days after manipulation, is insufficient for the

majority of invertebrate life cycles, meaning our samples after manipulation measured

recruitment rather than reproduction. Further studies should assess micro-invertebrate response

on a longer time scale to determine if there is a signal of increased reproduction or recruitment in

response to this floral nutrient pulse.  While we find no evidence of a response by micro-

invertebrate decomposers, it may be that microbial decomposers were responding. We did not

assess the microbial community, but previous studies on the mechanisms behind priming

underscore the importance of bacteria and fungi in priming dynamics (Kuzyakov et al. 2002,

Kuzyakov 2010). Microbes can respond to labile material much more rapidly than invertebrates.

A shift in the dominant microbial groups (e.g. via priority effects, where early arrival of a species

or group impacts the resultant community [Hiscox et al. 2015, Lin et al. 2015, Tláskal et al.

2016]) could also explain the increased decomposition rates seen after the flower petals had fully

decomposed.


The nutrients we measured in petals and scaled to a farm level were not inputs from

outside the system, unlike most fertilizer additions. Fertilizer additions are relatively small at our

study site, but at more intensified farms where fertilizer is used, suggested fertilizer application

rates would result in nutrient inputs far greater than those obtained from the mass bloom. For

reference, the annual recommended fertilization rates for coffee in Mexico, as per the Food and

Agriculture Organization of the United Nations, are 60 kg N/ha/year, 40 kg P/ha/year, and 15 kg

K/ha/year (FAO 2002). Our estimates of the contribution from petals are much lower, except in

the case of potassium, where we estimate the petals could represent more than the yearly budget of potassium, provided the potassium is in an available form. While the overall nutrient pools within the agroecosystem are not increasing with the addition of petals to the detrital pool, our results do provide evidence of accelerated cycling of nutrients cued by floral senescence.

Considering a mass bloom for its impacts beyond pollination is important in agroecosystems where yield is influenced by many factors, including nutrient availability. Here, we provide evidence that an annual pulse of senescent floral tissue is altering and increasing decomposition dynamics in a coffee agroecosystem. Our results suggest that, after a year of gradually reduced amounts of nutrients in the soils (due to uptake by coffee plants), the mass bloom could function to release those sequestered nutrients, potentially increasing relative nutrient availability if not relative nutrient totals, and allowing these nutrients to cycle within other pools. Isotopic methods could resolve the specific fate of petal nitrogen inputs. However, increasing management intensity of coffee systems is resulting in more exogenous fertilization (Lin et al. 2008) while climate change is reducing the synchronization of coffee blooms (Drinnan and Menzel 1995). In intensified coffee systems, and especially under climate change, the impacts of the coffee bloom may be reduced.

## 4.6 Acknowledgements

## 4.7 References

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## 4.8 Tables and figures

**Table 4.1** Flower petal nutrient data for nitrogen, phosphorus and potassium at multiple scales.

|  | Nitrogen (N) | Phosphorus (P) | Potassium (K) |
|---|---|---|---|
| **Average % / sample** | 2.492 | 0.218 | 2.532 |
| **Standard deviation** | 0.09471 | 0.016432 | 0.119875 |
| **Standard error** | 0.042356 | 0.007348 | 0.05361 |
|  |  |  |  |
| **grams / flower**[†] | 0.04605 | 0.000403 | 0.004679 |
| **grams / plant**[‡] | 3.647331 | 0.319068 | 3.705876 |
| **grams / hectare**[§] | 8753.595 | 765.7639 | 8894.102 |
| **grams / ha / season**[¶] | 26260.78 | 2297.292 | 26682.31 |
| **kilograms / ha / season** | 26.26078 | 2.0297292 | 26.68231 |

[†] average mass of a single flower is approximated at 0.1848 g
[‡] assuming average of 792 flowers per plant, per bloom
[§] assuming 2400 plants per hectare
[¶] assuming 3 major blooms per year
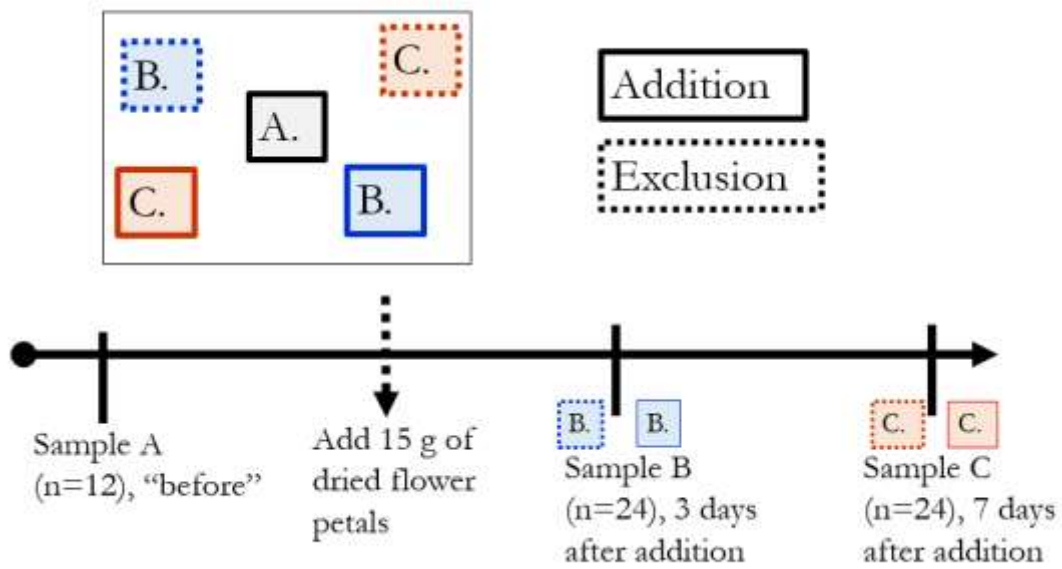
**Figure 4.1** Conceptual figure of the pitfall trap study design. Five pitfall traps were placed within each site. Sampling took place at 3 time points: before manipulation and 3 and 7 days after manipulation. For the additional manipulation, 15 g of dried flower petals were added around the edge of the pitfall trap; for the exclusion, all flower petals in the area were cleared.
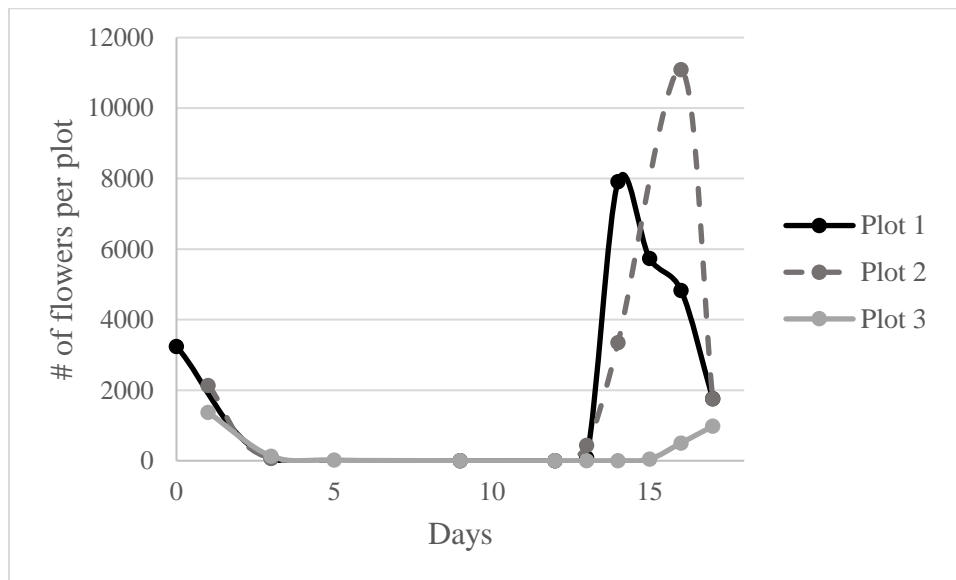


**Figure 4.2** Flowers per plot across the monitoring period. Flowers from all 12 plants in each plot were summed. Plot #3 did not have a mass bloom during the monitoring period.
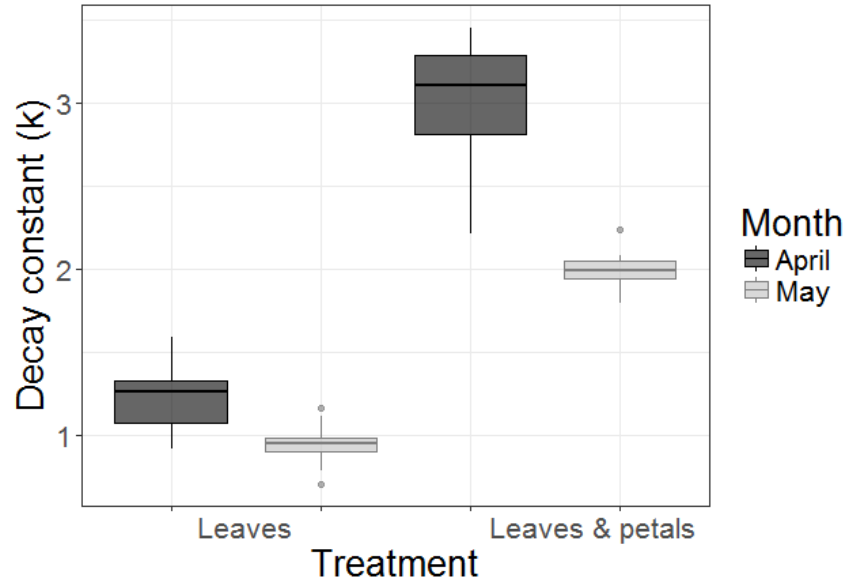
**Figure 4.3** Box plot of decay constant (*k*) by treatment (leaves, leaves and petals) and by time (April [one month], May [two months]).
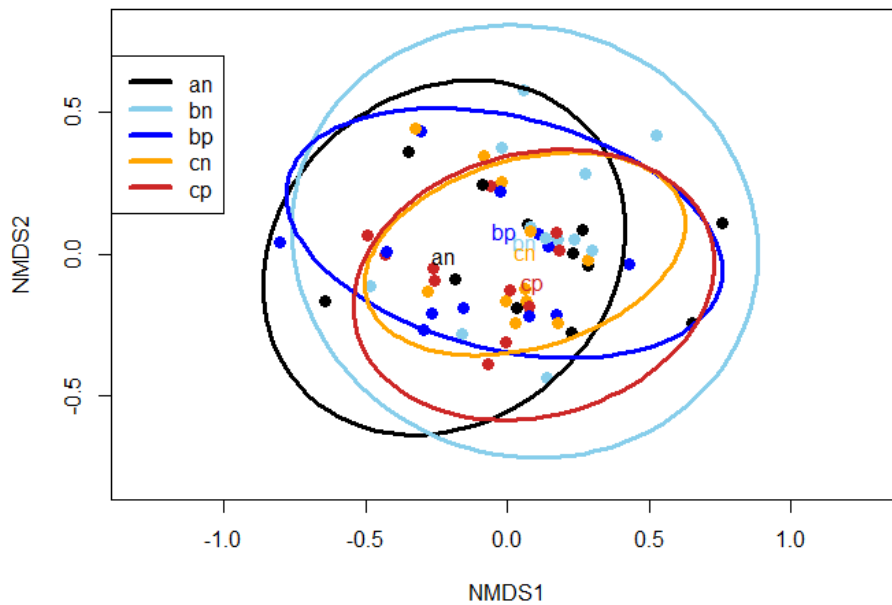


**Figure 4.4** NMDS plot that includes order-level data from pitfall traps. Analysis of similarity (ANOSIM), at the level of orders, indicated significant separation between treatments (*R*=0.066, *p*=0.02). The stress value was 0.186, indicating a good representation. Each color represents a different treatment (an=before manipulation, bn=3 days after, no petals added, bp=3 days after, petals added, cn=7 days after, no petals added, cp=7 days after, petals added) and the letters indicate the centroid of each treatment. Ellipses represent 95% confidence intervals; the overlap of ellipses indicates a lack of separation between treatments.

## Chapter 5: Evaluating Community Effects of *Azteca sericeasur* on *Inga micheliana* Leaf Litter Decomposition

### 5.1 Abstract

Our research examined the effect of *Azteca sericeasur,* a keystone arboreal ant, on the decomposition of leaf litter of the shade tree, *Inga micheliana,* in coffee agro-ecosystems. This interaction is important in understanding spatial heterogeneity in decomposition. We hypothesized that *A. sericeasur* could affect leaf litter decomposition by excluding other ants, which could release decomposers, like Collembola, from predation pressure. Determining the relative strengths of these interactions can illuminate the importance of *A. sericeasur* in decomposition and nutrient cycling processes.

We assessed the ant and arthropod communities surrounding 10 pairs of trees, where each pair included one shade tree with an established *A. sericeasur* nest. Tuna baits were used in conjunction with pitfall traps to assess the ant and arthropod community, and litterbags with *I. micheliana* leaf litter were used to assess rates of decomposition. The species richness of ants did not change in proximity to *A. sericeasur* nests, though the ant communities were distinct. Abundance of Collembola and community composition of other invertebrates did not change in the presence of *A. sericeasur* nests, and there were no differences in leaf litter decomposition rates. This contradicts past studies that suggest *A. sericeasur* reduces ant species richness in its territory. We suggest that other ants may avoid *A. sericeasur* by moving within and beneath the leaf litter. Our results indicate that there is no net effect of *A. sericeasur* on leaf litter decomposition.

**5.2 Introduction**

The activity of animals can have important impacts on decomposition dynamics, with accelerating or decelerating effects (Hättenschwiler et al. 2005, Gessner et al. 2010). Animals impose important controls on terrestrial decomposition, along with climate, litter chemistry and soil properties (Swift et al. 1979, Aerts 1997). The relative importance of each can vary based on context and scale (Aerts 1997, Zhang et al. 2008, Prescott 2010). Lavelle et al (1993) organizes the factors with climate first, followed by soil properties, litter chemistry and quality, and lastly, animal activity. Though lowest on the proposed hierarchy and highly context dependent, animal activity can have important impacts on decomposition dynamics.

Several reviews detail the ways in which predators and herbivores might influence nutrient dynamics directly and indirectly, across time scales, and in both accelerating and decelerating fashions (Wardle et al. 2002, Schmitz et al. 2010, Hunter et al. 2012). Direct effects include contributions to the detrital pool by way of cadavers, feces and urine (Carter et al. 2007) or alteration of the detrital pool where herbivores induce changes in plant tissue or convert that tissue to more labile forms, like frass or insect body tissue (Schmitz et al. 2010, Hunter et al. 2016). Indirectly, predators can mediate nutrient dynamics by altering the distribution, composition, abundance and behavior of herbivores (Hawlena et al. 2012, Hines and Gessner 2012). This has been shown experimentally when exclusion of spiders increased collembolan density and, in turn, decomposition rates (Lawrence and Wise 2000). Collembola and other grazers can increase microbial biomass which, consequently, accelerates decomposition rates (Hanlon and Anderson 1979, Seastedt 1984, Yang et al. 2012). Schmitz et al. (1997) provide a classical example of top-down control where differing hunting strategies by predatory spiders in old fields alter the behavior of the dominant herbivore, a grasshopper. This interaction results in

a distinct change in primary production and cascading effects on carbon lability and nitrogen mineralization in the old field system (Schmitz 2008).

Litter dwelling arthropods have been found to accelerate decomposition in some cases (Attignon et al. 2004, Hättenschwiler and Gasser 2005, Del Toro et al. 2015), while in others, they decelerate it (Hunter et al. 2003) or have no net effect (Gonzalez and Seastedt 2001), depending on which of the potential pathways is dominant. Predicting the effects of litter-dwelling arthropod trophic dynamics on decomposition is particularly challenging in tropical systems where leaf litter and litter communities are spatially and temporally heterogeneous (Kaspari and Yanoviak 2009). Ants (Hymenoptera: Formicidae) may play a key role in determining litter decomposition dynamics in tropical systems (McGlynn and Poirson 2012, Clay et al. 2013). In a mesocosm experiment, local biomass of ants was the primary factor regulating decomposition, exceeding the relative importance of soil chemistry where ants were present (McGlynn and Poirson 2012). In addition to heterogeneity in abundance and richness, ants also exhibit a range of foraging strategies, predating at varying trophic levels (Blüthgen et al. 2003, Tillberg et al. 2006, Platner et al. 2012, Roeder and Kaspari 2017). Thus, effects of ants on decomposition may depend strongly on the community context in which they are embedded.

The keystone ant species, *Azteca sericeasur* (formerly identified at this site as *Azteca instabilis* [Philpott et al. 2009, Mathis et al. 2011, Li et al. 2016]), provides a useful system for studying the impacts of arthropods omnivores on decomposition dynamics. While *A. sericeasur* nests in shade trees, it has a hemipteran mutualist, *Coccus viridis* (coffee green scale), on nearby coffee bushes, which it defends vigorously (Hsieh 2015). The aggressive nature of *A. sericeasur* can exclude other ant species (Ennis 2010) and other arthropods (Vannette et al. 2017). *A. sericeasur* is a keystone species with a proven capacity to alter community composition via

competitive exclusion and predatory effects (Vandermeer et al. 2010; Perfecto and Vandermeer 2014), and we expected that these effects could have important implications for leaf litter decomposition.

Here, we assess the effects of *A. sericeasur* on the litter-dwelling community surrounding its nest. We sought to investigate the indirect effects of *A. sericeasur,* as a keystone omnivore, on decomposition as mediated by its impact on ground-dwelling ants and the litter invertebrate community, including Collembola, which are important decomposers (Yang et al. 2012). We hypothesized a net positive effect of *A. sericeasur* on decomposition processes (Figure 5.1). We predicted that this net positive effect would act through the following causal pathway:

A. *A. sericeasur* presence would decrease the species richness of ground-dwelling ants within close range of their nests, due to their aggressive exclusion of heterospecific ants.

B. Lower species richness and abundance of ground-nesting ants would be associated with higher collembolan abundance, as several important ground-nesting ant species (including *Pheidole* spp.) are predators of Collembola.

C. An increase in Collembola, and possibly other decomposers, would lead to increased mass loss in *I. micheliana* leaf litter since Collembola are important leaf litter detritivores.

## 5.3 Methods

*Study system*

*Azteca sericeasur* has been well-studied in coffee agro-ecosystems, where it nests in mid-canopy trees (Philpott 2010). *Azteca sericeasur* (Hymenoptera: Formicidae: Dolichoderinae) are

found in wet forests and mature colonies can be polydomous (Longino 2007). *Azteca sericeasur* has a mutualistic relationship with *Coccus viridis,* the coffee green scale. As is often the case in ant-hemipteran mutualisms, the ants defend the scale and feed on the sugary honeydew excreted by the scale. *Azteca sericeasur* provides defense from predators of the green coffee scale (Hsieh 2015) and facilitates a faster growth rate of scale populations (Jha et al. 2012). *Azteca sericeasur* is omnivorous, relying on the honeydew from *C. viridis*, sugar from extrafloral nectaries and arthropod prey (Philpott & Armbrecht 2006, Livingston et al. 2008). They exclude other ants (Ennis 2010), alter the ant community (Philpott 2010), exclude flying insects (Vannette et al. 2017) and lower the total abundance of arthropods on coffee plants around their nests (Vandermeer et al. 2002). Further, *A. sericeasur* can serve as biocontrol, reducing the number of coffee berry borers and other pests on defended plants (Gonthier et al. 2013, Morris et al. 2015).

Previous research has demonstrated direct effects of ants in the *Azteca* genus on decomposition, as mediated by the inputs of refuse, including cadavers, feces, urine and pieces of carton nest (Clay et al. 2013). However, the *Azteca* species studied by Clay et al. is known for building large carton nests, whereas *A. sericeasur,* the species of focus here, only occasionally builds carton nests and more typically nests in the lower trunks of live and dead shade trees (Philpott 2005, Livingston et al. 2008).

We focused on the most common species of shade tree in the region, *Inga micheliana,* where nests are frequently found (Li et al. 2016). Trees in the *Inga* genus are ubiquitous as shade trees throughout coffee farms in the region, in part due to their ability to fix nitrogen (Grossman et al. 2006). At our study site, trees in the *Inga* genus make up more than half of all shade trees (Philpott and Bichier 2012). Nitrogen fixation—especially in young *Inga* trees—has been found to be relatively low, and advantages for weed control have been modest (Romero-Alvarado et al.

2000, Grossman et al. 2006). Nonetheless, *Inga* spp. remain a common choice due to these perceived advantages (Romero-Alvarado et al. 2000). In coffee systems, *I. micheliana* can host *Octolecaium* sp. scale and have extra-floral nectaries (Livingston et al. 2008).

*Study site*

      This study was conducted at *Finca Irlanda*, a 300-ha. organic shaded coffee farm in the Soconusco region of Chiapas, Mexico. Altitude ranges from 900-1200 m a.s.l. at the site and mean annual rainfall is approximately 4500mm (Li et al. 2016). The region has two distinct seasons: a rainy season from May through October and a dry season from November through April. Community sampling took place in June and July of 2016, during the rainy season. Litterbags were in the field for one year, from July 2016 until July 2017.

      Sampling was conducted at 10 locations, each of which included a pair of sites (n=20 sites) oriented around a focal *I. micheliana* shade tree. One site in each pair had an *A. sericeasur* nest that had been active for at least 2 years. The other site in the pair, the control, had not supported a nest during the previous 3 years. The paired sites were 30-100 m apart (see supplementary figure C5.1). Sampling took place in an area approximately 25 m$^2$, as described in detail below. There were no other *I. micheliana* trees in the sampling area, though there were coffee plants. Steep slopes and trees near pathways were avoided. *Azteca sericeasur* does not exhibit a strong affinity for nesting in particular shade tree species, so the location of the nests is correlated with the shade tree species abundance (Livingston et al. 2008). In all of our sites, *A. sericeasur* nests were located within the trunk of the tree; none had a visible carton.

*Sampling methods*

**Ant baiting** was carried out at each site, around the focal tree, to determine the ant community. Four transects with 8 baits each, extending in each cardinal direction, were placed at each tree for a total of 32 baits per site. Baits were placed at 0.5 m increments from the base of the focal tree to 2.5 m away and at 1 m increments from 2.5 m to 4.5 m from the base of the focal tree. Thus, baits were sampled at 0, 0.5, 1, 1.5, 2, 2.5, 3.5 and 4.5 m from the focal tree (see supplementary figure C5.2). A pinch of canned tuna was placed as bait on a cleared patch of soil and allowed to sit for 20 minutes, so that ants could locate and recruit to the bait. Tuna baiting is a widely used method for assessing the ant community, including in coffee agro-ecosystems (Philpott et al. 2006). Ants at all baits were identified to species or morpho-species. Most ants were identified in the field, but in cases where an identification could not be made in the field, individual ants were collected and identified at the field station. Guides from published taxonomy resources were used first to make identifications (Bolton 1994, Fernandez 2003), followed by "antwiki.org." Reference specimens were collected when baiting to ensure identifications were standardized between baiting and pitfall samples.

**Pitfall traps** were used one week after baiting. Four traps were used at each site—two within the activity radius of *A. sericeasur* and two outside of their radius. The traps within the radius were placed 0.5 m from the focal tree, a radius at which *A. sericeasur* were recorded at all trees with nests. The traps outside the radius of *A. sericeasur* were placed 2 m from the focal tree, where no *A. sericeasur* was observed at the tuna baits (see supplementary figure C5.2). Pitfall traps were buried flush with the ground and shaded by a larger lid to prevent falling debris or rain from entering. Traps were left closed for 24 hours after burial to reduce disturbance effects. Once opened, the traps were left open for 48 hours before re-collection. We used this ant

data to complement the data from the tuna baits, since not all ant species are attracted to tuna (Philpott et al. 2006) and competition can reduce the co-occurrence of ant species at baits where competitively dominant species are found (Perfecto 1994). The ants in the pitfall traps were identified to species or morpho-species (supplementary table C5.1). We used guides (Bolton 1994, Fernandez 2003), as well as "antwiki.org" and the reference samples taken from the tuna baiting to ensure morpho-species identifications remained consistent. The ants collected were kept to create a reference collection, which is located at the University of Michigan (Ann Arbor, MI, USA). All other invertebrates in the pitfall traps were stored in ethanol and identified to order or family.

**Litterbags** were assembled using a homogenized batch of recently senesced *I. micheliana* leaves collected from the field site and dried in an oven at 50°C to a constant weight. Five-millimeter fiberglass mesh (Saint-Gorbain ADFORS, www.adfors.com) was used, which allows most decomposer invertebrates to access the leaf material (Bradford et al. 2002). A total of 8 litterbags were placed at each site, at a point 1 m from the focal tree. The distance of 1 m was chosen because that was within the range of *A. sericeasur* at each of our focal trees with a nest. Litterbags were collected from each site after 2 weeks, 1 month, 2, 4, 6, 8, 10 and 12 months. This time frame is appropriate in the tropics, as climatic conditions result in most leaf litter decomposing within the year (Powers et al. 2009). Collected litterbags were dried and weighed (+/- 0.2 g, using American Weigh Scale [Cumming, Georgia] 1 kg scale) to determine mass loss.

*Statistical methods*

We used the non-parametric Wilcoxon signed rank test to compare species richness of ants at the tuna baits, which was appropriate because a) the control and treatment sites were paired and b) the data was non-normal, with outliers, which violates assumptions of parametric tests. A one-sided test was used to test the hypothesis that there would be a lower species richness of ants at the sites with *A. sericeasur* nests. We also created a linear mixed-effects model using the "lmer" function within "*lmerTest*" package in R (Kuznetsova et al. 2017). This allowed us to test for differences in species richness by treatment while controlling for variation in local richness between pairs by using "pair' as a random effect.

We estimated species richness with rarefaction curves created with the "*vegan*" package in R and used the "rarefaction" method (Oksanen et al. 2007). We used the "adonis" function in the "*vegan*" package to compare communities (Oksanen et al. 2007). This is functionally equivalent to permutational multivariate analysis of variance (PERMANOVA). We calculated distances based on Bray-Curtis, which is appropriate for our count data, and ran 1000 permutations. This was repeated for the ant community data from the tuna baits and the pitfall traps. "Adonis" provides $R^2$ as an output (rather than pseudo-F values), which indicates the strength of the relationship. We pooled data at the level of the tree to avoid pseudo-replication, but for the pitfall traps, we also looked at the effect of distance at each tree (n=40).

The "adonis" function was used to look for differences in the arthropod community composition in the pitfall traps, and non-metric multidimensional scaling (NMDS) was used to visualize differences in these communities. NMDS was computed using the "metaMDS" function from "*vegan*" in R (Oksanen et al. 2007) with the Bray-Curtis dissimilarity index and with three dimensions (k=3) to reduce our stress values. The matrix was computed using the

arthropod data identified to order or family and without any environmental factors. We carried

out this analysis with data pooled by tree and distance and, to be conservative, by tree. We used

the non-parametric Kruskal-Wallis test to assess differences in collembolan abundance in the

pitfall traps because the residuals were not normally distributed, violating a key assumption of

parametric tests.

The decay constant ($k$) was used to assess decomposition rates and compared between

treatments, as is standard in the decomposition literature (Olsen 1963, Melillo et al. 1982). The

decay constant, $k$, comes from the exponential decay equation ($N_t = N_0 * e^{-k*t}$). We created a

linear mixed-effects model using the "lmer" function within "*lmerTest*" package in R

(Kuznetsova et al. 2017) to assess the effect of time, *A. sericeasur,* Collembola and other ants

within the radius of *A. sericeasur* and the interaction of time and the presence of *A. sericeasur* on

the decay constant, $k$. To correct for non-normal residuals, $k$ was log-transformed. Site was

included as a random effect to control for site-based correlation.


## 5.4 Results

Sites with *A. sericeasur* nests had an average species richness of 32 ground-dwelling

ants, which was slightly greater than the 28 species found in sites without *A. sericeasur* nests.

However, this difference was not statistically significant (*V*=13, p=0.263). The presence of *A.

sericeasur* was not a significant predictor of species richness at the tuna baits (β= 0.8, df=9,

p=0.393). On average, there were 1-1.5 species of ant at the tuna baits, regardless of the bait's

distance from the focal tree (Figure 5.2). Estimated ant species richness from the pitfall traps was

lower in the traps near to (0.5 m) *A. sericeasur* sites compared to traps placed far from (2 m) the

nests, though all four treatments failed to reach an asymptote indicating we sampled a portion of the total ant community (Figure 5.3).

The ant community composition around *A. sericeasur* nests was distinct from the community composition at trees without *A. sericeasur* nests (tuna baits, $R^2=0.122$, p=0.003; pitfalls, $R^2=0.06$, p=0.004). Differences in the ant community composition at the pitfalls was not dependent on distance ($R^2=0.04$ p=0.09), nor was there a strong interaction between distance and treatment ($R^2=0.03$, p=0.39). Nevertheless, even after pooling traps across distances, the ant communities around focal trees with *A. sericeasur* were distinct from the communities around trees without *A. sericeasur* nests ($R^2=0.12$, p=0.01). The community of ants sampled through tuna baits and pitfall traps is reported in supplementary Table C5.1.

Despite statistically distinct ant communities, there is not visual separation in the overall communities found in the pitfall traps (Figure 5.4). Here the community, based on the pitfall trap samples, includes Diptera, Hymenoptera (divided into ants and non-ants), Arachnida, Coleoptera, Orthoptera, Isopoda, Hemiptera, Collembola and all others (see supplementary Table C5.2). The stress value for our NMDS visualization was 0.163 indicating good representation. The community of organisms in the pitfall traps did not differ based on distance to the tree (adonis; $R^2=0.032$, p=0.29) or presence of *A. sericeasur* ($R^2=0.024$, p=0.43), and there was no interaction between distance and *A. sericeasur* ($R^2=0.03$, p=0.26). Accordingly, there were also no differences in the overall community at the level of tree when pooling across distances ($R^2=0.04$, p=0.51).

On average, there were 64 Collembola in the sample taken 0.5 m from a focal *A. sericeasur* tree and 63 Colllembola at 2 m from focal *A. sericeasur* trees. Focal trees without *A.*

*sericeasur* averaged 64 Collembola in 0.5 m samples and 74 Collembola at 2 m. These differences were not statistically significant (Kruskal Wallis, chi-square=0.254, df=3, p=0.968).

Only time was a significant predictor of the decay constant *k* in our model (β= -0.01, df=125.2, p<0.001). The presence of an *A. sericeasur* nest at the focal tree (β= 0.005, df=68.9, p=0.975) the abundance of non- *A. sericeasur* ants (β= 0.0004, df=12.8, p=0.553) and abundance of Collembola (β= -0.0003, df=14.2, p=0.639) within the range of *A. sericeasur,* and the interaction term between *A. sericeasur* and time (β= 0.006, df=124.7, p=0.483) were all non-significant in our model.

**5.5 Discussion**

Our findings suggest that *A. sericeasur* may alter ant community composition and influence the litter community through higher-order interactions, rather than simple exclusion of other ants. We found weaker-than-expected effects of *A. sericeasur* on the invertebrate community around their nests and no effect of *A. sericeasur* on leaf litter decomposition.

Our finding that leaf-litter ant species richness was unchanged in close proximity to *A. sericeasur* nests contradicts most existing research that suggests *A. sericeasur* excludes other ants from the areas immediately surrounding their nests (Ennis 2010, Philpott 2010). However, not all studies have found an effect of *A. sericeasur* on the ant community. Philpott and colleagues (2004) found that the presence of *A. sericeasur* decreased colonization rates of common twig-nesting ant species but had no effects on rare species.

The discrepancy between our results and results from other studies focusing on *A. sericeasur* could be due to a potential behavioral adaptation of non-dominant ants to avoid *A. sericeasur.* Previous studies have documented the effect of *A. sericeasur* on other insects that

forage arboreally (Vandermeer et al. 2002, Philpott et al. 2004), rather than on the ground. Our focus was on the leaf litter layer, which is shown here to support other ant species within a small radius of *A. sericeasur* nests. Thus, the maintenance of leaf litter on the soil surface could support ant species richness, even where *A. sericeasur* is dominating arboreal ant communities. *Azteca sericeasur* are known to use twigs, leaf litter and other detritus for pathways, nearly always avoiding walking on the soil. While never tested explicitly, to our knowledge, this behavioral preference is anecdotally supported and underscored by an increase in *A. sericeasur* foraging where connectivity is artificially augmented by ropes and bamboo (Jimenez-Soto et al. 2019). If *A. sericeasur* avoids walking on soil, other ant species that may otherwise forage arboreally may shift their behavior in the presence of *A. sericeasur* to avoid encounters. This higher-order interaction could be responsible for the community differences found in our study between sites with and without nests both at the baits and in the pitfall traps.

Alternatively, it may be that we found no effect of *A. sericeasur* on leaf litter ant richness because *A. sericeasur* foraging activity at the time of sampling—the rainy season—was focused on shade trees rather than on coffee bushes. *Inga micheliana* have extra-floral nectaries and host *Octolecanium* sp. helmet scale (Livingston et al. 2008), both of which provide alternative sources of sugar for *A. sericeasur*. Moreover, during the rainy season, when the study was conducted, *C. viridis* has reduced sugar content (Rivera-Salinas et al. 2018), while extra-floral nectaries are more productive (Rico-Gray et al. 1998). Further, *C. viridis* occurs in lower densities around *I. micheliana* with *Octolecanium,* suggesting competition between the scale species and a preference of *A. sericeasur* for *Octolecaium* (Livingston et al. 2008). We did not monitor scale densities on the coffee bushes nor on the shade trees. A minimum level of ant activity was a pre-requisite for site selection, but it is not known where the ants were primarily foraging. If *A.*

*sericeasur* was primarily foraging in the crown of *I. micheliana* at the time of the study, its effects on ground-dwelling ants would be reduced.

Seasonal dynamics of scale insects (both *Octolecanium* sp. and *C. viridis*) may alter the food sources available to *A. sericeasur,* but the dietary needs of *A. sericeasur* also change between seasons. Past research has suggested that *A. sericeasur* are not sugar limited at the start of the rainy season, as they have not shown a preference for high density *C. viridis* patches (Rivera-Salinas et al. 2018). However, despite the complications that distinct seasonality presents, the sampling time of this work is consistent with past work on *A. sericeasur* where they have been found to exclude other ant species (Ennis 2010, Rivera-Salinas 2019).

Previously, *A. sericeasur* has been assumed to be a dominant keystone species. These results do not support this conclusion, in regard to brown food web or detrital dynamics, where the invertebrate community appears to be unchanged by the presence of *A. sericeasur.* Other ants, including those in the *Pheidole* genus, which are primarily predators, are more likely to have a strong effect on the brown food web (Wilson 2005, Shukla et al. 2013). We did not find support for our hypothesis that *A. sericeasur* was excluding other ant species and suggest that the presence of *A. sericeasur* might even be driving other ant species to spend more time within the leaf litter layer. Nonetheless, we find no evidence of an effect of *A. sericeasur* on the litter-dwelling community.

The lack of a net effect of *A. sericeasur* on decomposition is not unexpected, given the similarity in the detritivore communities around trees with and without *A. sericeasur* nests. Our study relied on site choice, rather than direct manipulation, to evaluate the effects of ants and litter-dwelling detritivores on decomposition. Because of the unexpectedly weak effects of *A. sericeasur* on ant and litter-dwelling detritivores, we could not disentangle the effects of ants and

the effects of micro-arthropods. In a study conducted in Costa Rica, decomposition decreased where ants were excluded but micro-arthropods were allowed access to the litter, suggesting a cascade in which ants prey upon micro-arthropods that are grazing upon decomposer microbes (McGlynn and Poirson 2012). Studies that use litterbags of varying mesh sizes to control access to the litter are needed, as are studies that directly manipulate abundances and community composition.

The results from this study suggest that top-down effects of predators on decomposition dynamics are weak in this system. However, we caution that these results may not be widely applicable and instead highlight the context dependency of such top-down effects. In this case, *A. sericeasur* did not influence decomposition dynamics nor did it appear to influence the invertebrate community or abundance of other ants, but it did change the community composition of ants. Further research is needed to determine if this is due to temporally and spatially specific constraints on *A. sericeasur* dominance or resilience in the decomposer community.

**5.6 Acknowledgements**

## 5.7 References

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## 5.8 Figures
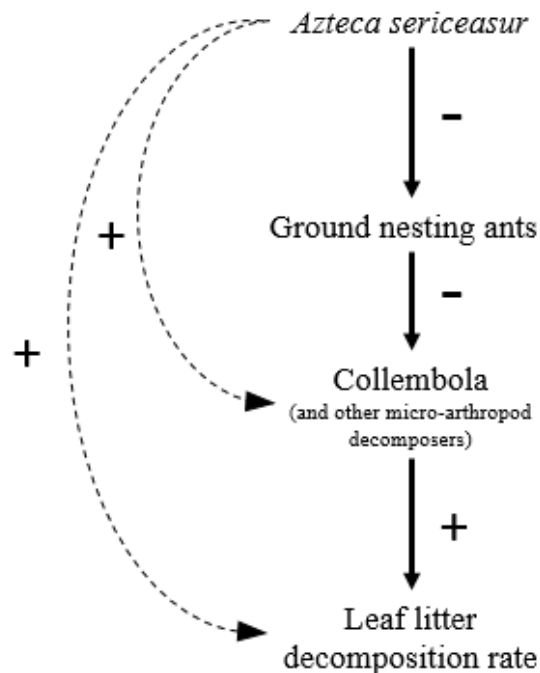


**Figure 5.1.** Model illustrating hypotheses. We expect *A. sericeasur* will reduce the diversity of ground-nesting ant species, due to their aggressive nature, which would release Collembola, a micro-invertebrate decomposer, from predation pressure and potentially increase leaf litter decomposition. Thus, we predict *A. sericeasur* will have a net positive effect on leaf litter decomposition rate, as mediated through ground-nesting ants.
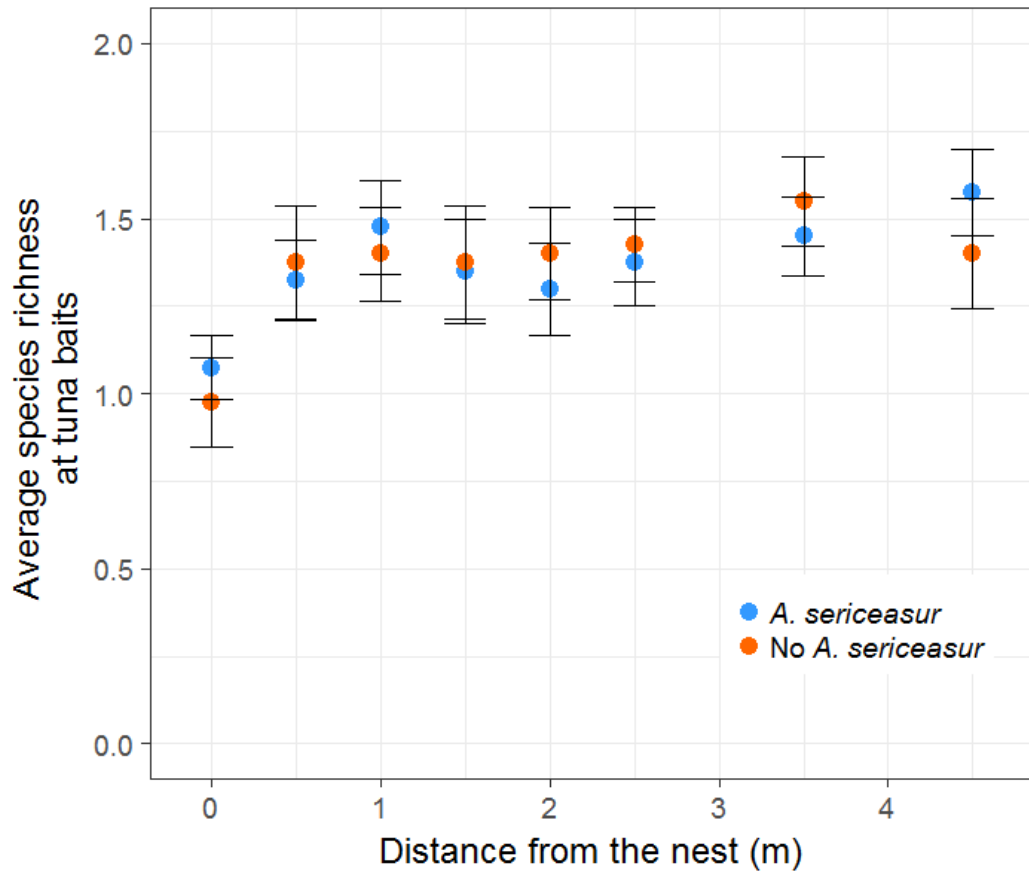
**Figure 5.2** Average species richness at tuna baits. Data from sites with *A. sericeasur* nests are shown in blue and data from sites without *A. sericeasur* nests are shown in orange. Error bars represent standard error.
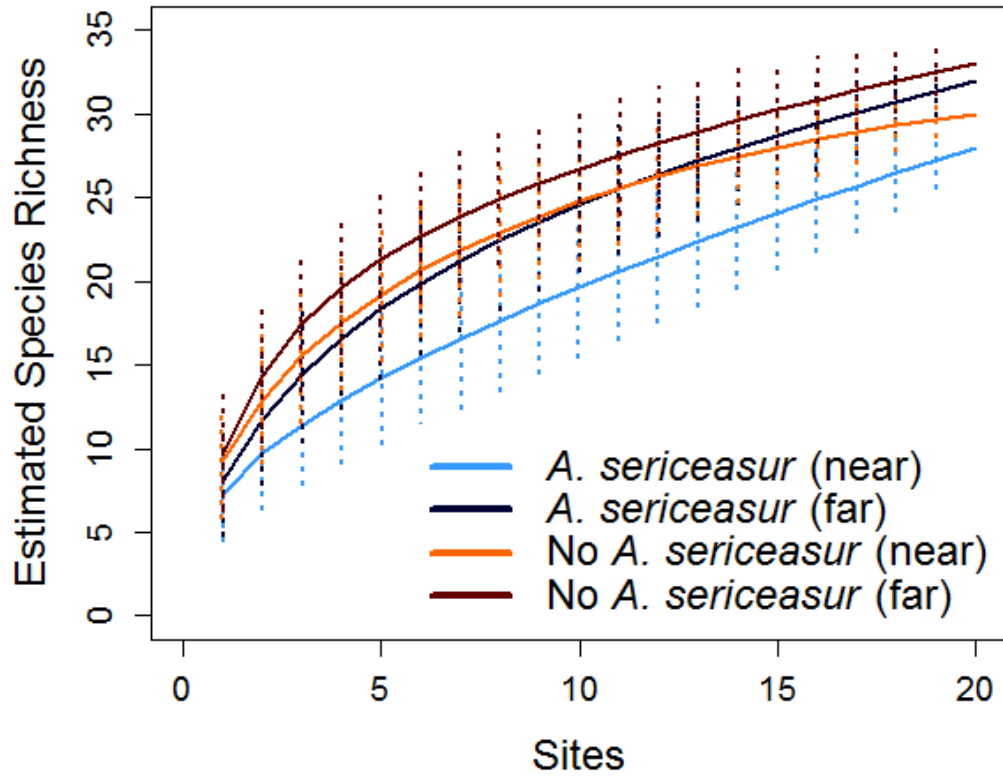
**Figure 5.3** Rarefaction curves for the four treatments (with *A. sericeasur* and without *A. sericeasur*, at distances near [0.5m] and far [2m] from the tree). Dashed lines indicate a 95% confidence interval.
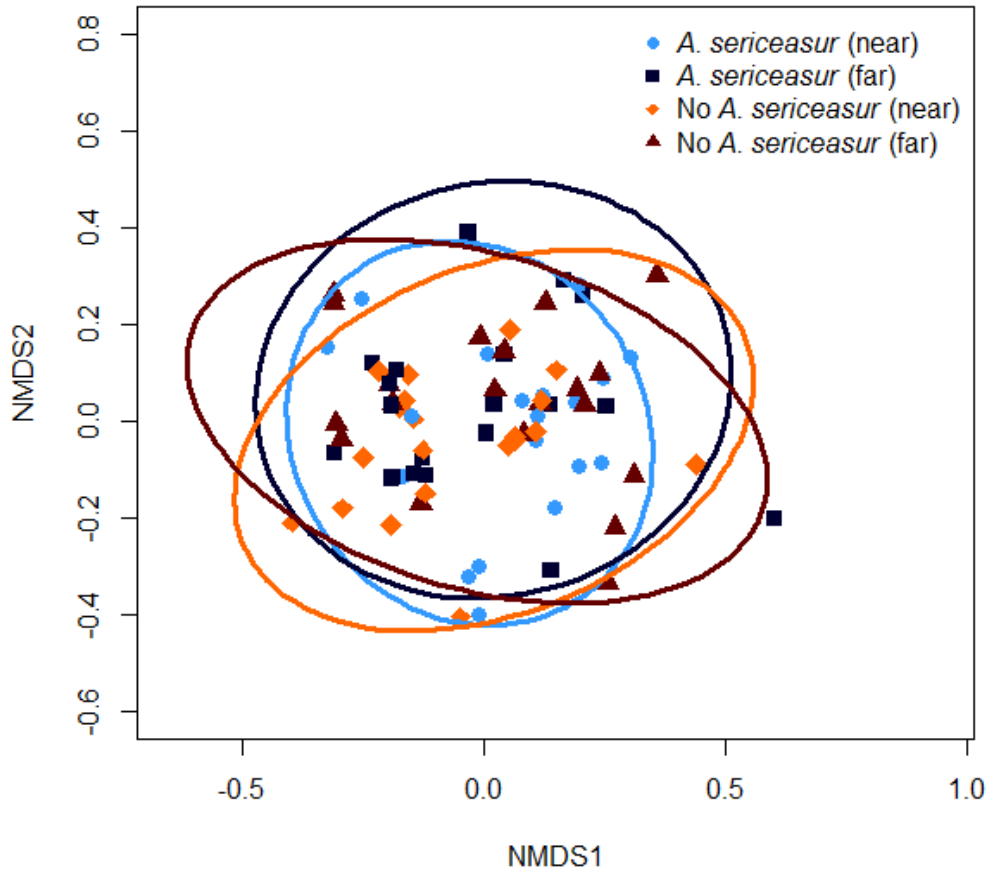
**Figure 5.4** NMDS plot of arthropods in pitfall traps at sites with *A. sericeasur* nests (A) and without nests (N). Traps were placed 0.5 m from the tree (near) and 2 m from the tree (far) to compare communities within and outside the range of *A. sericeasur*. Ellipses represent 95% confidence intervals and the final stress value was 0.163.

# Chapter 6: Conclusions

## 6.1 Synthesis

Increased understanding of the biotic drivers of decomposition is essential, and challenging, given the vast number of direct and indirect linkages between biota and decomposition processes (Bardgett and Wardle 2010, Rouifed et al. 2010, Boyero et al. 2014, García-Palacios et al. 2016). This dissertation builds upon existing work that highlights the context dependent nature by which biota influence decomposition in managed and unmanaged ecosystems. I have used a variety of methods to assess linkages between decomposition and primary producers and upper trophic levels, with explicit consideration of spatial and temporal variability.

## Chapter 2: Leaf litter decomposition of *Coffea arabica* and *Coffea robusta* at home and away: short term home-field advantage.

Home-field advantage is a popular ecological theory which posits that detritus will decompose more quickly in the environment where it is from, it's "home," compared to other "away" environments (Ayres et al. 2009). In this chapter, I tested the strength of home-field advantage for two commercially cultivated coffee species, *Coffea arabica* and *Coffea robusta.* I used a reciprocal transfer experiment, quantifying the decay rate of both species in their home environments, a con-generic away environment, and a forested-away environment in a six-week tethered line experiment and a year-long litterbag study. I found evidence of home-field advantage in the shorter, tethered line study, but no difference in day rates between species or

environments in the year-long time frame. Nonetheless, home-field advantage acting on a short time scale could provide an ecologically important pulse of nutrients.

**Chapter 3: Interactive effects of *Inga micheliana* and *Alchornea latifola* shade trees on mixed litter decomposition.**

When leaf litter from multiple species is decomposing in combination, non-additive effects can result in overall faster or slower decomposition than would be predicted based on the decomposition of the species in isolation (Gartner and Cardon 2004, Hättenschwiler et al. 2005). Here I focused on the decomposition of *Coffea arabica* and two common shade tree species, *Inga micheliana* and *Alchornea latofolia,* to investigate if and how shade tree species choice influenced decay dynamics. Of the three species tested, only *C. arabica* consistently decomposed more quickly in mixture. As predicted, based on its nitrogen-fixing ability, being in mixture with *I. micheliana* provided the largest acceleration to litter decay. Despite *C. arabica'*s low carbon to nitrogen ratio, the same acceleration of the decay rate was not seen in other species when combined with *C. arabica*, perhaps because some of the foliar nitrogen was tied up in the secondary defensive compound, caffeine. One of several potential mechanisms for the dispersal of decomposers was tested, with pairwise comparisons tested uphill and downhill to determine if micro-topography was important in mediating mixture effects. No effects of micro-topography were found, suggesting that decomposer microbes are likely dispersing through other means. In sum, these results add to the existing body of research that finds non-additive effects of multiple species in mixture are highly context dependent.

**Chapter 4: Synchronous flowering of *Coffea arabica* accelerates leaf litter decomposition.**

Mass blooms, where flowering occurring synchronously, can be an important source of temporal variation in nutrient availability because flowers tend to be nutrient rich and preferred by decomposers, relative to other litter types (Whigham et al. 2013). In this chapter I quantified the magnitude of a *Coffea arabica* mass bloom and assessed its impacts on leaf litter decomposition and the decomposer community. The magnitude of the bloom provided ecologically relevant inputs of nitrogen, phosphorus and potassium, though the fate of the petal nutrients within the ecosystem is unknown. Neither the leaf litter community nor an important decomposer, Collembola, responded to manipulation of floral detritus on the time scale of days, but *C. arabica* leaves decomposing with petals decomposed more quickly than *C. arabica* leaves in monoculture. These results highlight the potential importance of flowering in the nutrient dynamics in coffee agro-ecosystems.

**Chapter 5: Evaluating community effects of *Azteca sericeasur* on *Inga micheliana* leaf litter decomposition.**

Upper trophic levels can influence decomposition through a suite of direct and indirect pathways (Hunter 2016). In this chapter, I explored the indirect effects of a keystone ant species, *Azteca sericeasur,* on the decomposition of litter from a shade tree, *Inga micheliana,* where *A. sericeasur* commonly nests. Contrary to previous research, I found no decrease in ant species richness around *A. sericeasur* nests, though the community did differ. Despite a shift in the ant community, I found no evidence of a shift in the leaf litter decomposer community and decomposition rates did not differ at sites with or without *A. sericeasur* nests. Decomposition

processes are retained, despite the presence of an aggressive, keystone ant species. A shift in ant community, but no change in decomposition processes suggests that there is a functionally redundant arthropod community and that niche partitioning is likely occurring in the presence of *A. sericeasur.*

## 6.2 Implications for basic ecology

On the whole, this dissertation highlights the importance of spatially and temporally variable biotic drivers in decomposition processes. While decomposition is often studied on longer time scales, my work also suggests that shorter time frames – even weeks – may be important to study, especially in tropical agro-ecosystems and. For example, in Chapter 4, decomposition of *C. arabica* leaf litter was faster at one month with flower petals – and those effects could be acting on an even quicker time scale.

My findings also underscore the potential role of secondary chemicals on decomposition. The results from the home-field advantage study are likely a result of variable chemistry between *Coffea* species as well as decomposer specialization. And, in Chapter 3, the initial carbon to nitrogen ratios in the three focal species did not accurately predict their effects in mixtures, perhaps due to caffeine, a nitrogenous secondary defensive compound in *C. arabica.* Secondary chemistry is the presumed mechanism driving these results, but more research is needed.

Reductionist studies that focus on single drivers of decomposition, like those presented here, are necessary to identify the direction and potential magnitude of biotic drivers. In this dissertation, I focused on singular pathways that might connect multiple biotic drivers to decomposition processes, but research that aims to test all potential pathways of a single driver are necessary for a holistic understanding of decomposition.

**6.3 Implications for agro-ecology**

All of the fieldwork in this dissertation was conducted on a single shaded, organic coffee farm in the Soconusco region of Chiapas, Mexico. Several results from this work provide actionable guidance for farmers and land managers in the region, and beyond. Importantly, the role of management decisions in driving ecosystem process and function is clear; management decisions, including which shade trees to plant, are key drivers of resultant decomposition function. Particularly in discussions of coffee management and development of certification schemes meant to incentivize sustainable management, decomposition is rarely considered as an important outcome, with more attention given to yields, biodiversity and pest control (Philpott et al. 2007, Blackman and Naranjo 2012). My work illustrates the numerous ways in which management decisions and biota can influence decomposition, which makes it important to integrate decomposition and nutrient cycling into decision making processes.

The keystone ant species, *Azteca sericeasur,* studied in chapter 5, is considered a nuisance species by farm workers, due to its aggressive nature that leads to disruptions to workers in close proximity to their nests (Philpott and Armbrecht 2006). However, other studies have found a positive effect of *A. sericeasur* on biocontrol and pest densities on farms (Jiménez-Soto et al. 2013) and here there is no deceleration of decomposition in the presence of nests. The mechanism tested here is just one of many potential pathways by which *A. sericeasur* could influence decomposition dynamics, and *A. sericeasur* is one of a many species that could alter decomposition dynamics. Evaluating the importance, benefits and risks of each of these pathways and more organisms is essential in making recommendations to farmers.

Biota in agro-ecosystems are often examined from a spatial and temporal lens. Herbivores, which can become pests, and the predators that can serve as biocontrol often have

clear seasonal variability (Ennis and Philpott 2019). Annual or semi-annual events like harvest

and pruning also provide obvious temporal variation in agro-ecosystem dynamics (Staver et al.

2001). In coffee agro-ecosystems, ant nests, streams and other sources of spatial variation have

also been studied and found to generate variation in the trophic webs and ecosystem services

(Vandermeer et al. 2008). Though much work remains, the results of this dissertation clearly

indicate a need to consider sources of spatial and temporal variation when managing agro-

ecosystems for important processes, including decomposition.


**6.4 Future directions**

Generalizing findings of any one study across systems is a perpetual challenge in

ecology. This is especially true for place-based field research, where many of the linkages are

context dependent. Perhaps the most significant limitation of this thesis is its focus on a single

coffee farm. The results of this work, and much of ecology, is limited by its lack of fine-scale

mechanistic understanding on one hand, and on the other hand, by its lack of generalizability

across farms, much less climates, soil types and ecosystems. Population and community ecology

dynamics of decomposing microbes are the presumed mechanism driving many of the patterns

seen in decomposition dynamics – including home-field advantage, non-additive mixture effects,

and priming of decomposition by labile materials, like petals. However, while tractable methods

for studying microbial community ecology are gaining traction, their use in field based studies

remains limited (Kuczynski et al. 2010, Hugerth and Andersson 2017). On the other end of the

spectrum, a relatively recent slew of decomposition-based meta-analyses have tried to unite

findings from place-based research, but context dependency has muddied any clear answers on

the mechanisms driving larger patterns in decomposition (Gartner and Cardon 2004, Ayres et al. 2009).

The challenge of scale—uniting fine scale mechanisms to broad scale patterns—is not unique to the study of biotic drivers of ecosystem function, or even ecology, but it is an essential problem to tackle, especially in light of global climate change and biodiversity losses. Decomposition processes are inextricably linked to abiotic conditions, namely temperature and precipitation, both of which are all but guaranteed to change with anthropogenic climate change. The understanding we do have of decomposition is at risk of crumbling with non-random species loss, collapse of trophic networks, changing abiotic conditions, and potential feedbacks associated with the release of storage of carbon dioxide in soils (Ives and Cardinale 2004, Ball et al. 2008, Santonja et al. 2014). It's essential that biotic drivers of decomposition be included as scientists and land managers seek to understand how decomposition will change.

Future work should continue at the intersection of basic and applied research. It is particularly important that agro-ecological research be framed in a way that provides useful, understandable and feasible recommendations for farmers and land managers. Agro-ecosystems provide a tractable model system with which basic ecological principles can be examined and tested – and are essential in building a sustainable future.

## 6.5 References

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## Appendices
## Appendix A: Supplementary Tables for Chapter 2

**Table A2.1** Pairwise contrasts for tethered line main effects (environment contrasts are provided in the main document) and interaction term. The interaction contrasts are labeled as "species, environment" versus "species, environment," where CA, CA – CR, CA refers to the contrast between *C. arabica* in *C. arabica* compared to *C. robusta* in *C. arabica.*

| Predictors | Estimates | Std. Error | df | t-value | P value |
|---|---|---|---|---|---|
| **A. Pairwise contrasts for species** | | | | | |
| ***C. arabica – C. robusta*** | **3.42** | **1.36** | **198** | **2.509** | **0.0129** |
| **B. Pairwise contrasts for species x environment interactions** | | | | | |
| Species: *C. arabica* | | | | | |
| **CA, CA – CR, CA** | **7.711** | **2.05** | **198.4** | **3.756** | **0.0031** |
| **CA, CA – CA, F** | **12.273** | **3.08** | **15.5** | **3.985** | **0.0118** |
| **CA, CA – CR, F** | **12.621** | **3.08** | **15.5** | **4.098** | **0.0095** |
| CA, CA – CA, CR | 5.091 | 2.68 | 36.5 | 1.902 | 0.4174 |
| CA, CA – CR, CR | 7.304 | 2.49 | 26.6 | 2.932 | 0.0671 |
| CA, F– CR, F | 0.348 | 2.75 | 197.1 | 0.126 | 1.000 |
| CA, F – CA, CR | -7.182 | 3.19 | 18.4 | -2.250 | 0.2631 |
| CA, F – CR, CR | -4.949 | 3.03 | 15.1 | -1.639 | 0.5875 |
| CA, CR – CR, CR | 2.213 | 2.23 | 200.3 | 0.993 | 0.9198 |
| Species: *C. robusta* | | | | | |
| CR, CA – CA, F | 4.562 | 3.04 | 14.7 | 1.498 | 0.6705 |
| CR, CA – CR, F | 4.910 | 3.04 | 14.7 | 1.613 | 0.6034 |
| CR, CA – CA, CR | -2.620 | 2.64 | 34.0 | -0.994 | 0.9167 |
| CR, CA, - CR, CR | -0.407 | 2.45 | 24.5 | -0.166 | 1.000 |
| CR, F – CA, CR | -7.53 | 3.19 | 18.4 | -2.359 | 0.2212 |
| CR, F – CR, CR | -5.317 | 3.03 | 15.1 | -1.754 | 0.5203 |

**Table A2.2** Linear mixed model output for full carbon to nitrogen ratio model. Wheel was nested with time as a random variable in the model.

| Predictors | Estimates | Std. Error | df | t-value | P value |
|---|---|---|---|---|---|
| **Linear mixed model output** | | | | | |
| (Intercept) | 19.231 | 0.706 | 99.47 | 27.25 | <0.005 |
| Species | 1.064 | 1.037 | 99.30 | 1.026 | 0.307 |
| Environment | | | | | |
| *C. robusta* | 0.663 | 1.033 | 100.88 | 0.642 | 0.522 |
| **forest** | **2.33** | **1.095** | **99.26** | **2.126** | **0.036** |
| **Time** | **-0.084** | **0.027** | **91.70** | **-3.036** | **0.0031** |
| Species x environment | | | | | |
| *C. robusta* x *C. robusta* | -0.855 | 1.483 | 99.25 | -0.577 | 0.565 |
| *C. robusta* x control | 0.107 | 1.573 | 99.19 | 0.068 | 0.945 |
| Species x time | 0.024 | 0.038 | 99.20 | 0.620 | 0.536 |
| Environment x time | | | | | |
| *C. robusta* x time | -0.004 | 0.042 | 89.70 | -0.096 | 0.923 |
| Forest x time | 0.0023 | 0.042 | 93.12 | 0.055 | 0.956 |
| Species x environment x time | | | | | |
| *C. robusta* x *C. robusta* x time | -0.0128 | 0.0564 | 99.86 | -0.228 | 0.820 |
| *C. robusta* x forest x time | -0.084 | 0.058 | 99.15 | -1.454 | 0.149 |

**Table A2.3** Pairwise contrasts for litterbag main effects and interaction term. The interaction contrasts are labeled as "species, environment" versus "species, environment," where CA, CA – CR, CA refers to the contrast between *C. arabica* in *C. arabica* compared to *C. robusta* in *C. arabica.*

| Predictors | Estimates | Std. Error | df | t-value | P value |
|---|---|---|---|---|---|
| **A. Pairwise contrasts for species** | | | | | |
| *C. arabica – C. robusta* | 0.2 | 0.779 | 123 | 0.257 | 0.7979 |
| **Pairwise contrasts for environment, by species** | | | | | |
| Species: *C. arabica* | | | | | |
| *C. arabica* – forest | -0.4905 | 1.36 | 45.2 | -0.361 | 0.9310 |
| *C. arabica – C. robusta* | -0.2772 | 1.31 | 40.3 | -0.211 | 0.9757 |
| Forest- *C. robusta* | 0.2133 | 1.37 | 46.3 | 0.155 | 0.9869 |
| Species: *C. robusta* | | | | | |
| *C. arabica* – forest | 0.1450 | 1.36 | 44.3 | 0.107 | 0.9938 |
| *C. arabica – C. robusta* | -0.0373 | 1.31 | 40.3 | -0.028 | 0.9996 |
| Forest- *C. robusta* | -0.1824 | 1.37 | 45.5 | -0.133 | 0.9903 |
| **B. Pairwise contrasts for species x environment interactions** | | | | | |
| Species: *C. arabica* | | | | | |
| CA, CA – CR, CA | -0.0920 | 1.30 | 122.1 | -0.071 | 1.000 |
| CA, CA – CA, F | -0.4905 | 1.36 | 45.2 | -0.361 | 0.9991 |
| CA, CA – CR, F | 0.0531 | 1.36 | 44.3 | 0.039 | 1.000 |
| CA, CA – CA, CR | -0.2772 | 1.31 | 40.3 | -0.211 | 0.9999 |
| CA, CA – CR, CR | -0.1293 | 1.31 | 40.3 | -0.098 | 1.000 |
| CA, F– CR, F | 0.5436 | 1.42 | 123.3 | 0.383 | 0.9989 |
| CA, F – CA, CR | 0.2133 | 1.37 | 46.3 | 0.155 | 1.000 |
| CA, F – CR, CR | 0.3612 | 1.37 | 46.3 | 0.263 | 0.9998 |
| CA, CR – CR, CR | 0.1479 | 1.33 | 122.1 | 0.112 | 1.0000 |
| Species: *C. robusta* | | | | | |
| CR, CA – CA, F | -0.3986 | 1.36 | 45.2 | -0.293 | 0.9997 |
| CR, CA – CR, F | 0.1450 | 1.36 | 44.3 | 0.107 | 1.0000 |
| CR, CA – CA, CR | -0.1853 | 1.31 | 40.3 | -0.141 | 1.0000 |
| CR, CA, - CR, CR | -0.0373 | 1.31 | 40.3 | -0.028 | 1.0000 |
| CR, F – CA, CR | -0.3303 | 1.37 | 45.5 | -0.240 | 0.9999 |
| CR, F – CR, CR | -0.1824 | 1.37 | 45.5 | -0.133 | 1.0000 |

**Table A2.4** ANOVA output for litterbag decay data at the one-month collection point. The assumption of normality was not met, as discussed in the methods section, so residuals should be interpreted with caution.

| | df | Sum Sq. | Mean Sq. | F-value | p-value |
|---|---|---|---|---|---|
| **Location** | 2 | 0.000933 | 0.0004665 | 0.727 | 0.494 |
| **Species** | 1 | 0.000218 | 0.0002179 | 0.304 | 0.565 |
| **Location x species** | 2 | 0.001816 | 0.0009080 | 1.416 | 0.262 |
| **Residuals** | 24 | 0.015394 | 0.0006414 | | |

# Appendix B: Supplementary Tables for Chapter 3

**Table B3.1** Average decay constants for all treatments in the 2017 tethered line study. Averages are presented with standard error

| Location | Leaf species | Treatment | Decay constant (k) | Standard error |
|---|---|---|---|---|
| Forest | *A. latifolia* | alone, in monoculture | 10.988 | 1.996 |
| | *A. latifolia* | with *C. arabica* | 9.823 | 0.939 |
| | *A. latifolia* | with *I. micheliana* | 10.337 | 0.946 |
| | *A. latifolia* | with *C. arabica* and *I. micheliana* | 11.610 | 1.284 |
| | *C. arabica* | alone, in monoculture | 9.979 | 0.869 |
| | *C. arabica* | with *I. micheliana* | 15.965 | 1.570 |
| | *C. arabica* | with *A. latifolia* | 9.591 | 0.784 |
| | *C. arabica* | with *A. latifolia* and *I. micheliana* | 12.448 | 1.371 |
| | *I. micheliana* | alone, in monoculture | 8.581 | 1.541 |
| | *I. micheliana* | with *C. arabica* | 12.520 | 1.652 |
| | *I. micheliana* | with *A. latifolia* | 9.602 | 1.442 |
| | *I. micheliana* | with *C. arabica* and *A. latifolia* | 6.444 | 0.694 |
| Farm | *A. latifolia* | with *C. arabica* | 5.559 | 0.383 |
| | *A. latifolia* | with *C. arabica* and *I. micheliana* | 9.282 | 0.939 |
| | *C. arabica* | with *A. latifolia* | 9.456 | 0.670 |
| | *C. arabica* | with *I. micheliana* | 13.549 | 0.910 |
| | *C. arabica* | with *A. latifolia* and *I. micheliana* | 12.849 | 1.005 |
| | *I. micheliana* | with *C. arabica* | 10.789 | 0.993 |
| | *I. micheliana* | with *C. arabica* and *A. latifolia* | 7.103 | 0.589 |

**Table B3.2** Average decay constants for all treatments in the 2018 slope line study. Averages are presented with standard error.

| Leaf species | Position | Treatment | Decay constant (*k*) | Standard error |
|---|---|---|---|---|
| | monoculture | | 7.778 | 0.511 |
| | above | with *C. arabica* | 4.283 | 0.819 |
| *A. latifolia* | below | with *C. arabica* | 4.360 | 0.442 |
| | above | with *I. micheliana* | 6.358 | 0.522 |
| | below | with *I. micheliana* | 7.267 | 0.554 |
| | monoculture | | 7.487 | 0.482 |
| | above | with *A. latifolia* | 6.204 | 0.490 |
| *C. arabica* | below | with *A. latifolia* | 8.418 | 0.910 |
| | above | with *I. micheliana* | 10.636 | 0.869 |
| | below | with *I. micheliana* | 10.293 | 1.175 |
| | monoculture | | 4.764 | 0.476 |
| | above | with *A. latifolia* | 6.101 | 0.500 |
| *I. micheliana* | below | with *A. latifolia* | 5.401 | 0.475 |
| | above | with *C. arabica* | 4.687 | 0.481 |
| | below | with *C. arabica* | 4.852 | 0.629 |

**Table B3.3** Pairwise comparisons of focal species below and above other species in the 2018 slope study.

| Focal species | Mixture species | Degrees of freedom | F-value | *p-value* |
|---|---|---|---|---|
| *C. arabica* | *I. micheliana* | (1, 314) | 0.1464 | 0.7022 |
| *C. arabica* | *A. latifolia* | (1, 314) | 6.118 | 0.0139 |
| *I. micheliana* | *C. arabica* | (1, 314.9) | 0.0407 | 0.8401 |
| *I. micheliana* | *A. latifolia* | (1, 314.7) | 0.6600 | 0.4172 |
| *A. latifolia* | *C. arabica* | (1, 314.9) | 0.0273 | 0.8688 |
| *A. latifolia* | *I. micheliana* | (1, 315.3) | 0.9251 | 0.3369 |

## Appendix C: Supplementary Tables and Figures for Chapter 5

**Table C5.1** Ants sampled in pitfall traps and at tuna baits. The samples categorized as "unknown" were too damaged for identification. The reference collection is available at the University of Michigan (Ann Arbor, MI, USA).

| Subfamily | Genus | Morphospecies | Pitfalls | | Tuna Baits | |
|---|---|---|---|---|---|---|
| | | | *A. sericeasur* | No *A. sericeasur* | *A. sericeasur* | No *A. sericeasur* |
| **Agroecomyrmecinae** | *Tatuidris* | *tatusia* | 0 | 1 | 1 | 1 |
| **Dolichoderinae** | *Azteca* | *sericeasur* | 430 | 0 | 44 | 0 |
| | *Dolichoderus* | *lutosus* | 0 | 2 | 0 | 0 |
| **Dorylinae** | *Neocerapachys* | sp1 | 0 | 3 | 0 | 0 |
| | *Cheliomyrmex* | *morosus* | 0 | 123 | 0 | 0 |
| | *Eciton* | sp1 | 0 | 3 | 0 | 0 |
| | *Labidus* | *coecus* | 641 | 70 | 0 | 0 |
| **Formicinae** | *Brachymyrmex* | sp1 | 1 | 0 | 2 | 4 |
| | *Camponotus* | *festinatus* | 0 | 1 | 0 | 0 |
| | *Camponotus* | sp1 | 0 | 0 | 0 | 4 |
| | *Colobopsis* | *abditus* | 1 | 21 | 0 | 0 |
| | *Nylanderia* | sp1 | 1 | 1 | 0 | 0 |
| | *Nylanderia* | sp2 | 3 | 0 | 0 | 0 |
| | *Nylanderia* | sp3 | 1 | 0 | 0 | 0 |
| **Ectatomminae** | *Gnamptogenys* | *regularis* | 65 | 27 | 0 | 0 |
| | *Gnamptogenys* | *striatula* | 127 | 85 | 0 | 0 |
| | *Gnamptogenys* | sp1 | 0 | 0 | 39 | 42 |
| **Myrmicinae** | *Acromyrmex* | *coronatus* | 0 | 1 | 0 | 0 |
| | *Acromyrmex* | *versicolor* | 0 | 1 | 0 | 0 |
| | *Adelomyrmex* | sp1 | 1 | 0 | 0 | 0 |
| | *Apterostigma* | sp1 | 0 | 15 | 0 | 0 |
| | *Apterostigma* | sp2 | 3 | 0 | 0 | 0 |
| | *Carebara* | sp1 | 1 | 0 | 0 | 0 |
| | *Cephalotes* | sp1 | 1 | 0 | 0 | 0 |
| | *Cephalotes* | sp1 | 0 | 0 | 2 | 0 |
| | *Cyphomyrmex* | sp1 | 2 | 1 | 0 | 1 |
| | *Eurhopalothrix* | sp1 | 3 | 10 | 0 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Mycetomoellerius* | sp1 | 0 | 3 | 0 | 0 |
| | *Myrmicocrypta* | sp1 | 0 | 0 | 4 | 2 |
| | *Paratrachymyrmex* | sp1 | 3 | 3 | 0 | 0 |
| | *Pheidole* | *protensa* | 315 | 364 | 175 | 167 |
| | *Pheidole* | *simonsi* | 1 | 0 | 0 | 0 |
| | *Pheidole* | sp1 | 0 | 0 | 19 | 36 |
| | *Pheidole* | sp2 | 10 | 9 | 6 | 5 |
| | *Pheidole* | sp3 | 1 | 6 | 3 | 7 |
| | *Pheidole* | sp4 | 3 | 11 | 0 | 7 |
| | *Pheidole* | sp5 | 0 | 0 | 13 | 21 |
| | *Pheidole* | sp6 | 0 | 0 | 14 | 15 |
| | *Pheidole* | sp7 | 0 | 0 | 6 | 12 |
| | *Pheidole* | sp8 | 6 | 0 | 0 | 0 |
| | *Pheidole* | sp9 | 0 | 0 | 21 | 13 |
| | *Pheidole* | sp10 | 0 | 0 | 15 | 20 |
| | *Pheidole* | sp11 | 0 | 0 | 0 | 5 |
| | *Pheidole* | sp12 | 0 | 0 | 3 | 1 |
| | *Pheidole* | sp13 | 1 | 0 | 0 | 0 |
| | *Pheidole* | sp14 | 3 | 2 | 0 | 0 |
| | *Pheidole* | sp15 | 12 | 2 | 0 | 0 |
| | *Pheidole* | sp16 | 0 | 0 | 5 | 4 |
| | *Pheidole* | sp17 | 0 | 0 | 27 | 15 |
| | *Pheidole* | sp18 | 0 | 0 | 1 | 0 |
| | *Pheidole* | sp19 | 0 | 0 | 1 | 0 |
| | *Pheidole* | sp20 | 0 | 3 | 0 | 0 |
| | *Pheidole* | sp21 | 7 | 2 | 0 | 0 |
| | *Pheidole* | sp22 | 23 | 17 | 0 | 0 |
| | *Pheidole* | sp23 | 1 | 2 | 12 | 20 |
| | *Rogeria* | sp1 | 0 | 0 | 2 | 2 |
| | *Solenopsis* | *picea* | 0 | 14 | 0 | 0 |
| | *Solenopsis* | *geminata* | 72 | 97 | 0 | 0 |
| | *Solenopsis* | *aurea* | 0 | 12 | 0 | 0 |
| | *Solenopsis* | sp1 | 6 | 1 | 1 | 9 |
| | *Solenopsis* | sp2 | 18 | 13 | 0 | 0 |
| | *Solenopsis* | sp3 | 1 | 0 | 0 | 0 |
| | *Solenopsis* | sp4 | 0 | 0 | 7 | 18 |
| | *Stenamma* | sp1 | 0 | 3 | 0 | 0 |
| | *Strumigenys* | *gundlachi* | 1 | 1 | 0 | 0 |
| | *Wasmannia* | *auropunctata* | 0 | 1 | 0 | 0 |
| **Ponerinae** | *Cryptopone* | *gilva* | 1 | 0 | 0 | 0 |
| | *Cryptopone* | sp1 | 0 | 0 | 1 | 0 |
| | *Hypoponera* | *nitidula* | 2 | 6 | 0 | 0 |
| | *Hypoponera* | *opacior* | 0 | 0 | 0 | 2 |
| | *Hypoponera* | sp1 | 3 | 1 | 0 | 0 |
| | *Leptogenys* | sp1 | 1 | 16 | 0 | 1 |
| | *Neoponera* | *apicalis* | 1 | 0 | 0 | 0 |
| | *Neoponera* | *villosa* | 1 | 0 | 0 | 0 |
| | *Odontomachus* | *laticeps* | 19 | 43 | 0 | 0 |
| | *Odontomachus* | *meinerti* | 3 | 0 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Odontomachus* | sp1 | 0 | 0 | 20 | 24 |
| | *Pachycondyla* | *harpax* | 11 | 12 | 0 | 0 |
| | *Pachycondyla* | *impressa* | 0 | 12 | 0 | 0 |
| | *Pachycondyla* | sp1 | 1 | 1 | 0 | 2 |
| | *Pseudoponera* | *cognata* | 1 | 1 | 0 | 0 |
| **Pseudomyrmecinae** | *Pseudomyrmex* | *gracilis* | 0 | 1 | 0 | 0 |
| | *Pseudomyrmex* | *boopis* | 0 | 1 | 0 | 0 |
| **Unknown** | | | 45 | 117 | 0 | 0 |

**Table C5.2** Mean abundance and standard deviation for organisms in pitfall samples. Data show here is divided by treatment (presence of *A. sericeasur* nest) and distance from the focal tree (0.5 and 2 m).

| | *A. sericeasur* (0.5 m) | | *A. sericeasur* (2 m) | | No *A. sericeasur* (0.5 m) | | No *A. sericeasur* (2 m) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std dev | Mean | Std dev | Mean | Std dev | Mean | Std dev |
| Non-formicid hymenoptera | 3.1 | 2.5 | 3.7 | 7.5 | 3.0 | 2.4 | 2.8 | 2.6 |
| Formicid hymenoptera | 66.1 | 59.4 | 26.7 | 22.2 | 29.4 | 30.4 | 28.8 | 27.1 |
| Diptera | 28.8 | 21.1 | 18.9 | 15.2 | 20.1 | 15.1 | 23.6 | 22.0 |
| Colleoptera | 18.5 | 14.7 | 13.8 | 5.9 | 15.0 | 7.2 | 12.6 | 11.5 |
| Orthoptera | 4.5 | 3.4 | 6.8 | 6.7 | 4.5 | 3.9 | 6.9 | 4.2 |
| Arachnida | 5.5 | 3.9 | 4.0 | 4.0 | 5.0 | 3.3 | 6.2 | 5.7 |
| Hemiptera | 1.3 | 1.9 | 1.4 | 1.7 | 2.0 | 2.4 | 1.4 | 1.8 |
| Collembola | 64.1 | 47.0 | 63.1 | 56.2 | 63.5 | 49.6 | 73.8 | 65.4 |
| Isopoda | 0.15 | 0.47 | 0.1 | 0.31 | 0.3 | 0.73 | 0.3 | 0.80 |
| Other | 2.0 | 1.8 | 1.4 | 1.6 | 11.9 | 44.8 | 5.8 | 17.3 |
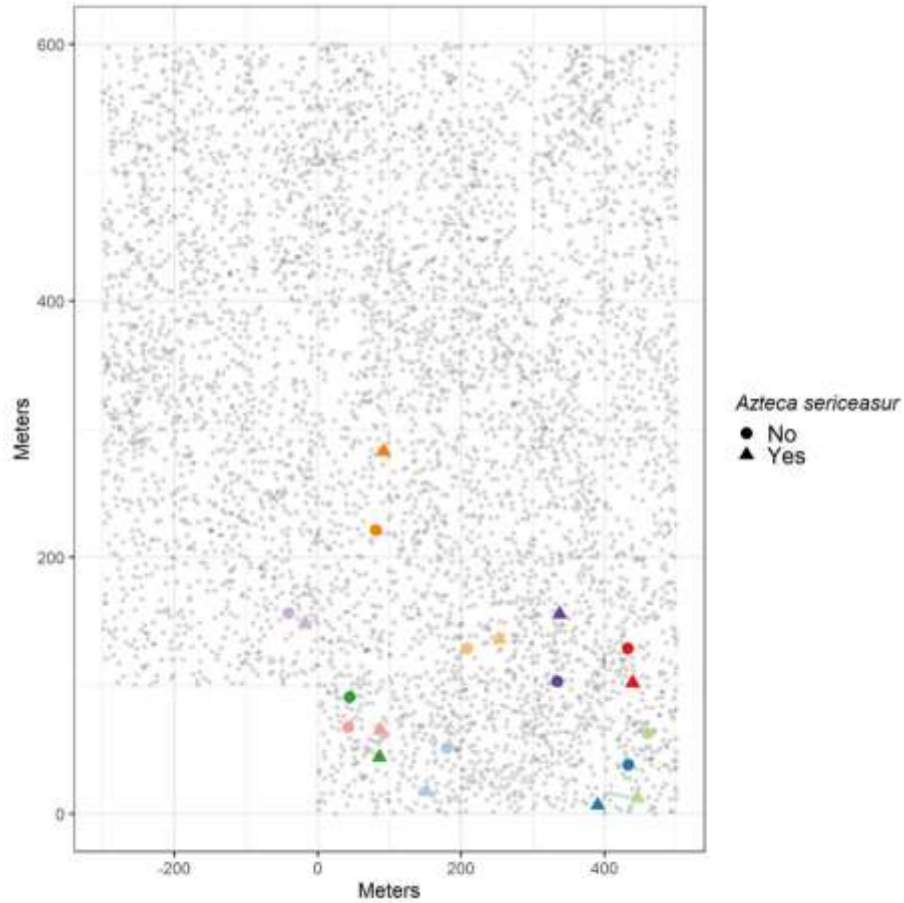
**Figure C5.1** Map of the 45 ha. plot where sampling took place. Each gray dot is an *I. micheliana* shade tree in the plot; shade trees of other species are not shown here. The paired sites, which were sampled on the same day, are denoted with different colors. There are a total of 10 locations; each location has one focal tree with *A. sericeasur* (indicated with a triangle) and one focal tree without *A. sericeasur* (indicated with a circle).
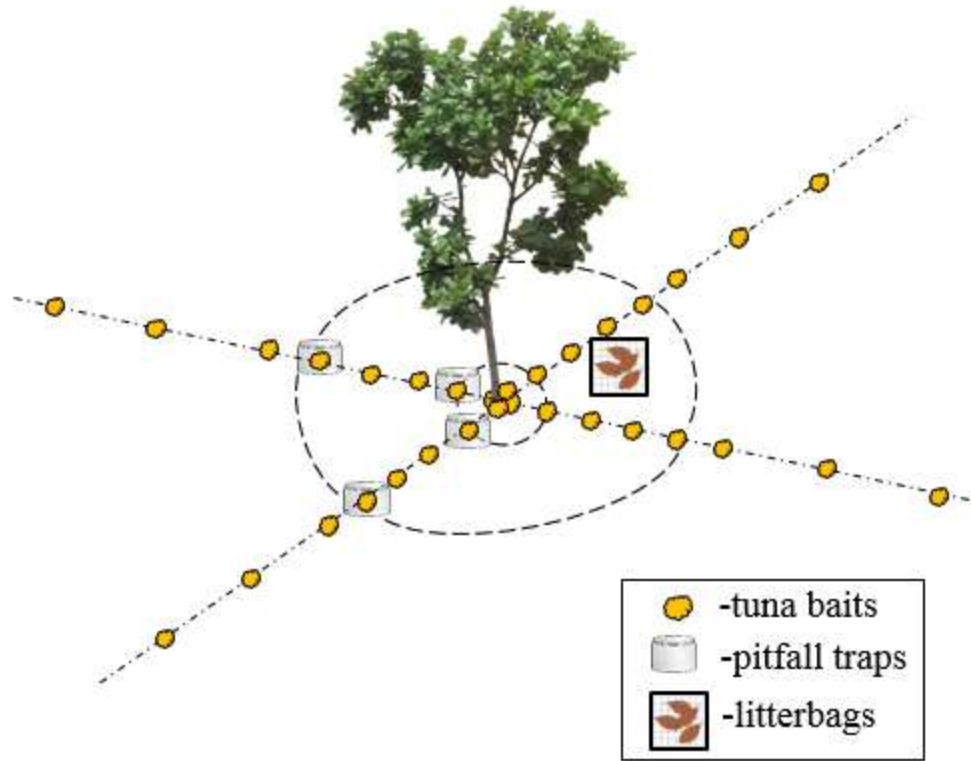
**Figure C5.2** Illustration of our sampling design, which includes tuna baits (0, 0.5, 1, 1,5, 2, 2.5, 3.5 and 4.5 m), pitfall traps (0.5 and 2 m) and litterbags (1 m). The inner dotted ring represents a 0.5 m radius and the outer ring a 2 m radius. Our sampling design was oriented at an *Inga micheliana* shade tree, shown at the center of the figure.