Effects of Quality of Leaf Litter on Ant Assemblages in a Shade-Grown Coffee Agroecosystem in Chiapas, Mexico

by

Bolívar Alexander Aponte Rolón

A thesis submitted in partial fulfillment
Of the requirements for the degree of Master's in Science
(School for Environment and Sustainability)
in the University of Michigan 2018

Thesis committee:

    Professor Ivette Perfecto, advisor

    Professor John Vandermeer, co-advisor

    Assistant Professor Jennifer Blesh

Abstract

Shade trees can provide important economic benefits by supporting productivity in coffee agroforestry systems through processes such as biological nitrogen fixation. Less is known, however, about the benefits shade trees offer to above- and belowground communities in coffee agroecosystems. A useful lens to evaluate the ecological benefits of shade trees is to assess how leaf-litter ant and detritivore communities respond to the quality of leaf-litter from established nitrogen-fixing tree species, such as *Inga micheliana*, and non-nitrogen fixing species, such as *Alchornea latifolia*, commonly planted in coffee agroecosystems. In this study we set out to answer the following questions: 1) how do C:N ratio differ between *I. micheliana* and *A. latifolia*, and 2) how do leaf-litter ant communities differ between low quality leaf litter (i.e. high C:N ratio) and high quality leaf litter (i.e. low C:N ratio). Twenty-eight randomly selected sites (14 *I. micheliana*; 14 *A. latifolia*) were established within a 45-hectare plot in a shaded organic coffee farm in Chiapas, Mexico. Three $1m^2$ quadrats within a 5m radius from the base of the selected trees were established and the leaf litter within the quadrants was removed and sieved. Ant specimens were extracted from leaf litter collected from quadrats using the mini-Winkler method and identified to genus and species or morphospecies level. Results indicate that *I. micheliana*, the nitrogen-fixing species, has a lower C:N ratio than *A. latifolia*. However, in spite of the differences in C:N ratios, no significant difference in abundance and richness of ant were detected. Results suggests that there may be unaccounted feedbacks from nitrogen and non-nitrogen fixing vegetation to brown food webs enabling them to sustain similar leaf-litter ant communities.

Table of Contents

Table of Figures and Tables

1. Introduction

Coffee farming constitutes the main source of income for millions of traditional farmers and serves as habitat for biodiversity (Perfecto et al. 2014, Perfecto and Vandermeer 2015). Coffee agroecosystems range from low-management (shade-grown) styles that promote high levels of biodiversity (Philpott et al. 2008a) to intensified agricultural plantations (sun-coffee) that result in low-biodiversity levels (Perfecto et al. 1996, Armbrecht et al. 2005). Scientific ecological literature in the last two decades has shown that shaded-coffee farms provide habitat for a wide variety of biodiversity (Perfecto et al. 1996, Moguel and Toledo 1999, Mas and Dietsch 2003), while coinciding geographically with biodiversity hotspots around the world (Moguel and Toledo, 1999; Hardner and Rice, 2002; Valencia et al., 2015). Planned biodiversity (e.g. coffee plants and shade trees) provides producers with direct sources of income, and, in conjunction with associated biodiversity, diverse coffee agroecosystems facilitate a set of valuable ecosystems services (e.g. biological pest control) and ecosystem functions (e.g. formation of soil organic matter) (Moguel and Toledo 1999, Tscharntke et al. 2005, Jha et al. 2011, Barnes et al. 2017).

Shade trees play an important role in coffee agroecosystems. By providing shade they maintain soil moisture, control weeds and reduce the probability of pest breakouts (Soto-Pinto et al. 2002, Morris and Perfecto 2016). Trees are also a good source of fuelwood and construction material (Peeters et al. 2003, García-Barrios et al. 2009, Valencia et al. 2014b). Tree species composition varies widely and it is highly dependent on the farmer's preference and management practice (Valencia et al. 2014c). Moreover, tropical soils are generally nutrient poor and have a low cation exchange capacity (Grubb 1995). In Central America, farmers managing shaded coffee agroecosystems address soil fertility issues by selecting nitrogen fixing trees,

particularly those from the *Inga* genus (Romero-Alvarado et al. 2002, Valencia et al. 2014, but see also Grossman et al. 2006), though this varies according to region and country. Non-N-fixing trees are also common given their multiples uses, (e.g. timber, fruits, light shade, etc.) (Peeters et al. 2003). Extensive efforts have been directed at understanding the role that N-fixing trees play in coffee production and maintenance of biodiversity, nonetheless many of these studies have focused on above-ground interactions (Philpott et al. 2004, Philpott and Bichier 2012, Hajian-Forooshani et al. 2016, Barrios et al. 2018). Less is known about how N-fixing trees influence below-ground food webs in coffee agroecosystems.

Due to the capacity of species in the genus *Inga* for biological nitrogen fixation (BNF) (Pennington et al. 1997) the trees generally have lower C:N ratios in their foliage, which produce high quality leaf-litter and have the potential to accelerate loss of lignin and soluble C (Talbot and Treseder 2012). High quality leaf-litter, either due to the ontogeny of leaves (i.e. C:N ratio) or addition of resources (e.g. necromass) can increase microbial activity and accelerate decomposition (Zhang and Zak 1995, Shik and Kaspari 2010, Talbot and Treseder 2012, Clay et al. 2013). Carbon to nitrogen ratio has been shown to alter microbial communities and affect potential synergisms in decompositions rates (Chapman et al. 2013), ultimately influencing nutrient cycling. Changes in microbial communities due to nutrient availability (e.g. C:N ratio) could potentially lead to increases in decomposition rates, which in turn can cause the loss of habitat space for soil arthropods (Shik and Kaspari 2010). While past studies have shown the effects that C:N ratio has on microbial communities and feedback loops between these (Beare et al. 1992, Coleman 2011) how C:N ratio in leaf-litter may influence soil arthropod communities, especially leaf-litter ant communities, remains elusive.

In this study, we investigate how leaf-litter from *Inga micheliana* (a N-fixing species) and *Alchornea latifolia* (a non-N-fixing species) influence leaf-litter ant communities. Ants (Hymenoptera: Formicidae) are highly abundant and diverse in tropical ecosystems (Hölldobler and Wilson 1990) and are regularly used to assess ecosystem responses to land management (Offenberg 2015). They also serve as ecosystem engineers (e.g. through soil bioturbation) (Bruyn 1999, Nkem et al. 2000, Vandermeer and Perfecto 2007) biocontrol agents (Perfecto and Castiñeiras 1998, Philpott and Armbrecht 2006, Vandermeer et al. 2010, Morris and Perfecto 2016) , and are good indicators of ecosystem health (Gero 2010). Ants respond to changes in local factors such as tree species richness, tree abundance and leaf-litter biomass (Armbrecht et al. 2005, Philpott and Armbrecht 2006, de la Mora et al. 2013). Shaded coffee farms have been shown to support a high diversity of ants and other leaf-litter arthropods (Perfecto et al. 1996, 2014, Philpott et al. 2004). However, little is known about how leaf-litter ant species, which range from generalist to highly specialized predator and even cryptic herbivores (Roeder and Kaspari 2017) respond to changes in C:N ratio from leaf litter in tropical agroecosystems (Philpott and Armbrecht 2006).

More specifically, here, we examined the abundance, richness and community composition of leaf-litter ant communities in leaf-litter from *I. micheliana* and *A. latifolia* in a shaded-coffee agroecosystem. Since the *I. micheliana* carries out biological nitrogen fixation (Pennington et al. 1997, Romero-Alvarado et al. 2002) we would expect a lower C:N ratio in the leaf-litter from it, as compared to *A. latifolia* that does not fix nitrogen. We specifically asked: (1) How does the C:N ratio vary between *I. micheliana* and *A. latifolia*? (2) How does C:N ratio from leaf-litter affects leaf-litter ant abundance, richness and community composition? (3) Are there any other environmental or site factors associated with the shade trees that affect ant

abundance, species richness and composition? We hypothesized that *I. micheliana* would have lower C:N ratio as compared to *A. latifolia* given its BNF capacity, and that increased nutrient availability influence leaf-litter ant abundance and richness, where ant abundance and richness would be greater in leaf-litter with lower C:N ratio.

2. Methods

2.1. Study site

We collected samples from a 45-hectare plot in *Finca Irlanda,* an organic shaded coffee farm in the Soconusco region in Chiapas, Mexico, located approximately 92º20´29´´W and 15º10´6´´N. Altitude in the plot ranges from 900-1200 m.a.s.l. (Li et al 2016). The region is characterized by two distinct seasons; a wet season from mid- late May to October and a dry season from November to April. Mean annual rainfall is 4500mm (De la Mora et al., 2013b; Philpott et al., 2008b)

2.2. Local site selection and environmental factors

To avoid potential competitive exclusion of leaf-litter ants (Ennis 2010) by keystone ant species, *Azteca sericeasur*, selected trees had to meet the following criteria: 1) free of *A. sericeasur* nests for the last 3 years; 2) neighboring trees within a 10m radius had to be free of *A. sericeasur*; 3) the pair trees had to be a minimum of 10m apart and a maximum of 100m from each other (*as per* de la Mora et al., 2013b); 4) diameter at breast height had to be equal or greater than 30 cm. Using the georeferenced map of the 45-hectare plot in *Finca Irlanda*, we selected 28 sampling sites: 14 of *Inga micheliana* tree, the most abundant N- fixing species, and 14 of *Alchornea latifolia* tree, the most abundant non-N- fixing species (Li et al., 2016). At each tree we set four 5 m transects from the base of the tree in a cross shape following cardinal

directions. Along the NS transect three 1 m^2 quadrats were established: one at the base of the tree and two at 5 m from the base of the tree. All transects were used to measure local environmental site factors (e.g. leaf-litter depth).

Local environmental site factor information was collected for each tree site to capture potential effects on ant abundance, richness and species composition. A total of 13 local factors were measured: diameter at breast height (DBH), number of coffee plants within the four 5 m transects, average leaf-litter depth (mm) within all 5 m transects, leaf-litter depth (mm) of within 1 m2 quadrat, slope cardinality (º), altitude (m), distance to edge (m) (edge of trails and roads in coffee farm), average percent canopy cover, pH, percent soil humidity, total percent N, total percent C and C:N ratio. Measurements of leaf-litter depth were taken at base of the tree, 2.5m and 5m from the base of the tree in all four cardinal directions. Leaf-litter depth in 1 m2 quadrats was measured at center and all four corners. Both, measuring along transects and within quadrats provided a measure of the mean leaf-litter volume under the tree canopy. Slope and altitude of sites was determined with a Garmin72h model (www.garmin.com). To determine average percent canopy cover, four measurements in cardinal directions were taken with Spherical Crown Densiometer, Model A (Forestry Suppliers, Inc.) at base of the tree. Average soil pH was determined by colorimetric method (Lovibond Soil pH Test Kit, www.forestry-suppliers.com) by taking one soil sample at each of the three 1 m^2 quadrats that were established within the NS transect of selected trees for arthropod extraction. Soil humidity was assessed through the gravimetric method by taking 4-6 grams of soil from each of the 1m^2 quadrats in NS transect of selected trees. Soil samples were dried for a minimum of 72 hours at 50 ºC until no further mass loss was recorded. An additional leaf-litter 1m^2 quadrat was collected for chemical analysis, which was conducted at the University of Michigan (UM).

2.3. Ant sampling protocol

Leaf litter was collected from the three 1m^2 quadrants established at each tree and sifted from these quadrats using the Winkler method (Agosti & Alonso 2000). Sifted detritus was put in mini-Winkler extractors and left for 72 hours to collect ants and other leaf-litter arthropods in containers with 70% ethyl alcohol. Mini-Winkler extractors were equipped with a 50-75W incandescent light bulb to increase extraction efficiency. Ants were separated from the other arthropods and organisms and placed in vials with 70% ethyl alcohol for further identification. Specimens were identified to species and morphospecies level using the "Identification Guide to the Ant Genera of the World" (Bolton 1994).

2.4. Leaf-litter chemical analyses: C:N ratio

Leaf-litter collected at additional quadrat was weighted and dried for a minimum of 72 hrs at 50ºC until no mass loss was recorded. Subsequently, three 20 g subsamples were taken and brought to the University of Michigan, Ann Arbor, MI. The samples were grinded using a standard coffee grinder at the finest setting (Krups brand) until a fine powder form was obtained. Subsamples were analyzed for total C and total N content using a LECO Trumac CN combustion analyzer (LECO Corporation, 3000 Lakeview Avenue, Saint Joseph, MI 49085).

2.5. Data analyses

    2.5.1. Predicting ant abundance and richness

We computed a correlation matrix to examine how local environmental factors affected ant abundance and diversity and checked for multicollinearity among predictor variables. Highly correlated predictor variables: percent canopy cover, leaf-litter within 1 m2 quadrats and number of coffee plants within 5 m transects had correlation coefficient greater than 0.25 and were not

included in subsequent analyses. Principal Component Analysis (PCA) was used to evaluate underlying interactions between local factors that could potentially influence ant community composition. The PCA was computed with the default 'princomp' function in R software (R Core Team, 2013). To compute the PCA, the remaining 10 local environmental site factors were utilized: diameter at breast height (DBH), altitude (m.a.s.l.), slope cardinality, distance to edge (m), leaf-litter depth (mm), pH, per cent humidity, total percent N, total percent C and C:N ratio. Although, total percent N was highly correlated with C:N ratio, we still considered it relevant as it has been reported to be a significantly positive predictor of leaf-litter predators (Kaspari and Yanoviak 2009). To further investigate how local environmental factors, affect relative abundance and richness, we computed a Principal Component Regression (PCR) with the loadings from PCA that explained the most variance in the data. To test the significance of the effect of leaf-litter type on ant abundance and richness, we computed two generalized linear mixed model (GLMM) with a log transformation for ant abundance and a Poisson distribution for species richness. The same 10 local factors were set as fixed effects and sampling date and site identification was set as random effect in the models. Insignificant variables were removed stepwise through manual backward elimination until we reached a model that maximized model fit according to the number of significant variables and AIC values. GLMMs were computed with 'glmer' function in 'lme4' package in R software (R Core Team, 2013). Model R-squared values where computed with "MuMin" package in R software. An outlier *A. latifolia* site, with more than 2000 ant individuals was removed from analyses. Because all sites were uniquely paired, the corresponding *I. micheliana* was also removed from all analyses. For reported analyses we used a total of 26 sites (13 *I. micheliana* and 13 *A. latifolia*). Values of local

environmental site factors were tested with Shapiro-Wilk normality tests and all are normally distributed.

2.5.2. *Ant Species Richness and Community composition:*

We constructed species accumulations curves for observed species richness (SACs) with 95% confidence intervals (CI) using second order jackknife using "vegan" package in R software (R Core Team, 2013). Additionally, for the assessment of ant species composition and diversity, we first, calculated similarity matrix by using a Bray-Curtis similarity index conducted with 10,000 permutations. Second, we computed a non-metric multidimensional scaling (NMDS) to represent the rank order of similarity values from the Bray-Curtis similarity index. The NMDS was computed by creating a matrix of the abundance of each species in each treatment type. Finally, we used an Analysis of Similarities (ANOSIM) to statistically compare the similarities of species found at each treatment type site. The ANOSIM provides the benefit of producing a global P value to determine whether species composition from treatment types differ, while also conducting pairwise comparison between sites. All analyses for species composition were computed with 'vegan' package in R software (R Core Team, 2013). Abundance and richness values were tested with Shapiro-Wilk normality tests and meet parametric assumptions. Finally, mean ant abundance and richness was tested for significant differences between treatments using paired student T-tests (R Core Team, 2013), where ant abundance is the count of individuals and richness is the number of unique species.

3. Results

3.1. Site characteristics

Several local environmental factors were found to be significantly different between *I. micheliana* and *A. latifolia* sites (Table 1). DBH, soil pH, and C:N ratio were all found to be significantly greater under *A. latifolia* than *I. micheliana*, while total percent N was greater under the *I. micheliana* trees (Fig. 1 & Table 1). Tree sites did not differ for any of the other measured local environmental factors.
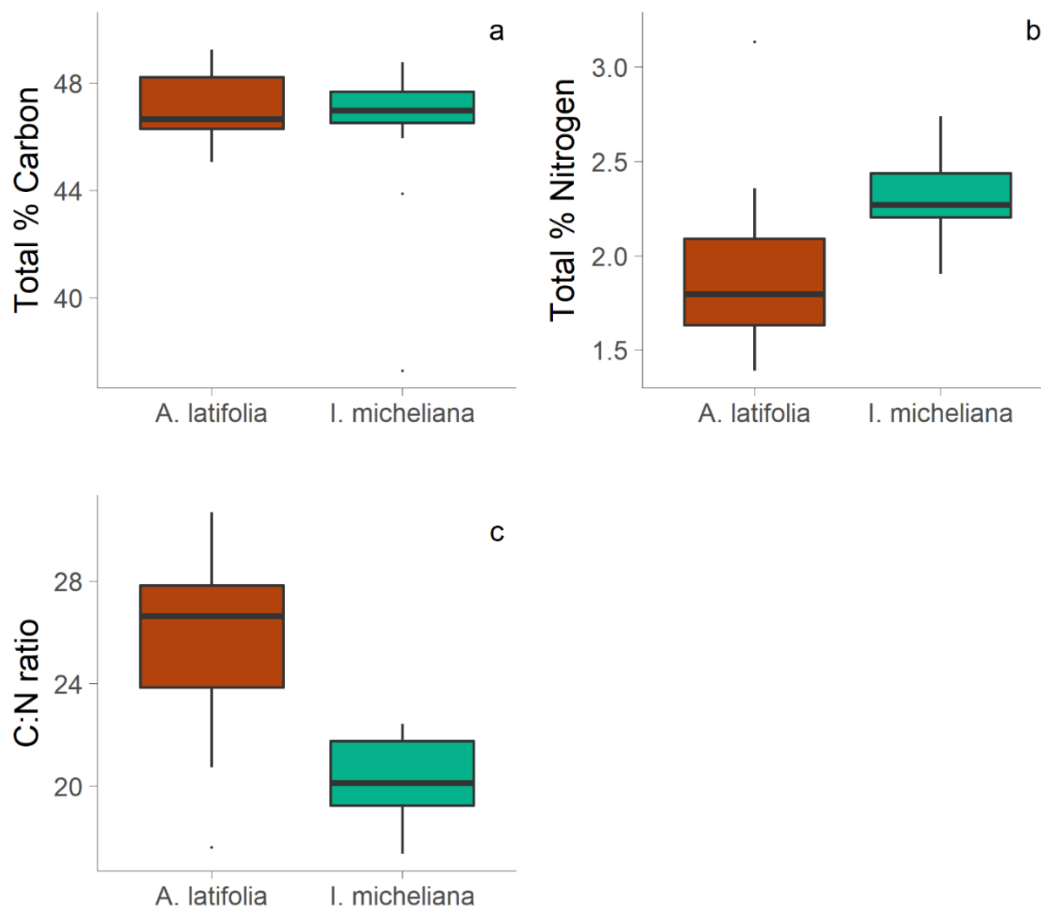

Figure 1 a) Total percent carbon (Paired t-test, p-value = 0.38); b) total percent nitrogen (paired t-test, p-value = 0.001); and c) C:N ratio of leaf-litter from *I. micheliana* and *A. latifolia* (Paired t-test, p-value < 0.001).

Table 1 Mean (± SE) values of local characteristics at I. micheliana and A. latifolia sites in coffee agroecosystem in Chiapas, Mexico.

Site characteristics	*Inga micheliana*	*Alchornea latifolia*	T-statistic	p-value
DBH (cm)	**102.59 ± 6.25ᵃ**	**188.39 ± 10.75ᵇ**	**-6.76**	**<0.001**
Canopy cover (%)	66.85 ± 9.09	72.00 ± 7.34	-0.44	0.33
Coffee plants	30.07 ± 2.91	23.15 ± 3.28	1.52	0.15
Altitude (m)	1039.77 ± 8.14	1032.39 ± 4.64	0.75	0.47
Slope cardinality	194.39 ± 26.13	224.00 ± 26.79	-0.97	0.35
Distance to edge (m)	12.71 ± 2.13	17.54 ± 4.65	-0.79	0.22
Leaf-litter depth (mm)	53.07 ± 5.68	58.95 ± 6.59	-0.933	0.18
Leaf-litter 1m² quadrat (mm)	55.41 ± 3.02	59.78 ± 6.46	-0.67	0.51
Soil pH	**6.00 ± 0.09ᵃ**	**6.36 ± 0.11ᵇ**	**-1.95**	**0.04**
Soil humidity (%)	80.95 ± 9.82	80.51 ± 4.17	0.04	0.52
C:N ratio	**20.27 ± 0.45ᵃ**	**25.28 ± 0.99ᵇ**	**-6.47**	**<0.001**
Total % Nitrogen	**2.31 ± 0.06ᵃ**	**1.92 ± 0.13ᵇ**	**3.83**	**0.001**
Total % Carbon	46.28 ± 0.83	47.08 ± 0.35	-0.91	0.19

Values show mean and standard error. Small letters (a, b) show significant differences between treatments and * denotes degree of significance.

The Principal Component Analysis (PCA) computed with local environmental factors: altitude, aspect, DBH, distance to edge, leaf-litter depth, percent canopy, soil humidity, soil pH, total percent carbon, total percent nitrogen and C:N ratio separated sampling sites into two groups, nonetheless, with a considerable overlap of confidence intervals (Fig. 2). Our PC1 explains 25% of the variance in local environmental factors and was positively correlated with DBH, distance to edge, leaf-litter depth, slope cardinality, soil humidity, soil pH, total percent carbon and C:N ratio and negatively correlated with altitude and total percent nitrogen. Total percent nitrogen and C:N ratio are the local environmental factors that contribute the most to PC1 and are inversely related. The PC2 explains 18% of the variance, it is negatively correlated with slope cardinality, distance to edge, soil humidity, total percent carbon and C:N ratio and positively correlated with DBH, leaf-litter depth, soil pH, altitude and total percent nitrogen (Fig. 2). On the PC2, the local environmental factors that contribute the most are total percent carbon and soil pH.

The Principal Component Regression (PCR) computed from PCA loadings shows no significant correlation between PC1 or PC2 and either relative abundance or species richness of leaf-litter ants (PC1 vs. Abundance: y = 252.85 -7.12x, R^2: 0.005, p-value: 0.71; PC1 vs. Richness: y = 20.77 + 0.17x, R^2: 0.001, p-value: 0.87; PC2 vs. Abundance: y = 252.85 -6.05x, R^2: 0.003, p-value: 0.79; PC2 vs. Richness: y = 20.77 -0.34x, R^2: 0.003, p-value: 0.77).
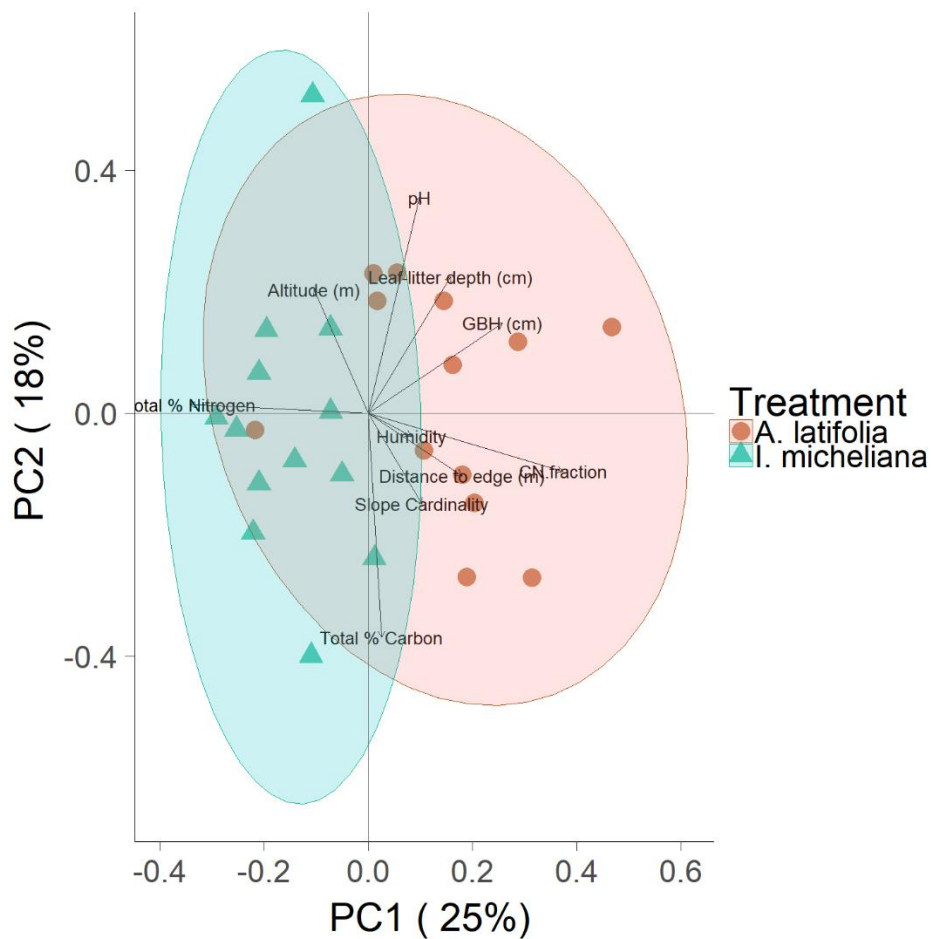
Figure 2 Principal component analysis of leaf-litter ant communities in leaf-litter from *A. latifolia* and *I. micheliana* and local factors: altitude, aspect, CBH, distance to edge, leaf-litter depth, percent canopy, soil humidity, soil pH, total percent carbon, total percent nitrogen and C:N ratio.

3.2. Site effects on ant abundance, richness and species composition

We sampled a total of 6,574 ant individuals from 8 subfamilies, 34 genera and 67 morpho-species and species (Table 2). Species accumulation curves indicate that our sampling effort captured the overall richness of leaf-litter ant species (Fig. 3). The overlap of the CIs of the species accumulation curves reveal no significant difference in observed species richness between leaf-litter types.

Figure 3 Species accumulation curves of leaf-litter ant communities under *A. latifolia* and *I. micheliana*. Method shows expected mean species richness.

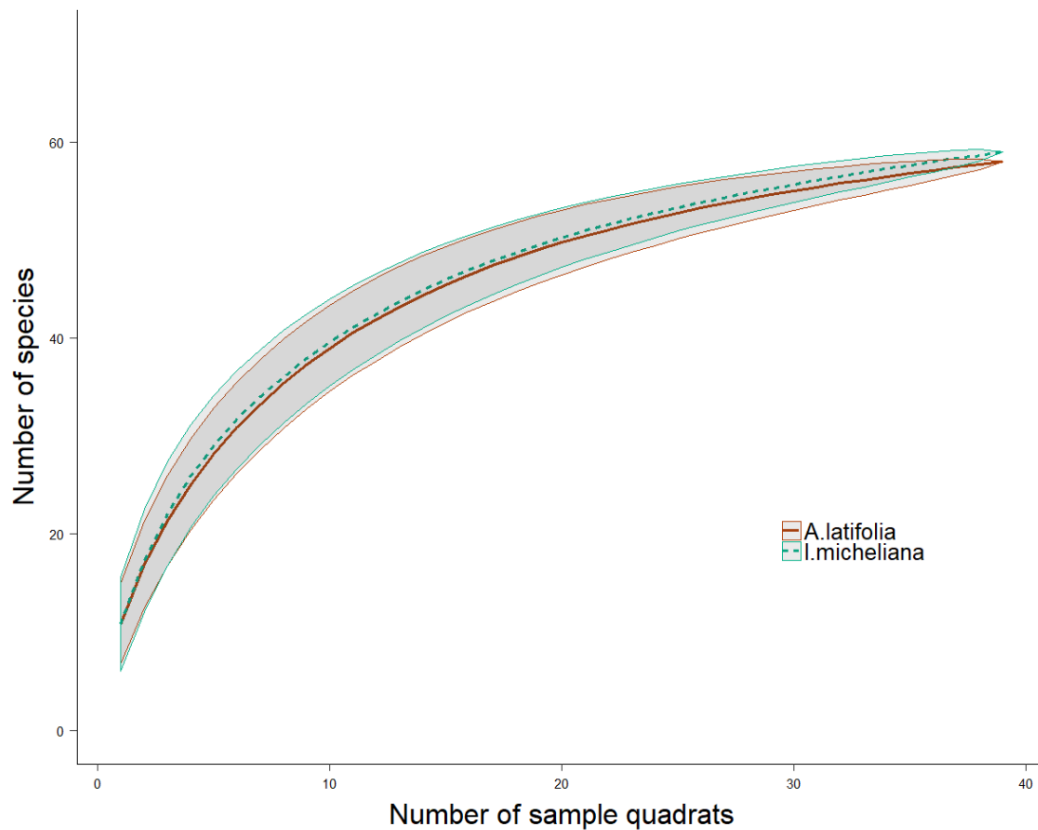**Table 2** Leaf-litter ants sampled in leaf-litter from *I. micheliana* and *A. latifolia* in shaded-coffee agroecosystem in Chiapas, Mexico.

Subfamily	Genus	Species/morphospecies	*I. micheliana*	*A. latifolia*	Shared species
Agroecomyrmecinae	*Tatuidris*	tatusia	18	11	
Dorylinae	*Cerapachys*	sp1biroi	11	8	
Ectatomminae	*Gnamptogenys*	sp1	36	2	
	Gnamptogenys	regularis	20	29	
	Gnamptogenys	striatula	156	105	
	Acropyga	sp1	6	13	
	Brachymyrmex	sp1	2	0	
	Camponotus	sp2s	1	2	
	Nylanderia	sp1	28	4	
	Nylanderia	sp2	92	27	
	Nylanderia	sp3	28	41	
	Nylanderia	sp4	3	6	
Myrmicinae	*Adelomyrmex*	sp1	4	9	
	Apterostigma	sp1	49	16	
	Apterostigma	sp2	2	12	
	Carebara	sp1	16	37	
	Cephalotes	basalis	1	0	
	Crematogaster	sp1	20	0	
	Crematogaster	sp2	16	1	
	Cyphomyrmex	sp1	31	13	
	Eurhophalotrix	sp1	269	245	
	Eurhophalotrix	sp2	172	19	
	Lachnomyrmex	sp1	16	6	
	Mycetosoritis	sp1	1	9	
	Nesomyrmex	sp1	1	1	
	Pheidole	sp1	2	0	
	Pheidole	simonsi	3	3	
	Pheidole	sp2	15	6	
	Pheidole	sp3	37	44	
	Pheidole	protensa	564	353	
	Pheidole	sp5	11	42	
	Pheidole	sp6	14	4	
	Pheidole	sp7	23	52	
	Rogeria	sp2	69	13	
	Rogeria	sp3	0	10	
	Rogeria	sp4	0	4	
	Rogeria	sp5	0	1	
	Solenopsis	geminata	4	6	
	Solenopsis	sp1	115	269	
	Solenopsis	minuscula	31	7	

	Solenopsis	*picea*	309	248	
	Solenopsis	*terricola*	586	924	
	Solenopsis	*zeteki*	307	7	
	Stenamma	sp1	0	13	
	Strumigenys	*biolleyi*	63	38	
	Strumigenys	sp4	24	23	
	Strumigenys	*gundlachi*	110	174	
	Strumigenys	*sazteca*	36	0	
	Trichomyrmex	sp1	0	1	
	Wasnmannia	*aurupuntacta*	0	3	
Ponerinae	*Cryptopone*	sp1	2	3	
	Hypoponera	sp1	7	19	
	Hypoponera	sp2	56	34	
	Hypoponera	*nitidula*	67	99	
	Leptogenys	sp2	16	2	
	Neoponera	*villosa*	1	0	
	Odontomachus	*meinerti*	11	5	
	Odontomachus	*laticeps*	5	5	
	Pachycondyla	*stigma*	0	1	
	Pachycondyla	*cognata*	14	9	
	Pachycondyla	*impressa*	0	1	
	Pachycondyla	*harpax*	4	10	
	Platythyrea	sp1	1	0	
Proceratiinae	*Discothyrea*	sp1	2	0	
	Proceratium	sp1	4	6	
Pseudomyrmecinae	*Pseudomyrmex*	sp1	1	0	
	Psuedomyrmex	*boopis*	5	0	
		TOTAL	**3518**	**3055**	**73 %**

Grey fill in Shared Species column denotes overlap of species.

The graphical representation of species composition from the NMDS shows no distinct leaf-litter ant communities in leaf-litter from *I. micheliana* and *A. latifolia* (Fig. 4). Analysis of similarity (ANOSIM) showed significant similarity in leaf-litter ant species composition between *I. micheliana* and *A. latifolia* sites (Global R = 0.045, p-value: 0.22).
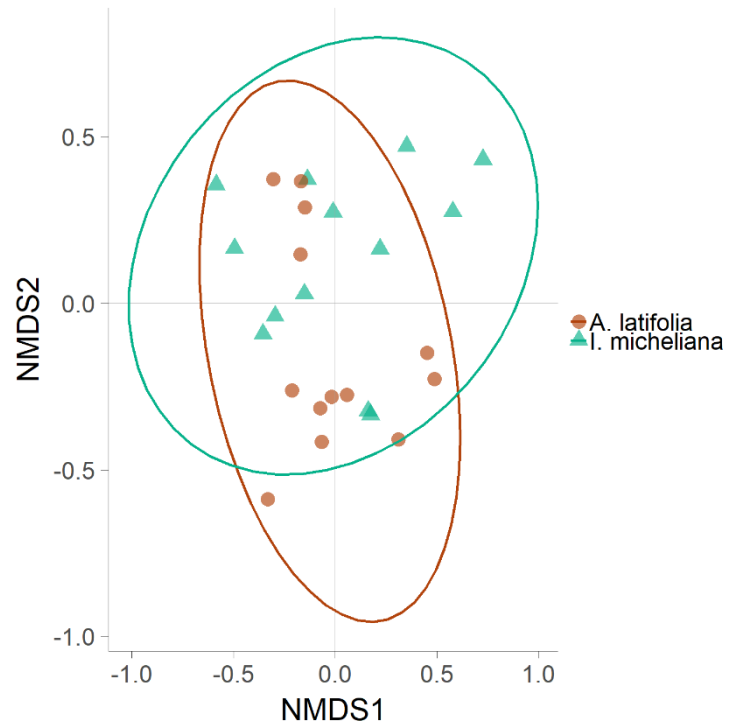

Figure 4. Non-metric multidimensional scaling (NDMS) of species community using distance from Bray-Curtis similarity matrix. Analysis of simmilarity of communities shows no significant difference (Global R = 0.065, P-value: 0.058). Points in brown circles are *A. latifolia and I. micheliana* are in green triangles.

Generalized linear mixed models (GLMM) reveal how significant local environmental factors are for predicting leaf-litter ant abundance and richness (Table 3). The best fit model, based on Akaike's Information Criterion (AIC) value, Model 1, for predicting ant abundance shows 4 of three initial 13 environmental site factors are significant predictors of leaf-litter ant abundance (marginal R^2: 0.35 and conditional R^2: 0.84). Tree species, DBH, and C:N ratio, as significant positive predictor variables, distance to edge is a significant negative predictors of ant abundance (Table 3). Our best-fit model, Model 2, based on AIC value, for predicting ant richness showed only distance to edge and total % Nitrogen as significant negative predictor variables (Table 3) (marginal R^2: 0.22 and conditional R^2: 0.70). Distance to edge is the only local environmental factor that is a significant predictor of both ant abundance and richness. Although significantly negatively correlated to both ant abundance and richness (Table 3).

Table 3 Generalized linear mixed models for predicting ant abundance and richness

Model 1: Abundance	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	2.653967	0.926361	2.865	0.00417 **
Tree species	0.965471	0.293898	3.285	0.00102 **
DBH (cm)	0.006021	0.002378	2.532	0.01134 *
Distance to edge (m)	-0.024668	0.005104	-4.833	1.35e-06 ***
C:N ratio	0.081100	0.027373	2.963	0.00305 **
Model 2: Richness	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	4.055952	0.326889	12.408	<2e-16 ***
Distance to edge (m)	-0.012529	0.004484	-2.794	0.000521**
Total % Nitrogen	-0.395358	0.139821	-2.828	0.000469**

* <0.05, **<0.01, ***<0.001 denotes level of significance.

4   Discussion

Leaf-litter from *I. micheliana* has significantly lower C:N ratio than *A. latifolia* (Fig. 1c,

Table 1). Tree species also differed significantly in DBH, soil pH and total % Nitrogen (Table 1).

These results are supported by the PCA, which shows C:N ratio and % nitrogen as the main

loading factors on the first principal component accounting for the clustering of tree species (Fig.

2). This aligns well with results from other studies that show that N-fixing trees produce leaf-

litter with low C:N ratio that can contribute to greater accumulation of soil organic matter (SOM)

and high inputs of N to soil may result in increased coffee production and agroecosystem

sustainability (Leblanc et al. 2006, López-Rodríguez et al. 2015). However, the differences in

C:N ratio and percent nitrogen detected in this study, although statistically significant, do not

seem to be strong enough to have an overall effect on the ant community, as we hypothesized.

The species accumulation curves show no significant differences in species richness in the leaf

litter ant community under *I. micheliana* versus *A. latifolia* sites (Fig. 3) and the NMDS plot

shows a strong overlap in species composition between the tree species (Fig. 4). On the other

hand, the results from our GLMM model 1 support the hypothesis that C:N ratio in leaf litter is

an important predictor of leaf-litter ant abundance, yet there is no significant difference in leaf-litter and abundance between tree species (data not shown), it is thus possible that the statistical significance in the model is not biologically relevant (Table 3). Our GLMM model 1 highlights tree species, DBH and distance to edge as other local environmental site factors that are significant predictors variables of leaf-litter ant abundance (Table 3). However, in the GLMM model 2 for predicting ant richness only distance to edge and total % Nitrogen are significant negative predictor variables (Table 3). Despite the fact results also show that total % Nitrogen in the leaf litter is significantly different between *I. michelania* and *A. latifolia*, there is no difference in species richness between the tree species (Fig. 3) This could be due to the possibility that the statistical difference in total % Nitrogen between the two species is not enough to be biologically significant (Fig. 1 and Table 3). Murnen et el (2013) report a small increase in colony growth and species richness after adding necromass to leaf litter and increasing its nutrient quality. Higher N content and lower C:N ratio in leaf-litter could potentially increase soil macrofauna abundance and richness, hence increasing availability of resources (e.g. prey) for leaf-litter ants. This pathway could result in the increases in abundance of generalist species (e.g. *Solenopsis* spp.) at the cost of lower species richness.

Distance to edge is a significant predictor variable for ant abundance and richness (Table 3). Here, distance to edge refers to distance to the nearest walking path (1-2 m wide) in the coffee farm, not the edge of a forest or agricultural system like it is commonly utilized (Majer et al. 1997, Silva et al. 2011). The light gap created by trails in the coffee farms has similar effects to gaps in natural forests (Perfecto and Vandermeer 1996, Majer et al. 1997), potentially allowing colonization of niche space and increased food resources (e.g. food disposal by farm workers) (*personal observations* by Aponte-Rolón), for genera like *Solenopsis, Pheidole*, and

Wasmannia auropunctata (Roger 1863), which have a high capacity for recruitment of workers and can built large colonies (Hölldobler and Wilson 1990). However, the presence of these dominant species can have a negative effect on other ants therefore reducing species richness (Ennis and Philpott 2017).

The present results contrast other studies that report a strong correlation of ant abundance and richness with humus mass and leaf-litter depth (Sabu et al. 2008, de la Mora et al. 2013). Rather, our results support reports from Shik and Kaspari et al. (2010) which show no differences in leaf-litter ant abundance or richness in experimental plots. Another study by Kaspari et al. (2010), reported a homogeneous ant richness along a topographic and nutrient gradient (e.g. NPK additions). Similar results were reported by Murnen et al. (2013) when they compare ant communities between forest, sun-coffee and shaded-coffee habitats, and found that habitat type did influence ant abundance and richness, but not food addition. Shik and Kaspari (2010), reported results partially support the "more food, less habitat" hypothesis, where leaf-litter decomposes faster due to increased microbial activity hence preventing observable differences in ant abundance and richness. While this study did not look at differences in decomposition rates of leaf-litter types, there is reason to believe that there are differences in decomposition rates of leaf-litter that could influence leaf-litter ant communities (*unpublished data* L. Schmitt).

Overall we found that the leaf litter produced by *I. michelania* (the N-fixing tree) had a statistically lower C:N ratio and pH and higher percent total nitrogen than *A. latifolia* (the non-N-fixing tree) (Table 1). However, these differences do not seem to be biologically important to distinguish the ant community living in leaf-litter under these species. More specifically, we could not detect any significant differences in the abundance, richness or species composition of

the ant community under these tree species. Nonetheless, using GLMMs we did find that, C:N ratio, total percent nitrogen and pH were significant predictors of ant abundance with abundance increasing with lower C:N ratio, lower pH , and higher percent total nitrogen. Likewise, we found that percent total nitrogen is a significant predictor of species richness, with ant richness declining with higher percent total nitrogen. Finally, distance to edge (i.e. distance to a trail or road) was a significant predictor of both ant species abundance and richness, with ant abundance increasing and richness declining the closest they are to the edge. Although all these variables were found to predict ant species abundance and or richness, we could not detect differences in the leaf-litter ant community below the N-fixing tree versus the non-N-fixing tree. This is likely because the differences in these variables between the two species are not strong enough to be of biological significance and to the fact that other variables that were not significantly different between the two species, like distance to the edge, strongly influence the ant community.

Further studies should focus on manipulative experiments that isolate the effects of leaf-litter type and get at the potential priority effects on ant community composition. As well as others that focus on comparing changes in microbial activity with ant community composition due to leaf-litter type. Results suggest that shaded a coffee agroecosystem can support high levels of ant biodiversity regardless of shade tree species preferred on site. Shade trees have positive effects on ant biodiversity, although the how specific factors contribute to this is not biologically clear.

Literature Cited

Armbrecht, I., L. Rivera, I. Perfecto, U. Valle, and D. De Biolog. 2005. Reduced diversity and complexity in the leaf-litter ant assemblage of Colombian coffee plantations. Conservation Biology 19:897–907.

Barnes, A. D., K. Allen, H. Kreft, M. D. Corre, M. Jochum, E. Veldkamp, Y. Clough, R. Daniel, K. Darras, L. H. Denmead, N. Farikhah Haneda, D. Hertel, A. Knohl, M. M. Kotowska, S. Kurniawan, A. Meijide, K. Rembold, W. Edho Prabowo, D. Schneider, T. Tscharntke, and U. Brose. 2017. Direct and cascading impacts of tropical land-use change on multi-trophic biodiversity. Nature Ecology and Evolution 1:1511–1519.

Barrios, E., V. Valencia, M. Jonsson, A. Brauman, K. Hairiah, P. E. Mortimer, and S. Okubo. 2018. Contribution of trees to the conservation of biodiversity and ecosystem services in agricultural landscapes. International Journal of Biodiversity Science, Ecosystem Services & Management 14:1–16.

Beare, M. H., R. W. Parmelee, P. F. Hendrix, W. Cheng, C. David, D. a Crossley, S. E. Monographs, and N. Dec. 1992. Microbial and Faunal Interactions and Effects on Litter Nitrogen and Decomposition in Agroecosystems. Ecological Monographs 62:569–591.

Bolton, B. 1994. Identification Guide To the Ant Genera of the World. Harvard University Press.

Bruyn, L. A. L. De. 1999. Ants as bioindicators of soil function in rural environments 74:425–441.

Chapman, S. K., G. S. Newman, S. C. Hart, J. A. Schweitzer, and G. W. Koch. 2013. Leaf Litter Mixtures Alter Microbial Community Development: Mechanisms for Non-Additive Effects in Litter Decomposition. PLoS ONE 8.

Clay, N. A., J. Lucas, M. Kaspari, and A. D. Kay. 2013. Manna from heaven: Refuse from an arboreal ant links aboveground and belowground processes in a low land tropical forest. Ecosphere 4:art141.

Coleman, D. C. 2011. Understanding soil processes: One of the last frontiers in biological and ecological research. Australasian Plant Pathology 40:207–214.

Ennis, K. K. 2010. Ground-foraging ant diversity and the role of an aggressive ant (Azteca instabilis) in coffee agroecosystems. University of Michigan.

Ennis, K. K., and S. M. Philpott. 2017. Strong influences of a dominant, ground-nesting ant on recruitment, and establishment of ant colonies and communities. Biotropica 49:521–530.

García-Barrios, L., Y. M. Galván-Miyoshi, I. A. Valsieso-Pérez, O. R. Masera, G. Bocco, and J. Vandermeer. 2009. Neotropical Forest Conservation, Agricultural Intensification, and Rural Out-migration: The Mexican Experience. BioScience 59:863–873.

Gero, B. 2010. Review article Ants and sustainable agriculture . A review 30:191–199.

Grossman, J. M., C. Sheaffer, D. Wyse, B. Bucciarelli, C. Vance, and P. H. Graham. 2006. An assessment of nodulation and nitrogen fixation in inoculated Inga oerstediana, a nitrogen-fixing tree shading organically grown coffee in Chiapas, Mexico. Soil Biology and Biochemistry 38:769–784.

Grubb, P. J. 1995. Mineral Nutrition and Soil Fertility in Tropical Rain Forests. Pages 308–330 *in* A. E. Lugo and C. Lowe, editors.Tropical Forests: Management and Ecology. Springer New York, New York, NY.

Hajian-Forooshani, Z., I. Saraeny, R. Salinas, I. Perfecto, and J. Vandermeer. 2016. Biological Control – Microbials Impact of Regionally Distinct Agroecosystem Communities on the Potential for Autonomous Control of the Coffee Leaf Rust 0:1–6.

Hardner, J., and R. Rice. 2002. Rethinking Green consumerism. Scientific American 286:88–95.

Hölldobler, B., and E. O. Wilson. 1990. The ants. The Belknap Press of Harvard University Press, Cambridge, Mass.

Jha, S., C. M. Bacon, S. M. Philpott, R. A. Rice, V. E. Méndez, and P. Läderach. 2011. A Review of Ecosystem Services, Farmer Livelihoods and Value CHains in Shade Coffee Agroecosystems. Page *in* W. B. Campbell and S. López Ortiz, editors. Integrating Agriculture, Conservation and Ecoturism: Examples from the Field, Issues in Agroecology-Present Status and Future Prospectus. Springer Science+Business Media B.V.

Kaspari, M., B. S. Stevenson, J. Z. Shik, and J. F. Kerekes. 2010. Scaling community structure: how bacteria, fungi, and ant taxocenes differentiate along a tropical forest floor. Ecology 91:2505–2511.

Kaspari, M., and S. P. Yanoviak. 2009. Biogeochemistry and the structure of tropical brown food webs. Ecology 90:3342–3351.

de la Mora, A., C. J. Murnen, and S. M. Philpott. 2013. Local and landscape drivers of biodiversity of four groups of ants in coffee landscapes. Biodiversity and Conservation 22:871–888.

Leblanc, H. A., P. Nygren, and R. L. McGraw. 2006. Green mulch decomposition and nitrogen release from leaves of two Inga spp. in an organic alley-cropping practice in the humid tropics. Soil Biology and Biochemistry 38:349–358.

López-Rodríguez, G., D. Sotomayor-Ramírez, J. A. Amador, and E. C. Schröder. 2015. Contribution of nitrogen from litter and soil mineralization to shade and sun coffee (Coffea arabica L.) agroecosystems. Tropical Ecology 56:155–167.

Majer, J. D., J. H. C. Delabie, and N. L. McKenzie. 1997. Ant litter fauna of forest, forest edges and adjacent grassland in the Atlantic rain forest region of Bahia, Brazil. Insectes Sociaux 44:255–266.

Mas, A. H., and T. V Dietsch. 2003. An Index of Management Intensity for Coffee Agroecosystems to Evaluate Butterfly Species Richness Published by : Ecological Society of America AN INDEX OF MANAGEMENT INTENSITY FOR COFFEE

AGROECOSYSTEMS TO EVALUATE BUTTERFLY SPECIES RICHNESS. Ecological Applications 13:1491–1501.

Moguel, P., and V. M. Toledo. 1999. Biodiversity conservation in tradicional Coffee systems of Mexico. Conservation Biology 13:11–21.

Morris, J. R., and I. Perfecto. 2016. Agriculture , Ecosystems and Environment Testing the potential for ant predation of immature coffee berry borer (Hypothenemus hampei) life stages 233:224–228.

Murnen, C. J., D. J. Gonthier, and S. M. Philpott. 2013. Food Webs in the Litter : Effects of Food and Nest Addition on Ant Communities in Coffee Agroecosystems and Forest. Environmental entomology 42:668–676.

Nkem, J. N., L. A. L. De Bruyn, C. D. Grant, and N. R. Hulugalle. 2000. The impact of ant bioturbation and foraging activities on surrounding soil properties 44:609–621.

Offenberg, J. 2015. Ants as tools in sustainable agriculture. Journal of Applied Ecology 52:1197–1205.

Peeters, L. Y. K., L. Soto-Pinto, H. Perales, G. Montoya, and M. Ishiki. 2003. Coffee production, timber, and firewood in traditional and Inga-shaded plantations in Southern Mexico. Agriculture, Ecosystems and Environment 95:481–493.

Pennington, T. D., R. Linklater, K. Royal Botanic Gardens, and F. R. P. (Great Britain). 1997. The genus Inga: botany. Royal Botanic Gardens, Kew.

Perfecto, I., and A. Castiñeiras. 1998. Deployment of the Predaceous Ants and their Conservation in Agroecosystems. Pages 39–54 *in* P. Barbosa, editor.Conservation Biological Control. Elsevier Inc.

Perfecto, I., R. A. Rice, R. Greenberg, and M. E. Van Der Voort. 1996. Shade Coffee: a Disappearing Refuge for Biodiversity. BioScience 46:598–608.

Perfecto, I., and J. Vandermeer. 1996. Microclimate changes and indirect loss of ant diversity in a tropical agroecosystem. Oecologia 108:577–582.

Perfecto, I., and J. H. Vandermeer. 2015. Coffee agroecology: a new approach to understanding agricultural biodiversity, ecosystem services, and sustainable development. First edition. Routledge, London, [England] ; New York, New York.

Perfecto, I., J. Vandermeer, and S. M. Philpott. 2014. Complex Ecological Interactions in the Coffee Agroecosystem. Annual Review of Ecology, Evolution, and Systematics 45:137–158.

Philpott, S. M., W. J. Arendt, I. Armbrecht, P. Bichier, T. V. Diestch, C. Gordon, R. Greenberg, I. Perfecto, R. Reynoso-Santos, L. Soto-Pinto, C. Tejeda-Cruz, G. Williams-Linera, J. Valenzuela, and J. M. Zolotoff. 2008a. Biodiversity loss in Latin American coffee landscapes: Review of the evidence on ants, birds, and trees. Conservation Biology 22:1093–1105.

Philpott, S. M., and I. Armbrecht. 2006. Biodiversity in tropical agroforests and the ecological

role of ants and ant diversity in predatory function. Ecological Entomology 31:369–377.

Philpott, S. M., and P. Bichier. 2012. Effects of shade tree removal on birds in coffee agroecosystems in Chiapas, Mexico. Agriculture, Ecosystems and Environment 149:171–180.

Philpott, S. M., R. Greenberg, P. Bichier, and I. Perfecto. 2004. Impacts of major predators on tropical agroforest arthropods: Comparisons within and across taxa. Oecologia 140:140–149.

Philpott, S. M., I. Perfecto, and J. Vandermeer. 2008b. Effects of predatory ants on lower trophic levels across a gradient of coffee management complexity. Journal of Animal Ecology 77:505–511.

Roeder, K. A., and M. Kaspari. 2017. From cryptic herbivore to predator: Stable isotopes reveal consistent variability in trophic levels in an ant population. Ecology 98:297–303.

Romero-Alvarado, Y., L. Soto-Pinto, L. García-Barrios, and J. F. Barrera-Gaytán. 2002. Coffee yields and soil nutrients under the shades of Inga sp . vs . multiple species in Chiapas , Mexico. Agroforestry Systems 54:215–224.

Sabu, T. K., P. J. Vineesh, and K. V Vinod. 2008. Diversity of forest litter-inhabiting ants along elevations in the Wayanad region of the Western Ghats. Journal of Insect Science 8:1–14.

Shik, J. Z., and M. Kaspari. 2010. More food, less habitat: How necromass and leaf litter decomposition combine to regulate a litter ant community. Ecological Entomology 35:158–165.

Silva, P. S. D., A. G. D. Bieber, M. M. Corrêa, and I. R. Leal. 2011. Do Leaf-Litter Attributes Affect the Richness of Leaf-Litter Ants ? Neotropical entomology 40:542–547.

Soto-Pinto, L., I. Perfecto, and J. Caballero-Nieto. 2002. Shade over coffee: Its effects on berry borer, leaf rust and spontaneous herbs in Chiapas, Mexico. Agroforestry Systems 55:37–45.

Talbot, J. M., and K. K. Treseder. 2012. Interactions between lignin, cellulose, and nitrogem drive litter chemistry - decay relationships. Ecology 93:345–354.

Team, R. C. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies. 2005. Landscape perspectives on agricultural intensification and biodiversity - Ecosystem service management. Ecology Letters 8:857–874.

Valencia, V., L. Garc??a-Barrios, P. West, E. J. Sterling, and S. Naeem. 2014a. The role of coffee agroforestry in the conservation of tree diversity and community composition of native forests in a Biosphere Reserve. Agriculture, Ecosystems and Environment 189:154–163.

Valencia, V., L. Garc??a-Barrios, P. West, E. J. Sterling, S. Naeem, L. García-barrios, P. West, E. J. Sterling, S. Naeem, L. Garc??a-Barrios, P. West, E. J. Sterling, and S. Naeem. 2014b. The role of coffee agroforestry in the conservation of tree diversity and community

composition of native forests in a Biosphere Reserve. Agriculture, Ecosystems and Environment 189:154–163.

Valencia, V., L. García-barrios, P. West, E. J. Sterling, and S. Naeem. 2014c. Agriculture , Ecosystems and Environment The role of coffee agroforestry in the conservation of tree diversity and community composition of native forests in a Biosphere Reserve. "Agriculture, Ecosystems and Environment" 189:154–163.

Valencia, V., P. West, E. J. Sterling, L. Garcia-Barrios, S. NAEEM, A. Arrios, and V. E. T. Al. 2015. The use of farmers ' knowledge in coffee agroforestry management : implications for the conservation of tree biodiversity. Ecosphere 6:1–17.

Vandermeer, J., and I. Perfecto. 2007. 19 The diverse faces of ecosystem engineers in agroecosystems. Ecosystem Engineers : Plants to Protists 4:367–385.

Vandermeer, J., I. Perfecto, and S. Philpott. 2010. Ecological Complexity and Pest Control in Organic Coffee Production: Uncovering an Autonomous Ecosystem Service. BioScience 60:527–537.

Zhang, Q., and J. C. Zak. 1995. Effects of Gap Size on Litter Decomposition and Microbial Activity in a Subtropical Forest. Ecology 76:2196–2204.