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Between two trees: Environmental effects of *I. micheliana* and *A. latifolia* on leaf litter ants in a coffee agroecosystem

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Abstract

Shade trees provide important ecological services that support productivity in coffee agroforestry systems. Processes such as biological nitrogen fixation play a key role in this. Less is known, however, about potential indirect mechanisms by which nitrogen fixation supports coffee productivity. One potential route for this to occur is by providing ecological benefits to other above- and belowground organisms that enrich the overall function of agroecosystems. A useful lens with which to evaluate the ecological benefits to these communities under shade trees is to assess how ground-dwelling ant communities respond to the quality of leaf litter from established nitrogen (N)-fixing tree species. Here we use two trees commonly planted in coffee agroecosystems: *Inga micheliana*, a N-fixing species, and *Alchornea latifolia*, a non-N fixing species. In this study, we set out to answer the following questions: (1) How does the leaf litter environment differ between *I. micheliana* and *A. latifolia*? (2) Do differences in environmental factors between *I. micheliana* and *A. latifolia* correlate with differences in ant abundance and species richness? (3) Do differences in environmental factors between *I. micheliana* and *A. latifolia* correlate with differences in ant community composition? 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 1 m² quadrats within a 5 m radius from the base of the selected trees were established and the leaf litter within the quadrats 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 N fixing species, has a lower C:N ratio than *A. latifolia*. Differences in C:N ratios, are correlated with ant abundance but not with ant species richness. Distance to edge (m) has significant effects on leaf litter ant abundance, richness, and species composition. Results

suggests that there may be unaccounted feedbacks from N and non-N fixing vegetation to brown food webs enabling them to sustain similar ground-dwelling ant communities.

Introduction

Coffee agroecosystems have been established in biodiversity hotspots around the world and constitute the livelihood of millions of traditional farmers in the tropics (Perfecto and Vandermeer 2015; Perfecto, Vandermeer, and Philpott 2014). Ecological literature in the last three decades has evidenced the array of coffee management practices and their impacts on biodiversity: from low-management (shade-grown) styles that promote high levels of biodiversity (Philpott et al. 2008) to intensified agricultural plantations (sun-coffee) that result in low-biodiversity levels (Perfecto et al. 1996; Armbrrecht et al. 2005; Moguel and Toledo 1999; Mas and Dietsch 2003). Planned biodiversity (e.g., coffee plants and shade trees), in conjunction with associated biodiversity, facilitates 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; Barnes et al. 2017; Tschardt et al. 2005; Jha et al. 2011).

Shade trees play an important role in coffee agroecosystems. They maintain soil moisture, control weeds, alleviate pest breakouts (Morris and Perfecto 2016; Soto-Pinto, Perfecto, and Caballero-Nieto 2002), and provide a good source of fuelwood, fruits, and construction material (García-Barríos et al. 2009; Valencia, et al. 2014; Peeters et al. 2003). Nonetheless, shade trees are not always present in coffee systems (i.e., sun-coffee agroecosystems). In shade-coffee farms tree species composition can vary widely and it is highly dependent on the farmer's management practice (Valencia, et al. 2014). Farmers in Central

America manage highly weathered and nutrient poor soils in coffee agroecosystems by favoring N fixing trees, particularly those from the *Inga* genus (Valencia, et al. 2014; Romero-Alvarado et al. 2002; Grossman et al. 2006). The practice varies according to region and country, especially when use of non-N fixing trees is 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 interactions taking place in the arboreal component of the agroecosystem (Hajian-Forooshani et al. 2016; Barrios et al. 2018; Philpott and Bichier 2012; Philpott et al. 2004). Less is known about how N fixing trees influence ground level and below-ground food webs in coffee agroecosystems.

Due to the capacity of species in the genus *Inga* for biological nitrogen fixation (BNF) (Pennington 1997) the trees generally have a lower carbon to nitrogen ratio (herein C:N ratio) in their foliage, which produces high quality leaf litter and has the potential to accelerate loss of lignin and soluble C in the leaf litter (Talbot and Treseder 2012). High quality leaf litter, either due to the identity of leaves (i.e., specific C:N ratio) or addition of resources (e.g., necromass) can increase microbial activity and accelerate decomposition (Talbot and Treseder 2012; Clay et al. 2013; Shik and Kaspari 2010; Zhang and Zak 1995). Differing C:N ratios have been shown to correlate with altered microbial communities and to affect potential synergisms in decompositions rates (Chapman et al. 2013), ultimately influencing nutrient cycling. Changes in microbial communities due to nutrient availability could potentially lead to increases in decomposition rates, which in turn could cause the loss of habitat space for soil arthropods, including leaf litter ants (Shik and Kaspari 2010). Nevertheless, increased decomposition rates could also lead to increases in soil macrofauna abundance and richness, especially fungi grazers

(i.e., Collembola) hence increasing availability of resources (e.g., prey) for ground-dwelling ants. While past studies have shown the effects that C:N ratio has on microbial communities and feedback loops between these (Coleman 2011; Beare et al. 1992), it remains elusive how the C:N ratio affects ground-dwelling ant communities.

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) (Vandermeer and Perfecto 2007; Nkem et al. 2000; Bruyn 1999) biocontrol agents (Vandermeer, Perfecto, and Philpott 2010; Morris and Perfecto 2016; Philpott and Armbrrecht 2006; Ivette Perfecto and Castiñeiras 1998), and are good indicators of ecosystem health (Benckiser 2010). Ants respond to changes in local factors such as tree species richness, tree abundance and leaf litter biomass (Armbrrecht et al., 2005; De la Mora et al., 2013; Philpott and Armbrrecht, 2006). Furthermore, shaded-coffee farms have been shown to support a high diversity of ants and other leaf litter arthropods (Perfecto et al. 1996; Philpott et al. 2004; Perfecto, Vandermeer, and Philpott 2014). However, little is known about how ground-dwelling ant species respond to changes in the C:N ratio of leaf litter in tropical agroecosystems (Philpott and Armbrrecht 2006).

We examined the abundance, richness, and community composition of ground-dwelling ant communities in leaf litter from *I. micheliana* and *A. latifolia* in a shaded-coffee agroecosystem. We specifically asked: (1) How does the leaf litter environment differ between *I. micheliana* and *A. latifolia*? (2) Do difference in environmental factors between *I. micheliana* and *A. latifolia* correlate with differences in ant abundance and species richness? (3) Do

differences in environmental factors between *I. micheliana* and *A. latifolia* correlate with differences in ant community composition? Since the *I. micheliana* carries out BNF (Pennington 1997; Romero-Alvarado et al. 2002) we predicted a lower C:N ratio in the leaf litter that accumulates beneath its crown, as compared to *A. latifolia* that does not fix N. Furthermore, we predicted that increased nutrient availability influences ground-dwelling ant abundance and richness, with greater ant abundance and richness in leaf litter with a lower C:N ratio.

Methods

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 at 92°20'29''W and 15°10'6''N. Altitude in the plot ranges from 900-1200 m asl (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., 2013; Philpott et al., 2008b).

Local site selection and environmental factors

To avoid potential competitive exclusion of ground-dwelling ants (Ennis 2010) by keystone ant species, *Azteca sericeasur*, trees selected met 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 paired trees were a minimum of 10m apart to avoid shared leaf litter content and a maximum of 100m from each other to ensure sampling was done in a similar area of the 45 hectare plot and sampled during the same day (e.g. sampling in overgrown or managed area not representative of the coffee farm as a whole) (De la Mora et al., (2013); (4) diameter at breast

height had to be equal or greater than 30 cm. Using a georeferenced map of the 45-hectare plot in *Finca Irlanda*, we selected 28 trees for sampling: 14 of *Inga micheliana*, the most abundant N fixing species, and 14 of *Alchornea latifolia*, 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 pattern following all cardinal directions. Along the NS transect, we established three 1 m² quadrats for arthropod extraction, for a total of 84 quadrats: 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 information (from now on “local factors”) 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) (cm), number of coffee plants within the four 5 m transects, mean leaf litter depth (mm) within all 5 m transects, leaf litter depth (mm) within each 1m² quadrat, slope cardinality (°), altitude (m asl), distance to edge (m) (edge of trails and roads in coffee farm), mean percent crown cover, pH, percent soil humidity, total percent nitrogen (% N), total percent carbon (% C) and C:N ratio. Measurements of leaf litter depth were taken at base of the tree, 2.5 m, and 5 m from the base of the tree in all four cardinal directions for total of 10 data points. Leaf litter depth within 1 m² quadrats was measured at the center and all four corners. Measuring along transects and within quadrats provided a measure of the mean leaf litter volume under the tree crown. Slope and altitude of sites was determined with a Garmin 72h model (www.garmin.com). To determine mean percent crown cover, four measurements in cardinal directions were taken with a Spherical Crown Densiometer, Model A (Forestry Suppliers) at the base of the tree. Mean soil pH was determined by colorimetric method (Lovibond Soil pH Test Kit, MPN number 694, www.forestry-

[suppliers.com](https://www.suppliers.com)). Briefly, we collected a core from the soil surface using a 2 mL vial tube at each of the three 1 m² quadrats established within the NS transects of selected trees for arthropod extraction. We then homogenized each core and proceeded following manufacturers' guidelines. Soil samples were processed in the field laboratory within 6 hours of being collected. Soil humidity was assessed through the gravimetric method by taking an additional 4-6 g of soil from each of the 1 m² quadrats in NS transects of selected trees. Each sample was weighed and then dried for a minimum of 72 hours at 50 °C until no further mass loss was recorded. All 13 local factors were measured on the same date as ant sampling occurred for each tree pair (see *Ant Sampling Protocol*). For C and N analyses, we collected leaf litter from an additional fourth 1 m² quadrat in proximity to NS transect ($n = 28$). Collecting from an additional quadrat ensured that leaf litter was not disturbed by previous measurements and sampling efforts (see below for details).

Ant sampling protocol

Leaf litter was collected from the three 1 m² quadrats established at each tree and sifted from these quadrats using the Winkler method (Agosti et al. 2000). All leaf litter inside the quadrat was sifted and placed 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 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).

Leaf litter chemical analyses: C:N ratio

All dried leaf-litter inside the fourth 1 m² quadrat was collected and dried for a minimum of 72 hours at 50 °C until constant weight was obtained. After which we homogenized and subsampled 20 g of leaf litter and placed in plastic bags (Ziploc, 16.5 cm x 14.9 cm) for chemical analyses. Subsamples were pulverized using a Krups brand coffee grinder (model GVX212) in the finest setting. Approximately 0.2-0.3 g of ground sample was analyzed for C and N content using a LECO Trumac CN combustion analyzer (LECO Corporation, 3000 Lakeview Avenue, Saint Joseph, MI 49085). Chemical analyses were conducted at the laboratory of Dr. Jennifer Blesh at the University of Michigan (Ann Arbor, Michigan, USA). Data from this fourth quadrat is considered representative of the chemical composition of leaf litter of *I. micheliana* and *A. latifolia* in this study and statistical analyses.

Statistical analyses

Ant Species Abundance, Richness and Community Composition.

To assess the effectiveness of our sampling efforts at capturing ant richness, we computed species accumulations curves (SACs) for observed species richness. We used the “BiodiversityR” package (Kindt and Coe 2005; Kindt 2022) with parameters set to 100 permutations with the “exact” method and second order jackknife with 95% confidence intervals (CI). Additionally, mean ant abundance and richness was tested for significant differences between treatments using paired student *t*-tests, where ant abundance was the count of individuals and richness was the number of unique species.

Local environmental factors

To address our first question, we computed paired *t*-tests for all the local factors of *I. micheliana* and *A. latifolia* sites: DBH, altitude, slope cardinality, distance to edge, number of coffee plants within all 5 m transects, leaf litter depth, leaf litter depth within 1 m² quadrats, pH, percent soil

humidity, total % N, total % C, and C:N ratio. We computed simple linear regression to explore the correlation between significantly different local factors and ant abundance and richness in the leaf litter of both tree species.

Predicting ant abundance, richness, and community composition

To address our second question, we computed Generalized Linear Mixed Models (GLMMs). We computed the variance inflation factors (VIF) with the *vif* function in the “car” (Fox, Weisberg, and Price 2022) to examine multicollinearity among all local factors: DBH, number of coffee plants within the four 5 m transects, mean leaf litter depth within all 5 m transects, leaf litter depth within each 1 m² quadrat, slope cardinality, altitude, distance to edge (edge of trails and roads in coffee farm), mean percent crown cover, pH, percent soil humidity, % N, % C, and C:N ratio. Computation was performed by building two initial GLMMs for abundance and richness using the *glmer* function in “lme4” package and optimized parameters with the *bobyqa* method (Bates et al. 2015). Local factors with a VIF greater than 5 were considered highly correlated. Although total % N was highly correlated (VIF >5) with C:N ratio in both initial GLMMs, 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 ensure the best fit possible, we scaled and centered the continuous local factors using the *scale* function in R software (R Core Team 2022) and computed the GLMMs with a Poisson distribution for ant abundance and species richness. Due to the high number of local factors, we did not model interaction effects, this allowed us to avoid convergence issues in our GLMMs. We consider our models are a reasonable subset of the truly maximal model. All local factors were set as fixed effects in the models. We set sampling date (8 dates during June-July 2016) and site identification (ID) as random effects in our models for predicting ant abundance to capture changes in other environmental changes not measured

throughout the season. We eliminated site ID as a random effect to avoid model singularity in our GLMM for predicting ant species richness. To select the most parsimonious model we used the “buildmer” package (Voeten 2022) to perform a stepwise backward elimination of insignificant variables until we reached a model that maximized model fit. The same optimizer parameters were applied. Best fit model residuals were visually evaluated to corroborate fit using function *qqPlot* from the “car” package (Fox, Weisberg, and Price 2022). Marginal and conditional R-squared values for best fit models were computed with “MuMin” package (Bartoń 2022). We removed an outlier *A. latifolia* site, with more than 2000 ant individuals from analyses. Because all sites were uniquely paired, the corresponding paired individual of *I. micheliana* was also removed from all analyses. For all response variables and local factors, apart from leaf litter depth, total % N, total % C and C:N ratio, we report data from a total of 78 quadrats (13 *I. micheliana* sites \times 3 quadrats and 13 *A. latifolia* sites \times 3 quadrats). Data for leaf litter depth, total % N, total % C and C:N ratio is reported on a per site basis ($n = 26$). Mean values and standard errors for local factors were compared and tested with paired Student’s *t*-tests.

Lastly, we computed a Permutational Multivariate Analysis of Variance (PERMANOVA) using *adonis2* function from the “vegan” package (Oksanen et al. 2022) to understand how differences in local factors between *I. micheliana* and *A. latifolia* correlate with differences in ant community composition (9999 permutations, method = “bray”). We used a permutational analysis of multivariate dispersion (PERMDISP) as companion to PERMANOVA to corroborate homogeneous dispersion of variances, a necessary assumption for this test, and exclude the possibility that any significant differences between leaf litter ant communities were caused by heterogeneous dispersion of variances. The PERMDISP was computed with *betadisper* and

tested for significance with *permutest* functions in the “vegan” package (Oksanen et al. 2022). To visualize how local factors correlate with leaf litter ant communities we computed a distance-based redundancy analysis (dbRDA) by applying the *capscale* function to a Bray-Curtis similarity matrix (Oksanen et al. 2022). The dbRDA and corresponding visualization appropriately illustrates the underlying patterns of compositional differences as it is considered analogous to PERMANOVA with non-Euclidean distance matrices (Legendre and Anderson 1999). We used the *simper* function in “vegan” to discriminate which species contribute the most to compositional differences between groups (Oksanen et al. 2022). All statistical analyses were performed using R Statistical Software (v4.2.1, R Core Team 2022).

Results

Sampling effort to capture ant abundance, richness, and community composition.

Our sampling of 78 quadrats (39 per tree species) resulted in a total of 6,574 ant individuals from 8 subfamilies, 34 genera and 67 morpho-species and species (Appendix S1: Table S1). Species accumulation curves estimated asymptotes indicate that our sampling effort captured the mean richness of leaf litter ant species (Fig. 1). The overlap of the CIs of the species accumulation curves revealed no significant difference in observed species richness between leaf litter types. We corroborated this with paired *t*-tests ($t = 0.049$, $df = 38$, $p = 0.96$). For overall ant abundance, no significant differences between tree species were observed as well (paired *t*-tests, $t = -0.73$, $df = 38$, $p = 0.47$). We found no significant differences in abundance for species present at more than five tree sites, except for *Nylanderia* sp1. ($p = 0.02$). Among species that were present in less than five tree sites we found significant differences for *Solenopsis zeteki* ($p = 0.01$), *Solenopsis* sp1. ($p = 0.01$).

Effects of local factors on ant abundance and richness.

Several local factors were found to be significantly different between *I. micheliana* and *A. latifolia* sites (Table 1). DBH (cm), and C:N ratio were significantly greater under *A. latifolia* than *I. micheliana*, while total % N and number of coffee plants was greater under the *I. micheliana* trees (Fig. 2 & Table 1). Quadrats at host tree sites did not differ for any of the other measured local factors. Exploratory simple linear regressions revealed, overall, no significant trends between significantly different local factors and ant abundance and richness (Fig. 3). We only observed a significant correlation for DBH and ant richness at *I. micheliana* quadrats ($p = 0.01$) (Fig. 3e).

Generalized Linear Mixed Models (GLMMs) revealed how local factors correlate with leaf litter ant abundance and richness (Table 2). The initial GLMM for predicting leaf litter ant abundance reports that distance to edge ($p = 0.001$); mean leaf litter depth within 1 m² quadrats ($p = 0.002$); pH ($p < 0.001$); percent soil humidity ($p < 0.001$); total % N ($p < 0.001$); total % C ($p < 0.001$); and C:N ratio ($p < 0.001$) are significant predictors (marginal R^2 : 0.44, and conditional R^2 : 0.99) (Fig. 4, Table 2). The most parsimonious model for ant abundance revealed that from the initial 13 local factors, only distance to edge (fixed effect) ($p = 0.0371$) with sampling date (random effect) were significant in predicting leaf litter ant abundance (marginal R^2 : 0.32, and conditional R^2 : 0.97). The GLMM model with all the factors for predicting leaf litter ant richness revealed that only distance to edge ($p = 0.003$) was significant (marginal R^2 : 0.24, and conditional R^2 : 0.57). The final parsimonious model for predicting richness of leaf litter ants revealed that distance to edge (fixed effect) ($p = 0.003$) and sampling date (random effect) are significant factors (marginal R^2 : 0.08, and conditional R^2 : 0.44). Distance to edge was the only local factor that had a significant negative correlation with both ant abundance and species richness in initial

and final (parsimonious) GLMMs (Fig. 4, Fig. 5, Table 2). We summarized the log mean estimates from the initial GLMMs' with 95% confidence intervals, to better illustrate the significant negative correlations for mean 1 m² quadrat leaf litter depth and mean soil and significantly positive correlations with mean soil pH, percent soil humidity, C:N ratio, total % N and total % C with and abundance (Fig. 4). The log means estimate visualization of the GLMM for ant richness highlights only distance to edge has a significant negative correlation.

The PERMDISP Tests revealed no significant differences in the centroids of the dispersions of leaf litter ant communities from *I. micheliana* and *A. latifolia* ($F = 0.4, p = 0.54$). Visualization of species composition with dbRDA showed overlap in the community structure as well as the associations between local factors with ant communities in *I. micheliana* and *A. latifolia* leaf litter (Fig. 6). The axes CAP1 and CAP2 of the dbRDA explain 21.2% and 19%, respectively, of 5.9% of the constrained variance (Fig. 6). Results from PERMANOVA revealed significant correlations for tree species ($R^2 = 0.03, p < 0.0001$); DBH ($R^2 = 0.02, p = 0.02$); altitude ($R^2 = 0.02, p = 0.02$); slope cardinality ($R^2 = 0.02, p = 0.01$); distance to edge ($R^2 = 0.03, p = 0.002$); and percent soil humidity ($R^2 = 0.02, p = 0.03$) (Table 3). Marginally significant correlations were observed for mean 1 m² leaf litter depth ($R^2 = 0.02, p = 0.06$) and total % N ($R^2 = 0.02, p = 0.06$) (Table 3). The species with the greatest cumulative contributions to compositional differences between groups are: *Solenopsis terricola* (0.17), *Pheidole protensa* (0.30), *Solenopsis picea* (0.37), *Eurhopalotrix* sp1. (0.44), *Solenopsis zeteki* (0.51), *Solenopsis* sp1. (0.58), *Gnamptogenys striatula* (0.62), *Strumigenys gundlachi* (0.66), *Hypoponera nitidula* (0.69), and *Eurhopalotrix* sp2. (0.72).

Discussion

Our study investigated two tree species of distinct chemical compositions and leaf trait morphologies and found that leaf litter from *I. micheliana* had significantly lower C:N ratio than *A. latifolia* (Fig. 2c, Table 1). Tree species also differed significantly in DBH, number of coffee plants, total % N, and C:N ratio (Fig. 2, Table 1). Results align well with reports from other studies showing that N-fixing trees produce leaf litter with low C:N ratio that can contribute to greater accumulation of soil organic matter (SOM) and higher decomposition rates. High inputs of N to soil may result in increased coffee production and agroecosystem sustainability (López-Rodríguez et al. 2015; Leblanc, Nygren, and McGraw 2006). The results from our initial GLMM models partially support our prediction that C:N ratio in leaf litter is an important predictor of leaf litter ant abundance, yet there was no significant difference in leaf litter ant abundance between tree species (data not shown). In other words, it is possible that the statistical significance in the model is not biologically relevant. The differences detected in this study do not support that C:N ratio is correlated to leaf litter ant richness (Fig. 4, Table 2). Other local factors that were significant predictors variables of leaf litter ant abundance were: distance to edge, mean 1 m² quadrat leaf litter depth, percent soil humidity, mean soil pH, total % N and total % C (Table 2). On the other hand, in the GLMM model for predicting ant richness, only distance to edge was a significant negative predictor variable (Table 2). Even though *t*-tests results also confirm that DBH, total % Nitrogen and number of coffee plants significantly different between *I. micheliana* and *A. latifolia*, it is not enough to be significantly correlated with leaf litter ant species richness between the groups (Fig. 2, Table 3). Again, this could be due to the possibility that the statistical difference of these local factors is not enough to be biologically relevant for the ant community (Fig. 1, Table 2). In a similar study, Murnen et al. (2013) reported a small increase in ant colony growth and species richness after adding

necromass to leaf litter and increasing its nutrient quality. Unfortunately, no data for % N or other nutrients is reported in this study. In general, we see no significant differences for ant species richness under *I. micheliana* compared to *A. latifolia* leaf litter as shown by the species accumulation curves (Fig. 1). Though we observe high overlap in species composition (Fig. 4), PERMANOVA results highlight that between group differences are correlated with distance to edge, DBH, altitude, slope cardinality, and percent soil humidity. We focus on results from our initial GLMMs to highlight the effects of the different local factors on ant abundance and richness (Fig. 4, Table 2). Our final parsimonious models also support the same general conclusion that distance to edge is an important predictor of ant abundance and richness (Fig. 5).

Although we did not collect data on soil macrofauna, it is likely that higher N content and lower C:N ratio in leaf litter could potentially increase their abundance and richness, thus increasing availability of resources (e.g., prey) for ground-dwelling ants. This pathway could result in increased abundance of generalist species (e.g., *Solenopsis* spp.) at the cost of lower species richness. Few other studies have investigated the specific role of C:N ratio from leaf litter in ant communities. Hence, comparison across studies is difficult and at times speculative. Studies focused on comparing decomposition of leaf litter from pioneer and old growth forests do report significant differences, where old growth forest litter host a greater abundance and diversity of leaf litter and soil invertebrate communities (Laird-Hopkins et al. 2017).

Distance to edge was a significant predictor variable for ant abundance and richness (Fig. 4, Table 2). It is also strongly correlated to species composition (Table 3). Here, distance to edge referred 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 (Silva et al. 2011; Majer, Delabie, and McKenzie 1997). The light gap created by trails in the coffee farms has similar effects to gaps in

natural forests (Perfecto and Vandermeer 1996; Majer, Delabie, and McKenzie 1997), potentially allowing colonization of niche space and increased food resources (e.g., food disposal by farm workers; Aponte Rolón, *personal observations*), for genera like *Solenopsis*, *Pheidole*, and for *Wasmannia auropunctata*, which have a high capacity for recruitment of workers and can build large colonies (Hölldobler and Wilson 1990). Nevertheless, the presence of these dominant species can have a negative effect on other ants therefore reducing species richness (Ennis and Philpott 2017).

Our results contrasted with other studies that report a strong correlation of ant abundance and richness with organic matter mass and leaf litter depth (De la Mora et al., 2013; Sabu et al., 2008). Rather, our results supported reports from Shik and Kaspari et al. (2010) which showed no differences in leaf litter ant abundance or richness in experimental plots and another study by Kaspari et al. (2010), that reported homogeneous ant species richness along a topographic and nutrient gradient (e.g., NPK additions). Results reported by Shik and Kaspari (2010), 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. Similar results were reported by Murnen, et al. (2013) when they compared ant communities among forest, sun-coffee, and shaded-coffee habitats, and found that habitat type did influence ant abundance and richness, but not food addition. Schmitt, et al. (2020) examined the decomposition of *I. micheliana* leaves but found no difference it was the presence of *A. sericeasur* that changed the leaf litter ant community composition. This points towards higher order ecological interactions influencing ants in the leaf litter.

Overall, we found that the leaf litter produced by *I. michelania* (the N fixing tree) had a statistically significant lower C:N ratio and DBH, and a higher total % N and number of

surrounding coffee plants than *A. latifolia* (the non-N-fixing tree) (Fig. 2, Table 1). However, these differences do not seem to be biologically important to distinguish the ant community living in leaf litter under these tree species. More specifically, we could not detect any significant differences in the leaf litter ant abundance and richness. In contrast, we see differences in species composition under these tree species are correlated multiple local factors. With GLMMs we found that distance to edge, mean 1 m² quadrat leaf litter depth, pH, percent soil humidity, C:N ratio, total % N and total % C were significant predictors of ant abundance. Abundance is positively correlated with percent soil humidity, C:N ratio, total % N and total % C, while distance to edge, mean 1 m² quadrat leaf litter depth is negatively correlated (Fig. 4, Fig. 5). Finally, distance to edge (i.e., distance to a trail or road) was a significant negative predictor of both ant abundance and species richness. Leaf litter ants decline in abundance and richness as distance to edge increases. A slightly different set of local factors correlates with ant species composition as shown by PERMANOVA results (Table 3) and dbRDA analysis (Fig. 6). Compositional differences between leaf litter ant communities are significantly correlated with DBH, altitude, slope cardinality, soil percent humidity, and distance to edge (Fig. 6, Table 3). Regardless of local factor's statistically significant differences, only distance to edge contributes to biologically important differences in leaf litter ant abundance, richness, and species composition.

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 focus on comparing changes in microbial activity due to leaf litter type and its effects on ant community composition. Results suggest that a shaded-coffee agroecosystem can support high levels of ant biodiversity regardless of which tree species, *I. micheliana* or *A. latifolia*, is planted by farmers.

Although, from this study, it is not clear at what scale (e.g., quadrat) local factors most contribute to observable biological differences in leaf litter ant abundance, richness, and species composition.

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Author Contributions

Bolívar Aponte Rolón and Ivette Perfecto are responsible for the conception of research questions and experimental design. BAR is responsible for fieldwork and data collection and analysis. BAR produced the first manuscript draft. Both authors agree on the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

All data and source code (Aponte Rolón 2023) are available from Zenodo:

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Table 1. Values (mean \pm SE) of local factor characteristics at *I. micheliana* and *A. latifolia* sites in coffee agroecosystem in Chiapas, Mexico.

Site characteristics	<i>Inga micheliana</i>	<i>Alchornea latifolia</i>	<i>t</i>	<i>p</i>
DBH (cm)	32.6 \pm 1.12	59.97 \pm 1.92	12.04	<0.001***
Altitude (m)	1039.77 \pm 4.58	1032.39 \pm 2.60	-1.33	0.19
Slope cardinality (°)	194.39 \pm 14.68	224.00 \pm 15.05	1.72	0.09
Distance to edge (m)	12.71 \pm 1.20	17.54 \pm 2.61	1.40	0.17
Leaf litter depth (mm)	53.07 \pm 3.19	58.95 \pm 3.70	1.66	0.11
Leaf litter 1 m ² quadrat (mm)	57.62 \pm 2.69	60.48 \pm 4.25	0.54	0.59
Crown cover (%)	66 \pm 5.11	72.00 \pm 4.12	0.79	0.44
No. coffee plants	30.07 \pm 1.63	23.15 \pm 1.84	-2.71	0.01**
Percent soil pH	6.00 \pm 0.15	6.36 \pm 0.11	1.72	0.09
Percent soil humidity	83.82 \pm 7.38	81.51 \pm 3.20	-0.29	0.77
C:N ratio	20.27 \pm 0.31	25.28 \pm 0.68	8.02	<0.001***
Total % N	2.32 \pm 0.03	1.92 \pm 0.06	-7.20	<0.001***
Total % C	46.61 \pm 0.36	47.01 \pm 0.29	0.83	0.41

Notes: Values show mean, standard error, and results from paired *t* tests (*n* = 78, *df* = 38). Values in boldface are significant.

** *p* < 0.01; *** *p* < 0.001.

Table 2. Generalized linear mixed models for predicting leaf litter ant abundance and richness.

Coefficient	GLMM: Ant Abundance				GLMM: Ant Richness			
	Log-Mean	SE	95% CI	<i>p</i>	Log-Mean	SE	95% CI	<i>p</i>
(Intercept)	4.47 ***	0.29	3.90 – 5.03	<0.001	2.27 ***	0.13	2.02 – 2.53	<0.001
Tree species	-0.30	0.43	-1.14 – 0.53	0.475	0.23	0.18	-0.11 – 0.58	0.188
DBH (cm)	-0.03	0.22	-0.46 – 0.41	0.909	0.10	0.08	-0.06 – 0.27	0.210
Altitude (m asl)	-0.12	0.15	-0.41 – 0.16	0.400	-0.03	0.05	-0.14 – 0.08	0.580
Slope cardinality (°)	-0.20	0.17	-0.54 – 0.13	0.239	-0.00	0.07	-0.14 – 0.14	0.993
Distance to edge (m)	-0.49 **	0.15	-0.79 – 0.20	0.001	-0.19 **	0.06	-0.32 – 0.07	0.003
Mean 1-m ² quadrat litter depth (mm)	-0.07 **	0.02	-0.11 – 0.02	0.002	-0.04	0.05	-0.14 – 0.06	0.428
Mean leaf litter depth (mm)	0.29	0.18	-0.06 – 0.65	0.107	0.11	0.08	-0.04 – 0.26	0.142
No. coffee plants	0.16	0.15	-0.15 – 0.46	0.314	0.07	0.06	-0.05 – 0.19	0.242
Percent crown cover	0.12	0.17	-0.22 – 0.45	0.486	-0.01	0.07	-0.14 – 0.13	0.939
Mean percent soil pH	-0.05 ***	0.01	-0.08 – 0.02	0.001	0.02	0.04	-0.05 – 0.10	0.553
Mean percent soil humidity	0.35 ***	0.03	0.30 – 0.40	<0.001	0.08	0.05	-0.01 – 0.18	0.090
C:N ratio	0.59 ***	0.10	0.39 – 0.80	<0.001	0.06	0.21	-0.35 – 0.46	0.783
% N	0.80 ***	0.12	0.57 – 1.02	<0.001	-0.05	0.21	-0.45 – 0.36	0.820
% C	0.12 ***	0.04	0.05 – 0.19	0.001	0.05	0.08	-0.09 – 0.20	0.476

Notes: Random effects for GLMM: Ant Abundance: $\sigma^2 = 0.01$; $\tau_{00} = 0.24_{ID}$; $\tau_{00} = 0.24_{\text{Sampling_date}}$; ICC = 0.97; N = 9_{Sampling_date}; N = 26_{ID}; Observations = 78; Marginal R² = 0.441; Conditional R² = 0.985. Random effects for GLMM: Ant Richness: $\sigma^2 = 0.09$; $\tau_{00} = 0.07_{\text{Sampling_date}}$; ICC = 0.43; N = 9_{Sampling_date}; Observations = 78; Marginal R² = 0.245; Conditional R² = 0.572. Values in boldface are significant.

** *p* < 0.01; *** *p* < 0.001.

Table 3. Permutational multivariate analysis of variance (PERMANOVA) of local factors and leaf litter ant community composition.

Characteristic	df	SS	R ²	F	p
Tree species	1	0.786	0.034	2.934	<0.001***
DBH (cm)	1	0.510	0.022	1.905	0.02*
Altitude (m asl)	1	0.510	0.022	1.902	0.02*
Slope cardinality (°)	1	0.543	0.024	2.027	0.01**
Distance to edge (m)	1	0.661	0.029	2.467	0.002**
Mean 1 m ² quadrat litter depth (mm)	1	0.430	0.019	1.604	0.061
Mean leaf litter depth (mm)	1	0.364	0.016	1.360	0.144
No. coffee plants	1	0.352	0.015	1.314	0.173
Percent crown cover	1	0.333	0.015	1.245	0.218
Mean percent soil pH	1	0.089	0.004	0.331	0.996
Mean percent soil humidity	1	0.465	0.020	1.736	0.034*
C:N ratio	1	0.230	0.010	0.857	0.631
% N	1	0.425	0.019	1.585	0.061
% C	1	0.300	0.013	1.119	0.315
Residuals	63	16.875	0.738		
Total	77	22.872	1.000		

Note: Values in boldface are significant.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure Captions

Figure 1. Species accumulation curves of leaf litter ant communities under *A. latifolia* and *I. micheliana*. Shaded areas represent 95 % CIs.

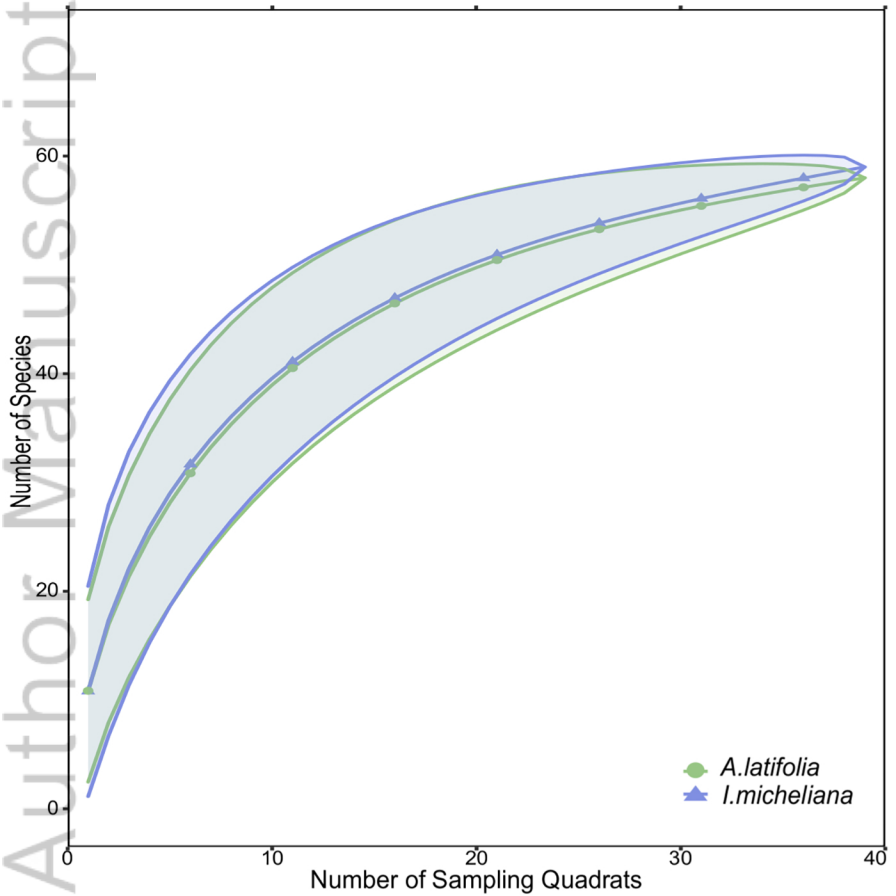
Figure 2. Boxplots of significantly different local factors at *I. micheliana* and *A. latifolia* sites: a) DBH ($p < 0.001$); b) total % N ($p < 0.001$); c) C:N ratio ($p < 0.001$); and d) number of coffee plants ($p = 0.01$). All significant values were computed with paired t -tests. Lines within boxes represent median values, boxes enclose 25th and 75th percentiles, and whiskers enclose 5th and 95th percentiles.

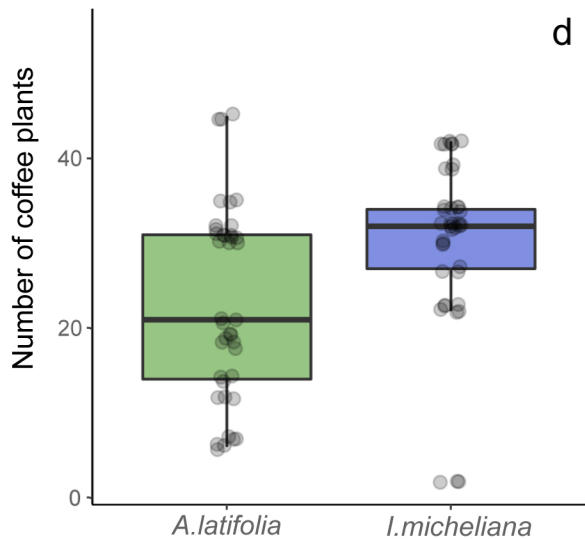
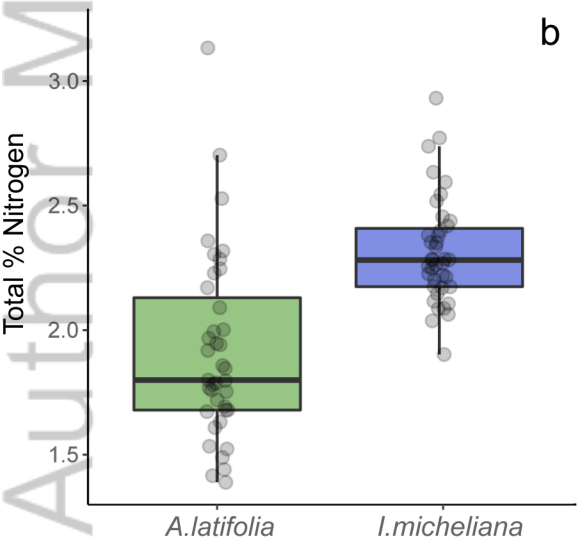
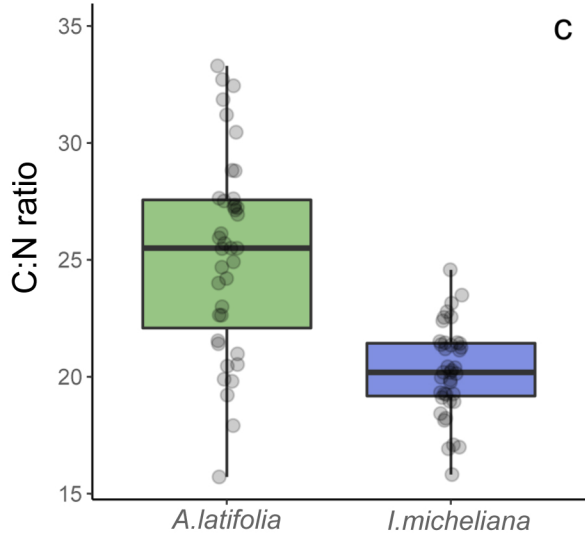
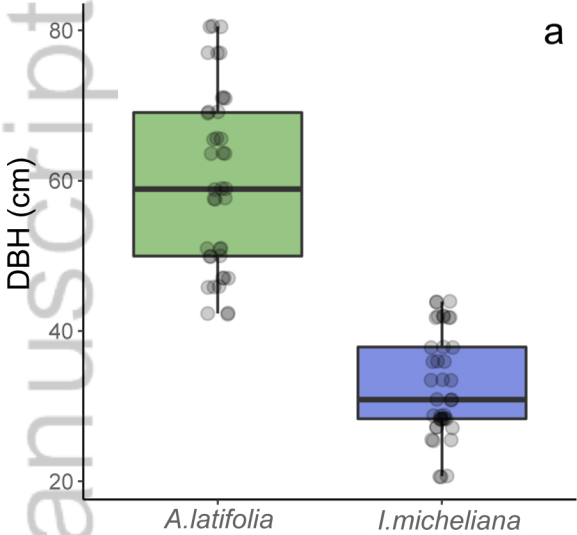
Figure 3. Simple linear regressions between significantly different local factors at *I. micheliana* and *A. latifolia* quadrats and ant abundance and richness in the leaf litter. Regression lines in black represent all data points ($n = 78$). Linear equation, r^2 value and p value correspond to all data points. Shaded areas represent 95 % CIs.

Figure 4. The effect of local factors on ant abundance and species richness in *I. micheliana* and *A. latifolia* leaf litter. A positive value indicates that local factors are positively correlated with ant abundance or species richness. Positive correlations in grey circles and negative correlations in black circles. Values represent log mean estimates of GLMMs. Bars represent 95 % CIs. Asterisks denote significance of effect size (** $p < 0.01$; *** $p < 0.001$).

Figure 5. Simple linear regressions between distance to edge (m) and *I. micheliana* and *A. latifolia* and ant abundance and richness in the leaf litter. Regression lines in black represent all data points ($n = 78$). Linear equation, r^2 value and p value correspond to all data points. Shaded areas represent 95 % CIs.

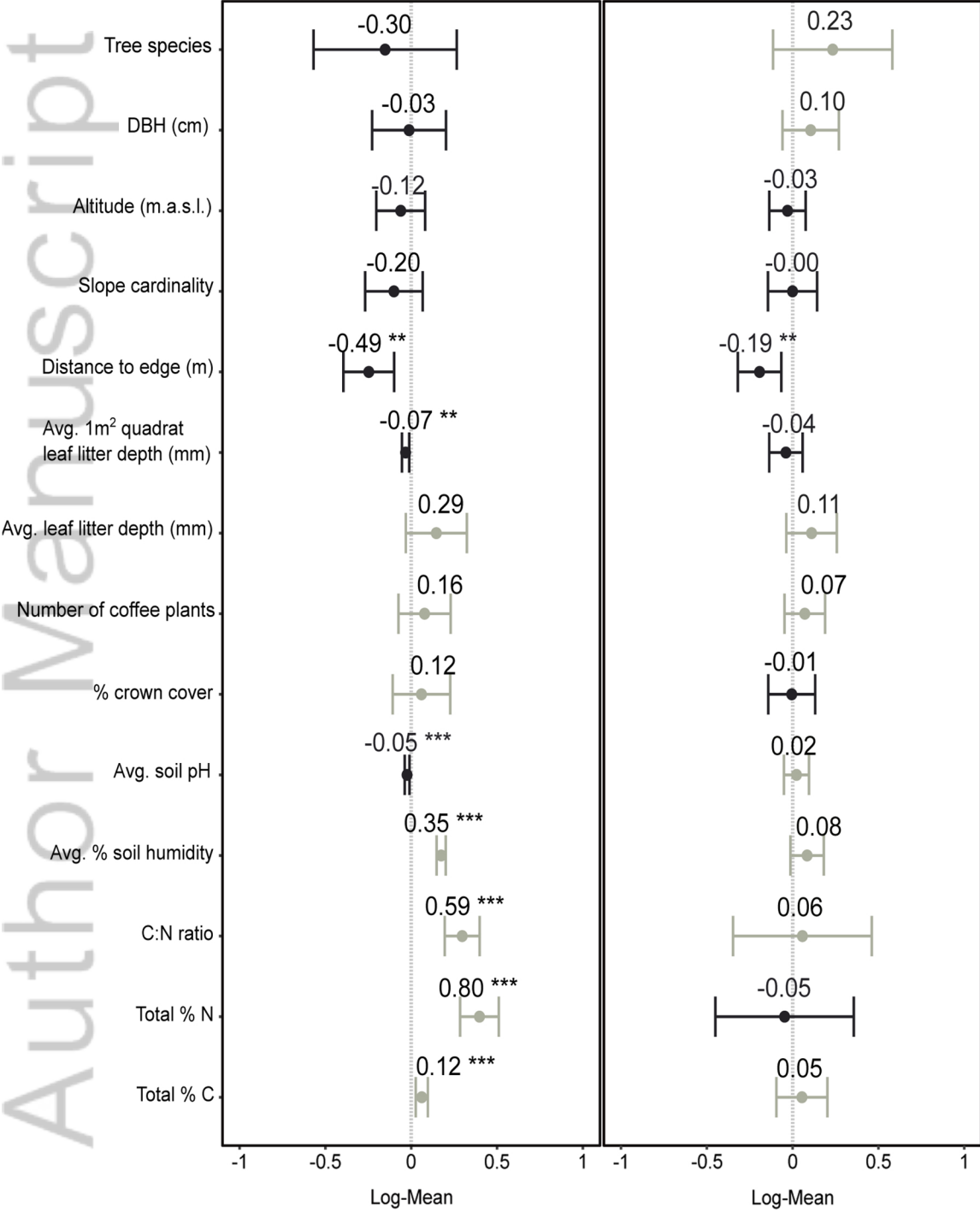
Figure 6. Leaf litter ant community composition associated with local factors. Ant community variation within and between sample quadrats ($n = 78$) under *I. micheliana* and *A. latifolia* trees from distance-based redundancy analysis (dbRDA) models constrained by local factors. Solid lines represent significant associations ($p < 0.05$). Each point represents leaf litter ant community sampled at quadrats.

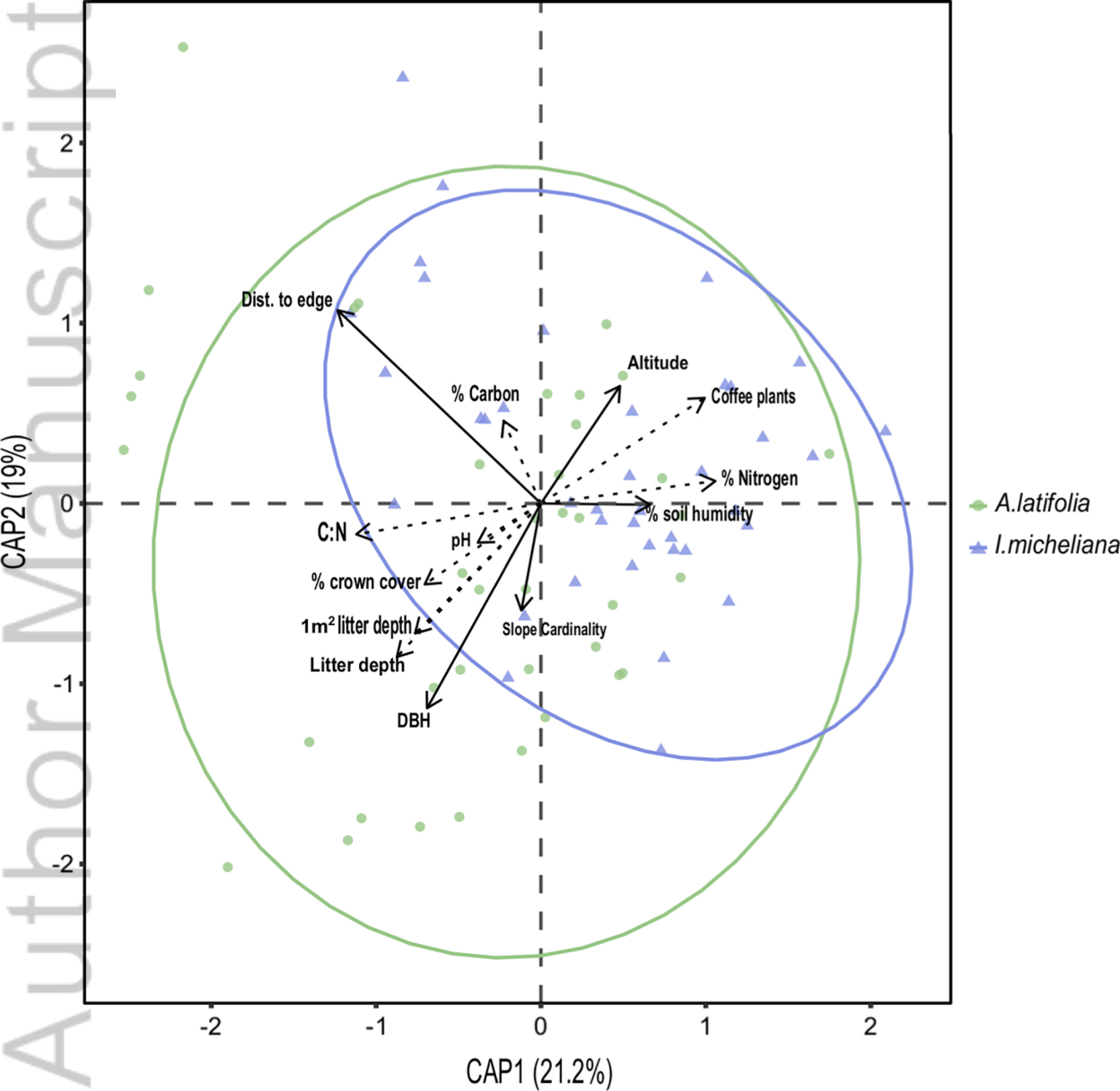


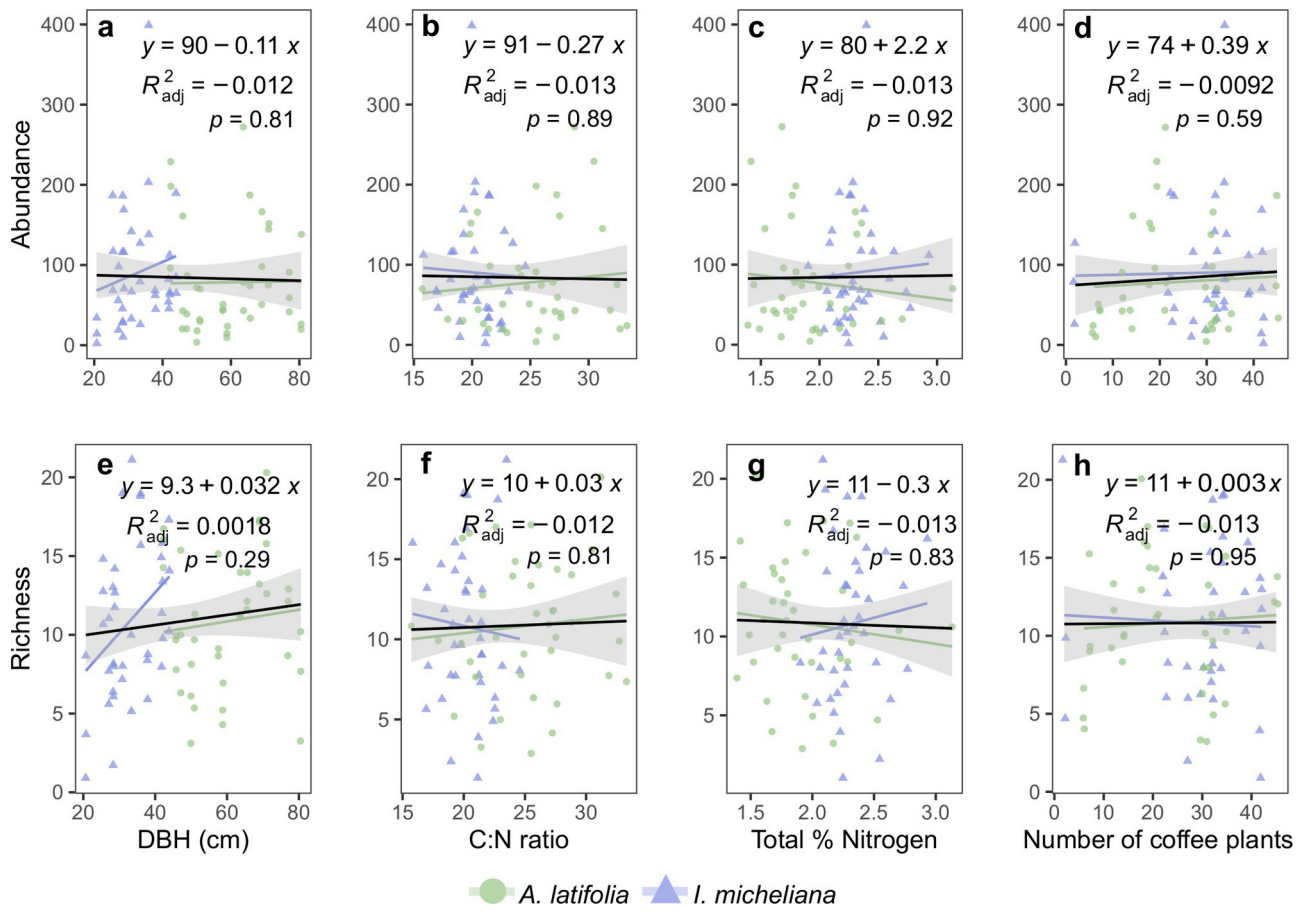


GLMM: Ant Abundance

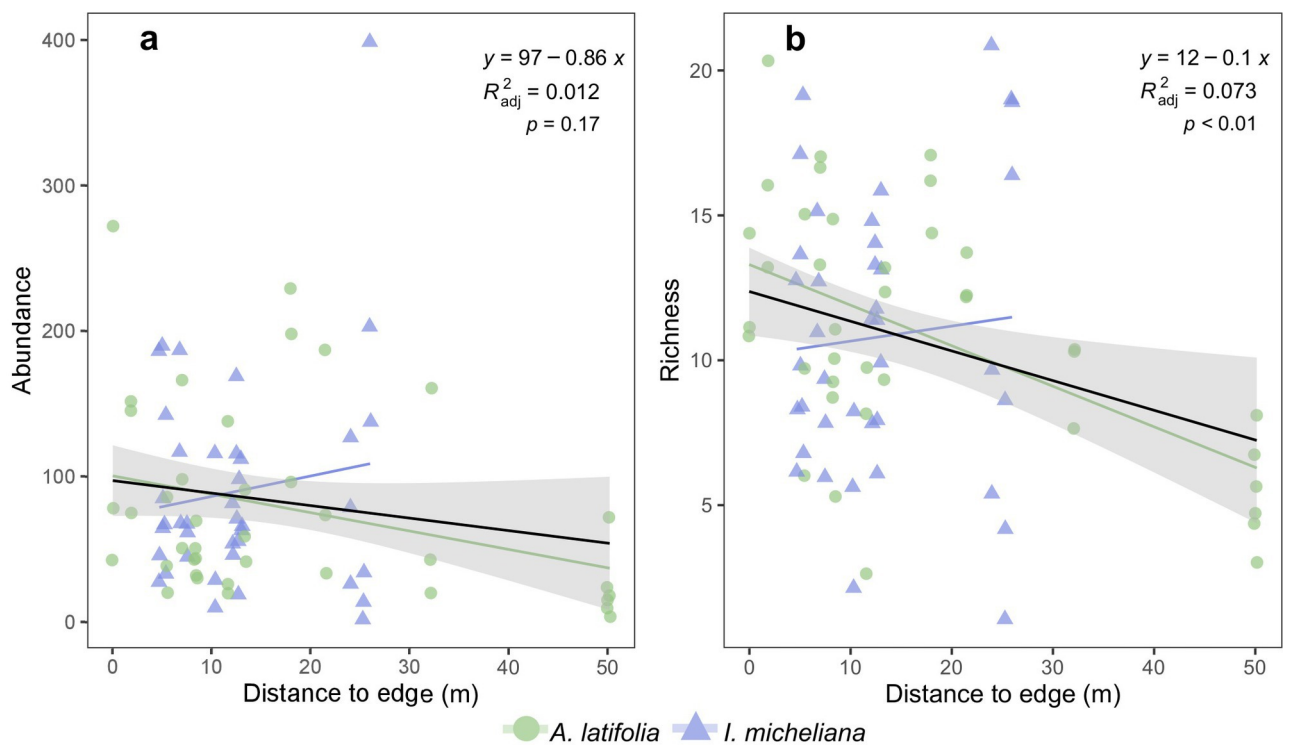
GLMM: Ant Richness







ecs2_4442_aponterolon_figure_3.eps



ecs2_4442_aponterolon_figure_5.eps