EFFECTS OF CANOPY CONNECTIVITY ON ANT COMMUNITY ASSEMBLY
ON A SHADED, ORGANIC COFFEE FARM

By

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Acknowledgements

ABSTRACT

Canopy connectivity influences foraging, dispersal, and competition in arboreal ant species, with implications for ant community assembly. Connectivity among the crowns of shade trees varies greatly with agricultural intensification in agroforestry systems, where some ant species have been shown to act as biological control agents against agricultural pests. Understanding how canopy connectivity affects arboreal ant communities could aid in the development of management practices that maximize biological control services from arboreal ant species. I used a manipulation of connectivity between the crowns of large shade trees to investigate the effects of canopy connectivity on arboreal ant species richness, composition, and co-occurrence rates in a coffee agroecosystem. Further, I examined the effects of the major dominant arboreal ant species, *Azteca sericeasur,* on ant species density and composition on trunks and crowns of upper shade trees.

A linear mixed-effects analysis showed that the number of species observed at baits set in tree crowns increased significantly after the crowns had been connected with nylon ropes ($p = 0.028$). In trees occupied by *A. sericeasur*, lower numbers of species were observed at baits even in the crown ($p = 0.067$). Crowns that were connected increased in similarity of ant species composition, particularly between adjacent connected crowns. Composition also significantly differed between both trunks ($P = 0.003$) and crowns ($P = 0.014$) that contained *A. sericeasur* nests and those that did not. Overall C-scores combined with an analysis of co-occurrence rates of individual pairs of species indicate that this arboreal ant community is not characterized by high rates of segregation, and pairwise competitive interactions are not among the most important forces structuring community assembly here.

In timed observations of connecting lines between tree crowns, only arboreal-nesting ant species were recorded, reinforcing the idea that canopy connections are most significant to strictly arboreal species. Connectivity may increase the number of species present in tree crowns by allowing ants to disperse and forage in the canopy while bypassing trunks with more aggressive, territorial species. While the keystone ant *A. sericeasur* makes heavy use of connections within lower vegetative strata, I found that other species, such as twig-nesting species, are more likely to make use of connections in the canopy above 11 m. Because some twig-nesting species in the upper crown have been shown to act as biological control agents, an increase in species density in tree crowns could have positive implications for agricultural pest control services.

INTRODUCTION

Canopy connectivity shapes arboreal ant communities by mediating movement and access to resources (Powell et al. 2011, Adams et al. 2016) and by influencing competitive interactions (Clay et al. 2010, Davidson et al. 1997, Ribeiro et al. 2013, Stanton et al. 1999), which in turn impacts diversity and composition in ant communities (Yanoviak 2015, Yanoviak and Schnitzer 2013, Yusah and Foster 2016). Because ants are cursorial, central-place foragers, they exhibit strong tendencies to make use of the most energy-efficient pathways to resources (Denny 2001, Loreto et al. 2016) and canopy connectivity allows arboreal ants to bypass travel to the ground, which represents a high energy expenditure and potential exposure to hazards on the ground (Yanoviak 2011). Vegetative connectivity has also been shown to be consequential to ant foraging area and nest site access (Gordon 2012, Loken et al. 2016, Tanaka et al. 2010). Because tree crowns tend not to grow in such a way that they make significant physical contact with one another, lianas are the major source of direct, persistent physical connections in many forest canopies (Yanoviak 2015).

Higher levels of canopy connectivity have been strongly linked to higher species richness and species density in crowns (Adams et al. 2016, Powell et al. 2011, Yanoviak and Schnitzer 2013), seemingly because it increases access to patchy resources and allows expanded foraging areas. Ease of movement has also been observed to favor ant species with certain traits, such as polydomous species with colonies that may "bud" and which benefit from being able to travel between nest sites (Gordon 2012, Tanaka 2010). The effects of connectivity on territorially-dominant arboreal ants (TDAAs), which have long been thought to play a role in structuring the "mosaic" of occurrence in tropical forest canopies (Dejean et al. 2015, Jackson 1984, Majer 1976), has been discussed in the literature with apparently contradictory conclusions. Dejean et al. (2015) and Yusah and Foster (2016) observed an increased abundance of territorially-dominant species in more connected canopy conditions, suggesting that TDAAs will expand territories to adjacent trees under connected conditions, possibly decreasing species richness. However, connectivity could also increase the cost associated with territorial defense (Ribeiro et al. 2013), causing less-connected canopy environments to favor TDAAs (Adams et al. 2016, Tanaka et al. 2011, Tanaka et al. 2012). An understanding of the relationship between connectedness and TDAAs in different environments could reveal the role of canopy connectivity in community assembly in environments where assembly is shaped by aggressive territorial interactions.

On biologically diverse, shaded coffee farms in Mexico, *Azteca sericeasur* is an important TDAA that acts as a keystone species within the arthropod community (Vandermeer et al. 2010, Philpott et al. 2010, Perfecto et al. 2014). *A. sericeasur* constructs carton nests in tree trunks and has polydomous colonies with clustered nest sites (Longino 2007, Vandermeer et al. 2008). Like many arboreal species, it avoids contact with the ground, giving preference to movement through connections in the shrub layer or even on branches and twigs on the ground

(I. Perfecto, personal communication). Connectivity at the shrub layer has been shown to increase *A. sericeasur*'s activity on connected plants (Jiménez-Soto et al. 2018), but little is known about this species' behavior higher in the canopy on the larger shade trees or the effects of tree crown connectivity on this species. *A. sericeasur* is a known biological control agent of *Hypothenemus hampei*, the coffee berry borer, on coffee farms (Morris et al. 2015, Perfecto and Vandermeer 2006, Vandermeer et al. 2009, Vandermeer at al. 2010) and factors driving the spatial patterning of its nests have inspired much study (Perfecto and Vandermeer 2008, Vandermeer et al. 2008, Perfecto and Vandermeer 2015, Li et al. 2016). *A. sericeasur* decreases the infestation rate of the *Hypothenemus hampei* (Morris et al. 2015), which destroys the coffee seed and is considered the most economically-damaging coffee pest worldwide (Damon 2000). Other ant species have also been shown to provide protection from *Hypothenemus hampei* (Gonthier 2013, Larsen 2010) indicating benefits from the ant community as a whole. *A. sericeasur* seems to play a role in structuring the arboreal ant community (Philpott et al. 2010) and changes in connectivity could have an effect on the community mediated by this species.

Due to the potential biological control services represented by the ant community on coffee farms (Gonthier at al. 2015, Morris et al. 2015, Vandermeer at al. 2010) the question of how canopy connectivity affects the diversity and composition of arboreal ant communities has potential implications for agroecosystem management. Vegetative complexity, including canopy structure, varies broadly on coffee farms across an intensification gradient. While forms of shaded production may involve several vegetative strata and an open or closed canopy above the coffee plants, a sun coffee monoculture, the most intensified form of production, involves no shade trees at all (Moguel and Toledo 1999). Studies have linked structural complexity of vegetation to maintenance of ants and other taxa that provide biological control services in coffee agro-ecological systems (Perfecto et al. 1996, Philpott and Bichier 2012), and have linked canopy structure to abundance and distribution of dominant ants and herbivores in unmanaged forests (Lourenco et al. 2015), but none have explored the effects of upper canopy connectivity on richness and composition of the arboreal ant community in agroforestry systems. Understanding potential structuring effects of canopy connectivity on arboreal ant community assembly in agroforestry systems could link management practices to arboreal ants and could lead to improved understanding of agroforestry coffee systems and unmanaged forest systems. In addition, research to date has provided a lot of information about which ant species dominate the smaller *Alchornea latifolia* and *Inga spp.* at the study site (Philpott 2005, Jiménez-Soto and Philpott, Philpott 2010, Philpott et al. 2006) but very little is known about ant activity and composition in the taller, less-accessible shade trees.

I investigated the effects of canopy connectivity on ant species richness and composition in trees on a biologically diverse, shaded coffee farm in Chiapas, Mexico. I asked whether canopy connectivity 1) increased local species density on individual shade trees or 2) caused changes in ant species composition. I focused on how *A. sericeasur* responded to increased connectivity and whether the presence of this species 1) decreased species density on trunks or

crowns and 2) changed the composition of ant species on trees with nests of this species. I also investigated co-occurrence patterns of ant species on trees to gain insight into the potential influences, such as competition or habitat filtering, that might be important to community assembly and that could help me identify the most likely mechanisms for differences in species density and composition.

I predicted that connectivity would increase species density on individual tree crowns by allowing some colonies to increase their foraging areas and by allowing more ants to access available resources. Because I expected an increased interchange between connected trees, I predicted that composition would become more similar among species in trees at each site at the crown level. Due to the aggressively territorial behavior of *A. sericeasur*, I expected to sample lower numbers of species on trees that contained an *A. sericeasur* nest, and I expected composition to differ between trees that had nests of this species and those that did not due to differential aggression levels *A. sericeasur* exhibits among other ant species (Jackson 1984, Blüthgen 2004). Because of the documentation of ant mosaics in simplified agricultural systems with low connectivity (Jackson 1984, Dejean 2003), the possibility that high connectivity prevents effective exclusion of other species by dominants (Ribeiro et al. 2013), and the tendency of connections to allow ant colonies to forage on trees other than their nest tree (Gordon 212), I predicted that segregation would be lower and co-occurrence rates would be higher after connection. I expected *A. sericeasur*'s activity to be higher on trunks and so predicted higher co-occurrence on trunks than on crowns. With connectivity, I predicted that pairwise co-occurrence patterns would be weakened because any segregation due to nest-site limitation should be relaxed if foragers have ability to access parts of the canopy off of their nest tree (Klimes et al. 2015, Tanaka et al. 2010).

METHODS

Study Site and Canopy Structure

This study was conducted on a 300-hectare organic coffee farm in the Soconusco region of the state of Chiapas, Mexico. The farm, *Finca Irlanda*, is located at 15º 11´ N, 19º 20´W in a rural, mountainous area northeast of the city of Tapachula. The area has an average annual temperature close to 20ºC and experiences about 5,000 mm of annual rainfall, though this average has recently been increasing under climate change (Richter 2016). Throughout most of the year, rains occur daily in the afternoon, but the area also experiences a dry season that lasts about two months (Richter 2016). Under the coffee classification system of Moguel and Toledo (1999), the farm is considered an organic, commercial polyculture. Data for this study was collected between 925 m and 1,075 m above sea level, between June 2017 and July 2018.

Although the farm has more than 100 species of shade trees (Perfecto and Vandermeer 2006), the majority of shade trees on Finca Irlanda are small, less than 15 m in height, and these are

dominated by species in the genus *Inga* (Vandermeer et al. 2008, Li et al. 2016). Larger shade trees, of about 25-30 m in height, form a second stratum in parts of the farm and common species include *Schizolobium parahyba*, *Ocotea spp.*, and *Albizia spp* (Vandermeer et al. 2008, Li et al. 2016). This study focused on the upper canopy layer in part to more closely mirror the height of an unmanaged forest and to target ant species that are strictly arboreal. The upper height at which *A. sericeasur*, an established biocontrol agent and territorially-dominant species, tended to forage and exploit resources had not been documented before this study. Management on *Finca Irlanda* currently includes periodic aggressive trimming of smaller trees, which reduces connectivity in the lower canopy layer. While upper canopy trees are not subject to the same trimming, lianas climbing their trunks are cut and removed, usually preventing lianas from growing to the extent that they could create canopy connections (personal observation).

Experimental Design

I artificially manipulated canopy connectivity using braided nylon lines. Because ants are likely to choose routes based on energy expenditure, I chose a surface comparable to lianas, small branches, or other natural crown connections (Clay et al. 2010). Using ropes to connect tree crowns allowed the experimental increase of physical connections without changing other environmental conditions, including microclimate.

Due to my interest in exploring the effect of canopy connectivity on *A. sericeasur*, I included one tree with an active *A. sericeasur* nest at each site. I selected trees that were in the upper stratum (~25-30 m in height) and that were growing close to each other but had no or very minimal physical contact between crowns. In order to allow for investigation of ant movement and effects over more than one crown connection, I selected groups of three trees, each with one tree occupied by *A. sericeasur* and two others that were not (the trees are referred to hereafter by their position in a site as "nest tree," "middle tree," and "end tree." Due to limitations of the number of trees on *Finca Irlanda* that fit these criteria, I included some trees whose canopy was lower than trees in the upper canopy. After selecting all tree triplets I randomly selected half of the sites to which to apply a connectivity treatment. I used one rope to connect the crowns of the nest tree and middle tree and a second rope to connect the middle tree and the end tree (Fig. M.1).

I collected a pre-treatment observation of all of the trees in the manipulation, and then connected the crowns of trees in the connectivity treatment immediately after this initial observation. I sampled all of the trees by baiting both crowns and trunks with two sugar-based baits (honey and jam) and one bait containing urea and salts (cotton balls soaked in mammal urine) (Arcila Hernández et al. 2012, Powell 2008). Response to nitrogenous wastes can be indicative of nitrogen-limitation in arboreal species (Davidson 1997, Yanoviak and Kaspari 2000). Baits high in both fat and proteins were avoided to decrease the chances of exclusion of species from baits before they were recorded (Perfecto 1994).

In order to gain initial access to selected trees to perform my observations, I used an arborist slingshot ("bigshot") to set lines reaching up to the crowns, above the point where main branches began to diverge from the trunk. After baiting the trunk of a tree at breast height with one cotton ball and one 8 x 8 cm patch of honey and one of jam applied directly to the bark, I ascended trees using single-rope technique (Perry 1978) to set the same set of baits in the crown on the center or main limbs at the height of the climb line. I waited for 30 minutes (this tends to be enough time to see recruitment to baits but not exclusion, I. Perfecto, personal communication). After 30 minutes, I recorded all ant species at baits, a categorical abundance value for each species, a value indicating the degree of sun and rain, the time of day, and notes on behavior. I also recorded species that did not feed on the baits but which passed within the baited area during the 30-minute timeframe. Then, I tied a nylon rope to the trunk or a main limb if the tree was in the treatment group. I also measured the distance from baits to the ground, distances between trunks of the adjacent trees in the plot, and the height of each tree crown.

In approximately 2-4 weeks' time from the initial observation at a given site, I returned to re-bait the trees and record species present, abundance, and behavior (I was not able to control variations in time but checked for effects of this variability in my analysis). Because this manipulation took place during the rainy season, I conducted all observations between 8 am and 2 pm, using only data that could be fully collected before the onset of rain.

From late June through early August of 2017, I established and successfully collected data from 24 trees at eight sites, four in the connectivity treatment and four unconnected control sites (Fig. M.2). In late June and July of 2018 I added and recorded data at twelve new trees in four additional sites and conducted a third sampling of all trees from the previous season that still had all trees and any ropes at a site intact. Two of the sites, one a treatment site and one a control site, had trees or ropes missing for which I was unable to collect data. All 36 of the trees in the experiment were sampled twice, most with between two and three weeks between the first and second sampling. Eighteen of the trees were sampled a third time, after one year, this sampling using three sets of baits. On the final observation of each of the sites I staged 10-minute observations of ant activity from both sides of the connecting ropes, recording how many of each species passed a selected point on the rope in either direction.

Other Variables Measured

In addition to the abundance and identity of ants arriving at baits, I collected data on a number of environmental variables with potentially confounding effects on diversity and composition. These include site, tree health, epiphyte cover, tree species, the height of baits from the ground, the presence of bees at baits, and weather. To minimize the effects of site, I paired sites in my design so that each site at which trees were connected was as close as possible to a site where the trees were unconnected. I estimated tree health based on a number of signals that most often indicate vigor or disease. These included bark health--whether patches were exfoliating or whether the bark was intact; signs of core rot such as water seeping out of the trunk; size and

number of dead branches, especially the presence of large dead limbs; and whether foliage had sustained large volumes of blight and herbivory. Trees scored a number from 1-5 based on these criteria, and when one of the trees was killed by a lightning strike it was scored at 0. Epiphyte cover was scored on a similar scale based on an estimate of the percent of trunk and branch surface covered and the size of the largest epiphytes. The recruitment of *Apis mellifera* to certain sugar baits caused me to record the presence of this species. Finally, weather was recorded in categories ranging from 0 to 6 based on the levels of precipitation and sunlight.

Statistics

Richness

In order to test for changes in species richness at baits, I first performed a paired t-test to compare initial observation species totals with second observation species totals in both the connected trees and the trees in the control group. Based on the significant differences I found in paired t-tests, I chose to analyze the richness values from baits in tree crowns with a linear mixed model in order to account for the effects of environmental variables and non-independences.

I used the nlme function in the lme4 package in R to run a linear mixed effects analysis (Bates et al. 2015). I constructed models including treatment group, weather ranking, epiphyte cover, tree species, height of baits, tree position, presence of bees, tree health, observation number, and application of the connectivity treatment as fixed effects and including site and the specific tree a sample was from as random effects. I selected the model with the lowest AIC value and ran a likelihood ratio test using the selected model and a version of the same model without the variable for applied connectivity as the null model. I confirmed that the response variable was normally distributed through inspection of quantile plots fitted to normal and other potential distributions. I confirmed assumptions of normality and homoscedasticity of residuals and lack of multicollinearity in the final linear mixed model through inspection of visual plots. I obtained an effect size by running my model in lme4 and derived significance values for all fixed effects included in the final model by performing a Wald test using the Anova() function from the "car" package in R (Fox and Weisberg 2011).

To estimate the total number of species in control and treatment sites, as well as at all sites sampled as a whole, I used sample-based incidence data to construct rarefaction curves based on the Mao Tau estimator using the "specaccum" function in the "vegan" package in R (Colwell et al. 2012, Oksanen et al. 2018). I used plots of these rarefaction curves to assess significant difference between species density values in treatment and control trees and in trunks and crowns based on overlapping confidence intervals (Colwell et. al. 2004).

Composition

I used non-metric multidimensional scaling to create plots that allowed me to visualize composition grouped by crown and trunk, treatment group, and tree position within different observations. I then used an analysis of similarities (ANOSIM) to test for significant

compositional differences using dissimilarity matrices created using the Sørensen dissimilarity index. ANOSIM allows for comparison of compositional differences between and within specified groupings using resampling to create a probability distribution (Oksanen et al 2013). I performed these analyses using the "metaMDS," "vegdist" and "anosim" functions in the vegan package in R (Oksanen et al. 2018). I set a seed for each of the ANOSIM null models at "23" to allow for replicable results, and ran the default of 999 permutations.

Co-occurrence

Rates of co-occurrence at sampled sites of pairs of species can be measured with a number of indices and these scores can be compared to null models generated by a number of similar methods of resampling from a matrix of species incidence by site (Gotelli 2000). I used the C-score metric (or "Checkerboard score," see Stone and Roberts 1990) and the SIM9 algorithm in the "ecosim" package in R to compare overall rates of co-occurrence in treatment and control groups, as well as on trunks and on crowns (Gotelli et al. 2015). I set a seed of 23 and ran 10,000 repetitions for all null models. I derived p-values for community-level co-occurrence rates from the one-tailed output comparing observed C-score to a null distribution created with SIM9. In order to compare the co-occurrence rates of individual species pairs and to calculate the probability that pairwise co-occurrence rates would be observed by chance, I used the function cooccur in the "cooccur" package in R with default settings (Griffith et al. 2016). This function uses a probabilistic model to calculate the likelihood of observed co-occurrence for individual pairs with sufficient data in the observed matrix (Griffith et al. 2016). Based on significantly high or low rates of co-occurrence between individual pairs, I searched through natural history information I had collected or had been locally reported that could explain interactions driving any non-random co-occurrence patterns between ant species.. I chose to examine individual species co-occurrences in crowns because the total number of species observed was smaller than the number observed on trunks, even though baits on crowns attracted about the same number of species per crown: the observation of fewer species in more samples provides more data for identifying exceptionally high or low pairwise co-occurrence. Ants on crowns were also more likely to be arboreally-nesting species rather than ground-nesting species, making inferences about potential species interactions simpler.

RESULTS

Number of Species per Tree

A paired t-test showed a significant difference in the mean number of species in connected tree crowns before and after connection (t = -3.6878, df = 17, p = 0.0018), but no significant difference between the means in the control crowns (t = -0.60924, df = 17, p = 0.5504). For the number of species found on baits set on trunks in trees with connected crowns,

there was no significant difference in the treatment group (t = 0.66886, df = 17, p = 0.5126) or in the control group (t = 0.10802, df = 17, p = 0.9152, Fig. R1).

In the linear mixed effects analysis of data from observations 1 and 2 (Table R.1), bee presence decreased the number of species observed at baits by 1.595 species, but this effect was marginally significant (p = 0.067). Tree health was positively associated with the number of ants at baits, with an effect size of 0.452 (p = 0.031). The observation (first or second) under which data was collected had an effect size of 0.418, with a higher number of ant species observed on the second observation, but this effect was not statistically significant (p = 0.261). A bait's presence on the end or middle tree at a site was marginally associated with a higher number of species, increasing the number of species per crown by 1.086 and 1.042, respectively (p = 0.067). The application of the connectivity treatment to a crown was associated with a 1.040 increase in the number of species observed (p = 0.028). A likelihood ratio test comparing full and null models showed that artificial connectivity affected richness at baits ($X^2(1)$ = 4.98, p = 0.0257), increasing it by 1.145 species per bait.

The same model had the lowest AIC values when data from the third observation was included. This model was significantly different than a null model excluding the connection variable (p = 0.03327 * in a likelihood ratio test). Tree health and the application of the connectivity treatment were still the only significant variables for a 95% confidence interval (Table R.1).

Species Density per Site

A comparison of sample-based rarefaction curves shows a significantly lower number of species present in the control versus treatment group during the first observation (before connectivity treatments were established in the treatment groups) with 32 species found at the control sites and 22 species found at treatment sites. The average number of species found per crown was 4.28 in the treatment crowns and 4.83 in the control. Rarefaction curves created from the second observation, however, show no significant difference between the number of species in crowns in the treatment versus control group (Fig. R.2). Total species counts on the second observation at the control and treatment sites totaled 30 and 27 species, respectively, with an average of 5.61 species in the connected crowns and 4.44 in the control crowns. The species accumulation curve for all baits used during the study uses information from 184 baits with a total of 102 morphospecies and begins to level off, signalling an approach toward an asymptote (Fig. R.3). The rarefaction curve for crowns in the study was closer to reaching an asymptote than the curve for trunks, with 57 species found in baits in crowns over the course of the study as opposed to 87 species found on trunks (Fig. R.4).

Composition

ANOSIM results showed that composition initially (before connection) differed significantly (R = 0.158, p = 0.047) between crowns in the connectivity treatment when grouped

by position as the tree with an *A. sericeasur* nest, trees in the middle (with a connection to both other trees), and end trees (connected only to the middle tree) (Table R.2). On the second observation, connected crowns did not differ significantly when grouped by position (R = -0.028 p = 0.603). Crowns in the control group, in contrast, showed no significant difference initially (R = 0.037 p = 0.300) but on the second observation did differ significantly (R = 0.179, p = 0.019) (Fig. R.5, Table R.1). On trunks, the difference in composition within the treatment group decreased only slightly (from R = 0.390, p = 0.001 to R = 0.243, p = .006) while dissimilarity in composition on trunks in the control group decreased very slightly and was not significantly different on the second observation (R = 0.149, p = .048 to R = 0.1315, p = .079, see Fig. R.5). In order to look into the pattern in the connected trees further, I compared individual dissimilarity indices calculated for each connected pair of trees before and after treatment, which indicate increased compositional similarity for fourteen out of eighteen pairs and decreased similarity in only four out of eighteen pairs (Table R.3).

Crown and trunk composition varied significantly on both the first (R = 0.127, p = 0.001) and second (R = 0.129, p = 0.001) observations. Trunks, as expected, had significantly different composition if the tree had an *A. sericeasur* nest (at observation 1, R = 0.214 and p = 0.003). Crowns in trees with *A. sericeasur* nests had significantly distinct composition as well (at observation 1 R = .0.176, p = 0.014), despite reduced dominance or even absence of *A. sericeasur* on crown baits. For an ordination illustrating the differences in composition between trunks and crowns and *A. sericeasur* presence and absence, see Fig. R.7.

Co-occurrence

On the second observation, ants on trunks in the control group exhibited a significantly high C-score (p = 0.046 for a one-tailed test), while the treatment trunks (after connection was applied) had a C-score near the mean of the distribution of the null model (p = 0.340) (Fig. R.8). However, the C-score for both the treatment and control trunks on the first observation was not significantly higher than the mean of the null models (Upper-tail p = 0.276 and Upper-tail p = 0.740, respectively) (Fig. R.8). The C-score values obtained for all crowns and all trunks did not differ significantly from null models collectively in observation 1 or 2.

Thirty-eight species total were found at baits in crowns on the second observation. In a test for pairwise co-occurrence values for the second observation, 109 out of 703 potential species pairs were analyzed (594 would have had an expected random co-occurrence rate < 1, so the data were insufficient to assess likelihood of observed co-occurrence rates) (Griffith et al. 2016). Two of these species pairs were associated positively, three were associated negatively, and 104 co-occurred within the expectations of random co-occurrence. *A. sericeasur* had a co-occurrence rate with *Crematogaster crinosa* significantly lower than would be expected if the two species co-occur randomly (p = 0.04629). *Cephalotes basalis* co-occurred less than expected with *Camponotus canescens* (p = 0.01336) and *Pseudomyrmex* sp. 1 (p = 0.03941). *Camponotus*

textor co-occurred more than expected with *Pseudomyrmex ejectus* (p = 0.008) and *Pseudomyrmex gracilis* (p = 0.031) (Fig. R.6).

DISCUSSION

Species Density

The significant increase in the average number of ant species found at baits in each tree crown between the first and second observation suggests an effect from the rope connections in treatment crowns. However, the paired t-test compares tree crowns, and not every one of the crowns was independent; connected trees are in fact likely to influence one another if the ropes used in the treatment group did indeed facilitate ant movement between crowns. Additionally, these results do not control for an effect of the second observation, and the number of average species at baits did rise very slightly in the control group, possibly indicating a change associated with the second observation such as a seasonal difference or a sampling difference. The results of the linear mixed model, which does control for the differences in samples associated by observation, with individual trees, and with tree position, confirm that connectivity does have an independent effect on species density.

The positive effect of artificially-increased connectivity on the number of ant species found per crown supports the hypothesis that increased connectivity increases the number of ant species occupying a tree crown. The effects of tree position should account for the separate and combined effects of differences in treatment based on the presence of *A. sericeasur* nests and the difference in the number of ropes tied to the crown, which was two for the middle tree and one for the two other trees. Both the "bees" and "tree position" variables improved the AIC values of the linear mixed model and were statistically significant in some of the models but not in the final models.

The positive association between number of species at baits and tree health was counter to my expectations; I had predicted that trees in poor health would harbor more ant species than healthy trees due to increased nest sites in tree cavities, dead branches, and peeling bark. The elevated number of species on healthy trees could represent a response to increased extrafloral nectaries. Extra-floral nectaries, a sugar source present on certain shade trees, are an important food source for many arboreal ant species and could lead to positive association between tree health and ant species (Blüthgen 2004, Livingston et al. 2008). Another major food source for arboreal ants, hemipteran honeydew (Blüthgen et al. 2003, Hunt 2003) could also be higher on trees growing more vigorously; the relationship between sap-feeding performance and tree stress is mixed in the literature, and likely conditional on environment, plant defensive strategies, and stress type (Galway et al. 2003). However, arboreal twig-nesting species studied at this field location are nest site limited (Jiménez-Soto et al. 2015) and nest-site limitation could make sensitivity to an increase in other resources less likely.

The analysis shows connecting ropes having a slightly smaller effect of the number of ant species found per crown when the data from the third sampling is included. The data collected on the third observation show an even smaller effect when compared to the first observation data, but this model is also unbalanced due to the fact that far fewer trees were observed on the third observation than on the first. While not conclusive, available data do suggest that the increase in species density with new connections could be temporary, tapering off slightly after a year's time.

The lack of significant change in the number of species sampled on trunks between the first and second observations in both the treatment and control groups indicates that the changes in species density measured by baiting in the crowns did not correspond with a change of the number of species on the trunk near ground level. Perhaps the most straightforward explanation for this is that gains in the species density in crowns were due to the presence of species that are more specialized to life in the tree crown and do not forage on or exploit resources on the ground or lower on the main trunk. For example, some of the species of twig-nesting ants found on *Finca Irlanda* stay farther out on branches and are less likely to be found on the trunks of smaller shade trees (Philpott 2010).

The rarefied species accumulation curve from all samples on trunks in both the treatment and control groups shows a much steeper accumulation than in crowns. The trunk curve levels off much less than the crown curve does, although neither curve reaches an asymptote. The fact that comparable mean numbers of species were found at baits on individual crowns and trunks, but trunks as a whole showed a larger species density, illustrates the much greater heterogeneity in composition on trunks. These trends are most likely driven by the fact that species found on trunk baits had high representation of both ground-nesting and ground-foraging species and more strictly arboreal species, while the species in crowns largely only represented arboreal species. The more stable, self-contained community in crowns, along with the fact that the effects of connectivity were more specific to tree crowns, caused me to focus my analysis on changes to diversity and composition in crowns.

The comparison between species accumulation curves for crowns in the treatment and control groups (Fig. R.2) is less straightforward. For the second observation, the confidence intervals overlap for smoothed species accumulation curves for connected and control crowns, indicating no difference in species density between the treatments. Yet, for the first observation, the species accumulation curves for the trees selected for treatment had non-overlapping confidence intervals beyond eight samples (Fig. R.2). This observed change in the shape of the species accumulation curve does not concord with my predictions; I did not expect the short-term presence of ropes in the canopy to increase the number of species present at a site and therefore did not predict an increase in the species density at the site level. The average number of species observed on each tree crown was predicted to increase if additional species were due to traffic from ants on adjacent trees, but an increase in the number of species found in all tree crowns in a treatment group would not result from interchange among the sampled trees. Because species

accumulation curves scaled to sample are a measure of species density, not a reliable estimate of the total number of species found throughout the tree crown, the increase in the total number of species could indicate that species that would otherwise have been elsewhere in the crown were drawn to the sampling areas. It is possible that placement of connecting ropes increased ant activity locally in the baiting area on each crown because baiting areas were near connecting ropes, which represent efficient pathways and a high-traffic area. This attraction effect could have increased the proportion of total species present that were found at baits.

However, the fact that the largest difference was in curves for the two treatment groups in the crowns before application of the treatment suggests that there could have been an asymmetry in my effective sampling effort. I observed very similar curves with overlapping confidence intervals for species found on trunks in the initial observation of both treatment groups, and there should have been no systematic differences between the two groups initially: sites in the two treatment groups were not clustered together, but rather spread out with pairs consisting of one treatment and one control site occurring close together in environmentally similar areas (Fig. M.2). One possible explanation for the observed difference in the initial curves is that trees in which ropes were tied were more disrupted during the climbing process, causing fewer species to come to baits: bringing anchored ropes into the tree crown was often a difficult process that involved disturbing tree branches and more movement within the tree.

The difference in the number of species observed per tree crown, which is the data used in the linear mixed models, differs between treatment groups much more strongly for the second observation (treatment mean = 5.61, control mean = 4.44) than on the first observation (treatment mean = 4.28, control mean = 4.83). Although the species accumulation curves for the two treatment groups do not differ on the second observation, indicating similar species density between each of the two treatment groups as a whole, the species density of individual crowns was over 25% higher in the connected crowns than in the control crowns. This difference in tree occupancy *within* sites combined with comparable species density *between* sites strongly supports the hypothesis that connectivity increases tree-level species density by facilitating exchange between trees. This increase in tree occupancy mirrors the results from a very similar manipulation of connectivity in the Brazilian cerrado (Powell et al. 2011).

Composition
Relatively few studies have looked at changes in compositional similarity between trees under connected and unconnected conditions. Klimes at al. (2012) found that there were actually more compositional differences between trees in a primary forest with a more connected canopy, although this was in comparison to a secondary forest with less environmental complexity and diversity overall. In this study, the change in homogeneity in connected crowns supports the hypothesis that increasing connectivity in the canopy would create more similarity in composition between connected crowns. However, the lack of significant difference in composition in the control crowns initially and unexplained variation in the R value leaves the

results inconclusive overall. A comparison of compositional similarity with these methods only between connected trees might be possible by modifying plots to contain larger numbers of connected trees. It is important to consider, for the tests of similarity by tree position, that the ANOSIM test is known to not be robust to heterogeneity, producing low p-values when dispersion is not equal between groups (Anderson and Walsh 2013), and the differences in the number of species found on trees in different positions may have created differences in dispersion between groups in these data.

Comparison of differences in composition by tree position (nest, middle, or end) was intended to allow the detection of potential homogenizing effects due to artificial connectivity. The decrease in the R value in the connectivity treatment (from R = 0.158 to -0.028) indicates that connectivity may have made composition more homogenous between connected crowns, erasing any effects of being on or adjacent to a tree with an *A. sericeasur* nest, which would be the main driver of difference in composition of trees by position.

In addition to examining compositional similarity by tree position, the similarities in composition between individual trees that were connected to each other can also be compared directly using the values from the dissimilarity matrix used with the ANOSIM function. Because the compositional similarity between most connected pairs of trees increased, the homogenization of composition in the nest, middle, and end tree groups that corresponded with connection seems more likely to be a result of increased species exchange over the connecting ropes. My field observations also support the idea that species counts resulted from interchange over the connecting ropes: I repeatedly noted species that had been found on only one crown on the first observation but were found on both on an adjacent crown and the connecting rope on the second observation. This observation reflects the Klimes et al. (2015) finding that trees in a primary forest with higher connectivity had greater occupancy in tree crowns in a lowland rainforest because foragers could access resources on trees they were not nesting in.

The significant difference in species composition on trunks and crowns found on the first and second observation demonstrates that trends in composition correspond with location on the trees. Visual inspection of the ordination for all samples from the first observation (Fig. R.7) shows that species found in crowns were also found on trunks, but not vice versa. This trend reflects the fact that samples on trunks (which were placed at approximately breast height) often attracted ground-nesting species, which did not tend to be found at baits placed in the tree crown. Some of these species normally forage on tree trunks, while a few may have been ground-foragers attracted to the low baits. Most species sampled in the crown, in contrast, were observed throughout the tree, even on the lower trunk. In Fig. R.7, gold lines branching from a centroid to each sample on a tree containing an *A. sericeasur* nest show the clustering of the 24 samples located on trees at which this species was nesting. Even though *A. sericeasur* was often not found at baits in nest tree crowns, the visible clustering of sample composition in *A. sericeasur* crowns and the significant compositional similarity suggests that the presence of an *A. sericeasur* nest significantly alters composition at the canopy level, although not to the degree

that it does on the lower trunk, where this species was almost always observed to be more abundant and more dominant at baits.

Co-occurrence

C-scores from the treatment and control trunks on the second observation appear to support the hypothesis that connectivity would cause less segregation or cause more random co-occurrence because the control trunks have a significantly high c-score and the trunks of connected trees do not. However, the fact that trunks in both the treatment and control group do not have significantly high scores before treatment suggests that significant segregation on all trunks may never have existed, as does the fact that combined data for the two groups did not have a significantly high or low C-score for crowns or trunks on the first or second observation. Overall the C-scores found here indicate that this is not a community characterized by high rates of mutual exclusion and non-overlap. While significant pairwise co-occurrence rates are not the norm for most communities, the literature on arboreal ant community assembly is replete with discussion of "mosaic" arrangements of mutually-exclusive territorially-dominant species and other examples of non-random co-occurrence (Dejean et al. 2015, Jackson 1984, Majer 1976). One proposal for explaining why some studies of arboreal ants have reported "mosaics" while others have not is that fierce competition may drive this pattern in the upper canopy but not in lower canopy (Ribeiro et al. 2013).

The lack of high C-scores in crowns as well as trunks does not support the prediction that co-occurrence rates would be higher in crowns due to nest site limitation and competition for nectar and honeydew. However, Ribeiro et al.'s prediction of increased territoriality in the upper canopy due to the presence of persistent resources like nectar and honeydew applies to a tropical forest with a closed canopy that keeps most sunlight in the upper stratum. The agroecosystem in which I conducted my fieldwork had high amounts of light reaching the lower strata, and honeydew and extrafloral nectaries are commonly exploited by ants in the shrub layer and on small trees and shrubs, making the mechanism Ribeiro et al. proposed to explain their findings likely not applicable in this system.

Non-random species co-occurrence for arboreal ants may be driven by environmental filtering, such as among species that tend to co-occur because they make use of similar resources (Fayle et al. 2015); it may also be driven by competition, in which case species would co-occur less than expected by chance independently of any environmental factors (Camarota et al. 2016, Ribas and Schoereder 2002). These two potential influences on co-occurrence patterns may also interact when resource use influences competition and cannot be inferred from co-occurrence rates themselves. Analysis of co-occurrence patterns, therefore, has the best potential to reveal evidence of community assembly rules when examined in conjunction with relevant natural history traits of species (Ribas and Schoereder 2002, Kohli et al. 2018). While I do not have the data to conduct a comprehensive analysis that would provide generalized information about what kinds of environmental filtering or biotic interactions contribute most to arboreal ant community

structure in the study agroecosystem as a whole, I used known or observed information about nesting habits, colony size, diet, and territorial behavior to attempt to identify credible causes in cases of particularly high or low individual pairwise co-occurrence rates.

In the case of the low co-occurrence rate of *A. sericeasur* and *C. crinosa* observed in this study, both species are territorially aggressive species that form large colonies and exploit extrafloral nectaries and hemipteran honeydew (Longino 2003, Longino 2007, Vandermeer et al. 2006), making them likely candidates for mutually-exclusive territorial behavior (Dejean and Corbara 2003). In addition, Philpott et al. (2018) found *C. crinosa* to occur significantly less on trees with *A. sericeasur* nests at same field site. *C. basalis and Pseudomyrmex. sp. 1*, which co-occurred less than expected, are both twig-nesting species (Yitbarek and Philpott, unpublished data) making competition for resources a plausible driver of this pattern (although during the study I always observed *C. basalis* nesting in tree cavities). Yitbarek and Philpott (unpublished data) found that twig-nesting ants from *Finca Irlanda* form dominance hierarchies over control of nest twigs in lab experiments, and while *C. basalis* was not one of the species ranked in the experiments, this species is so numerically dominant on trees it nests in (personal observations) that exclusion of species that could compete with it for nest sites is a possible explanation for low co-occurrence rates. For the remaining six species pairs that co-occurred more or less than expected by chance, I was not able to formulate a likely cause for high co-occurrence rates based on known behavioral or natural history traits, and based on my field observations these particular pairings seem likely to be random.

If a high number of co-occurrence rates between species pairs are tested for significant divergence from the expected range for random occurrence, one would expect the that a few of these pairs would fall outside of a 95% confidence interval by chance. (In fact, five pairs is almost exactly the 5% of 104 pairs that one would expect to deviate by chance, although most of the 104 pairs whose co-occurrence rates were tested were found in a small enough portion of the samples to make detection of any patterns difficult). According to the ant mosaic hypothesis, the predicted significant non-random pairwise interactions would likely be relatively common territorially-dominant species that I would have found often during sampling, such as *A. sericeasur* and *C. crinosa* (Jackson 1984).

Limitations

Testing whether composition varies by tree position is problematic because the independent variable does have a relationship to composition inasmuch as one tree was selected due to the presence of *A. sericeasur*. *A. sericeasur* is included in the ANOSIM tests and could not be omitted as it is sometimes the only species present for a sample. This means that the presence of *A. sericeasur* itself is confounded with any effects it may have on other species to change composition.

Due to restrictions in access to trees at certain times, the length of time that the trees were connected in this study could not be held constant. The shortest length of time that trees in a plot

were connected by the first measurement was 12 days, while a few trees were not observed until after 50 days had passed. However, the number of days that a rope was in a tree was not correlated with the change in the number of ant species per crown (Pearson's correlation coefficient < 0.005). Visible inspection of plots also showed no other discernible relationship between the amount of time trees had been connected by the second observation and the change in the number of species observed at baits. For this reason length of time of connection was not included as a variable in further analysis.

Because I placed first one and later three sets of baits in a central location in each tree crown, I was not able to construct species accumulation curves for each tree crown, which would have required very large numbers of baits placed throughout the tree crown, most of which was not accessible from a single anchor point in a major cleft of the tree. Like other similar studies (e.g. Adams et al. 2017) I constructed curves to estimate the number of species in the community occupying all of the study trees as a whole, but did not reach an asymptote with this sample size in the diverse environment of the study site. Because I do not have a precise count of the number of each species found at baits, the curves have not been re-scaled to individual ants and so they represent species density, not species richness at either the crown or the community level. While the species density measure is very useful, the lack of an estimate, based on species accumulation, of the total number of species in a crown limits my ability to make claims about an increase in total ant species richness on any of the tree crowns.

I had hoped for data from the third observation, which sampled sites that had been connected for an entire year, to shed light on any differences between the immediate and long-term effects of connectivity. Unfortunately, some original sites were unsuitable for continued data collection due to missing trees or ropes and the sample size remained relatively small. Due to the geographical arrangement of the sites where I was able to sample a third time, I also encountered a major challenge in data collection at these locations. A bee effect had to be added to the linear mixed model because of the vast increase in honey bee (*Apis mellifera*) activity at most of the original sites and the precipitous increase in recruitment of *A. mellifera* to ant baits in the middle of the second field season. This may have been due to movement of hives on the farm or a learned change in foraging behavior once the hive members learned that sugar-rich resources were being regularly placed in tree crowns. Once *A. mellifera* had recruited to the jam and honey baits they were observed excluding ants and also impaired my ability to see, collect, and record specimens among ants not excluded from baits. Attempts to create exclosures over baits that would not alter ant access to baits failed. Because bee activity interfered with data collection during most of the sampling events in the third observation (and because the third observation had fewer samples from mis-matched treatment and control site pairs), the analysis focuses on information collected during the two complete observations. The effect size of "bees" calculated from these two observations, which decreased the number of species observed in each crown by slightly over one and a half species, seems accurate based on my experience in the field.

Implications for management

  The response of the arboreal ant community to artificial connections in this study shows that the addition of direct, persistent connections at the crown level can cause an increase in species density in tree crowns and stimulate changes in composition by allowing colonies from neighboring trees to expand their foraging areas or networks of connected nests, in the case of polydomous species. At least two of the ant species most commonly counted in timed rope observations, *C. basalis* and *A. sericeasur*, are polydomous species with large colonies, and both are members of genera that make use of connections between trees to expand networks of nests within the canopy (Gordon 2012, Yanoviak and Schnitzer 2013). At the only site at which *A. sericeasur* was particularly active at the rope height, *A. sericeasur* appeared to use the rope to establish a new nest in the adjacent tree with heavy traffic in between the old and new nests. *C. basalis*, which has also been observed in other studies at the site along *A. sericeasur* foraging trails (Jimenez-Soto et al. 2018, Jonathan Morris, personal communication), appeared to also expand nest sites into the new tree and to share the connecting trail. In Panama, *Cephalotes maculatus* has been observed parasitizing pheromone trails of *Azteca trigona*; the heavily-armored morphology of *Cephalotes* provides immunity to the territorial aggression of *Azteca* (Adams 1990). I found evidence with this study that connectivity does allow large, polydomous colonies to expand their foraging areas and territories, in parallel with the results of a study by Tanaka et al. (2010) that found more polydomous species in trees with lianas or stranglers. A positive relationship between large, polydomous colonies and connectivity means that connectivity could increase the biocontrol services rendered by these species (Jimenez-Soto et al. 2018, Philpott et al. 2004).

  This study did not find that increased connectivity in the upper canopy facilitated the dominance of TDAAs. The specific TDAA investigated in this study, *A. sericeasur*, did not make use of lines above 10.5 m. The increase in species density associated with connectivity indicated that, at least in the short-term, connectivity between taller tree crowns did not facilitate exclusion by dominants. One key difference between the behavior of *A. sericeasur* and the TDAAs described in studies that propose a positive relationship canopy connectivity is that these studies were in intact rainforests (Dejean et al. 2015, Yusah and Foster 2016), while my fieldwork was conducted in a farm where lower vegetation layers were much more heavily insolated. If, as Ribeiro et al. (2013) proposed, competition is more intense where abundant sunlight makes plant-based sugars more available, then vegetative strata in which TDAAs are most dominant will vary with vegetation structure and light. Connectivity in the shrub layer and in tree crowns at heights below about 10.5 m do expand *A. sericeasur*'s foraging territory, but my research suggests that upper canopy connectivity is more consequential to other species.

  The increase in the number of species found at baits that followed crown connection suggest that connectivity itself has a role to play in boosting species density of canopy ants. Because diversity has been linked to the integrity of ecosystem services (Klein et al. 2003,

Balvanera et al. 2001), the persistent physical connections in the upper canopy may be one of the many components of vegetative complexity that enhances biological control (Perfecto et al. 1996, Philpott et al. 2006, Vandermeer et al. 2010). Because the keystone arboreal ant in this agroforestry system nests relatively low on the main trunk of large shade trees (Longino 2007, personal observations), connectivity may enhance occupancy in tree crowns by providing access to tree crowns that bypasses the aggressively-defended trunks on nest trees. Twig-nesting ants, which tend to be found in tree crowns, often have smaller colonies and are among the likely beneficiaries of connectivity in the upper canopy. Some twig-nesting ants are also among the biological control agents in coffee agroecosystems (Larsen 2010); therefore practices that increase ant diversity in tree crowns likely yield pest-control benefits.

Because the nylon ropes used in this manipulation were smooth-surfaced, fixed, and ran in a relatively straight line from one tree crown to another, they most closely mimicked the role that lianas play in the canopy, as opposed to contact between branches at the ends of tree crowns (Yanoviak 2015, Yanoviak and Schnitzer 2013). In order to receive the benefits of a connected upper canopy, some lianas could be allowed to grow up and pass between adjacent tree crowns. This management change would likely be easy and cost-effective, entailing a cessation or a decrease in cutting of liana stems that climb the base of taller shade trees. Allowing more growth of lianas, shade trees, and other non-crop species can help regulate temperature and moisture in agroecosystems; as climate change begins to impact global agriculture more and more drastically, agroecological approaches that preserve structural and species diversity on agricultural land seems more and more critical to resiliency (reviewed in Altieri et al. 2015). Such an agricultural shift also addresses mitigation: for instance, liana species richness was shown to have a positive relationship to carbon stocks in cacao agroforests in Indonesia (Kessler et al. 2012).

For forests, this research adds to the existing evidence that a connected canopy increases ant diversity locally (Adams et al. 2016, Klimes et al. 2015, Powell et al. 2011, Yanoviak 2015), suggesting that undisturbed forests with closed canopies and liana growth will be sites of greater ant species richness. Future studies in agroecosystems or in unmanaged forests should strive to describe the relationship between the effect of vegetation structure on alpha diversity and the implications of this for gamma diversity (as in Klimes et al. 2012) to better understand the implications of structural diversity both locally and at a forest level. This study shows how connections in the upper canopy allow additional ant species to access resources, perhaps most significantly in the crowns of trees housing a territorially-dominant species on their trunks. While the many mechanisms allowing the remarkable diversity and coexistence of ant species are still not fully understood (Andersen 2008), vegetation structure sets the context for the complex set of interactions that undergird that diversity (Adams 2016, Clay et al. 2010, Klimes et al. 2012, Ribeiro et al. 2013).
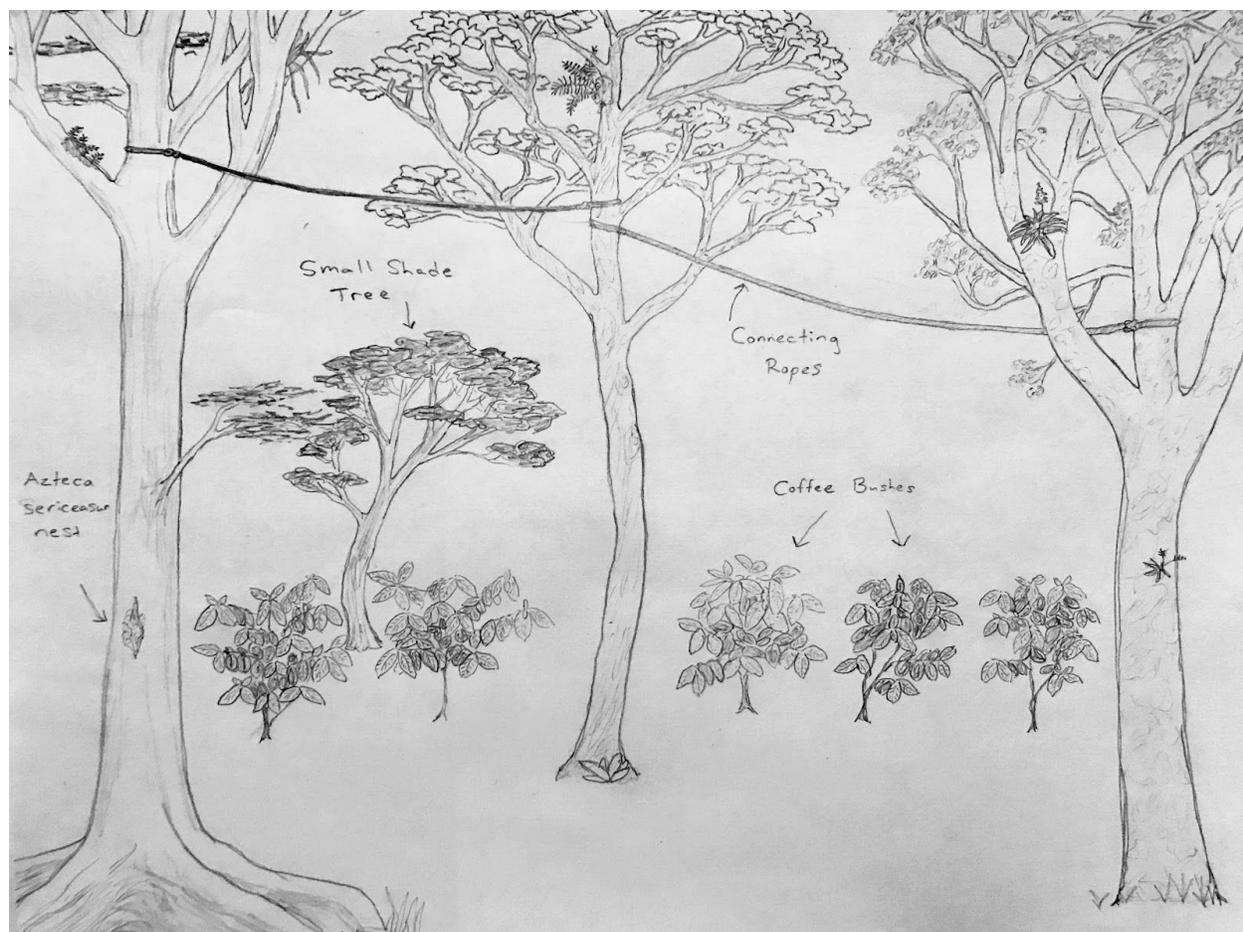
Tables & Figures

Fig. M.1: Diagram of a single site in the treated group with ropes connecting the crowns.

Fig. M.2: A bird's-eye view of two paired sites: a set of connected trees (red outline) and a set of three control trees (blue outline). All trees were climbed and sampled at the trunk and in the crown during an initial observation and follow-up observations. Each set of trees had one tree with an *Azteca sericeasur* nest (A), one tree in the middle with two connections (M), and a third (E) only indirectly connected to the nest tree.
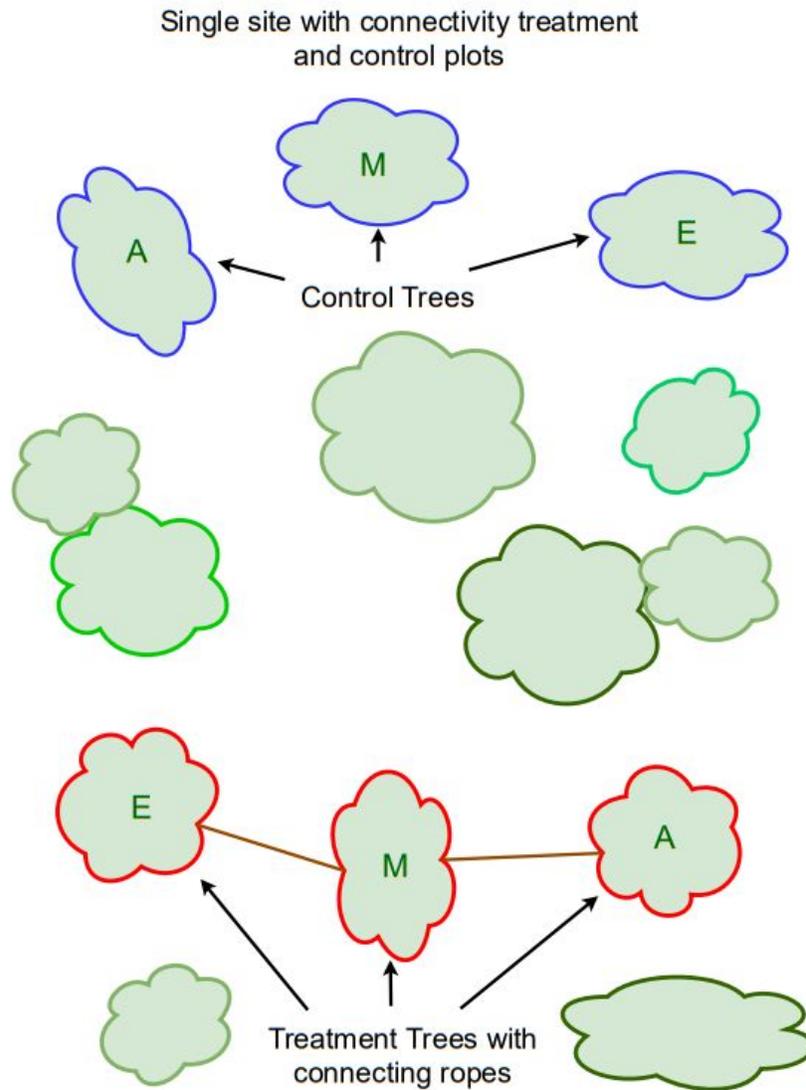
Table R.1: Results of a linear mixed effects analysis run in the lme4 package in R. The response variable for this test was the number of species found at all baits in each crown; effect size is in number of ant species. P-values are derived from the Anova() function from the "car" package.

Linear mixed model results from first two observations

Variable	Effect size	p value
Bees present	-1.5957	0.06663
Tree Position (end)	1.0863	0.06710
Tree Position (middle)	1.0416	0.06710
Tree Health	0.4524	0.03055 *
Observation	0.4187	0.26147
Connectivity applied	1.0397	0.02835 *

Linear mixed model results from data including the third observation

Variable	Effect size	p value
Bees present	-0.8367	0.10262
Tree Position (end)	0.8879	0.16952
Tree Position (middle)	0.7645	0.16952
Tree Health	0.4396	0.02941 *
Observation	0.3685	0.18802
Connectivity applied	0.8726	0.03524 *

Table R.2: Analysis of similarity values for composition by samples grouped by tree position (*A. sericeasur* nest tree, middle tree, and end tree) including the R statistic, which ranges from -1 to 1, with higher values indicating higher shared composition within as opposed to between groups.

	Observation 1	Observation 2
Treatment crowns	ANOSIM statistic R: 0.1584 Significance: **0.047***	ANOSIM statistic R:-0.0284 Significance: 0.603
Control crowns	ANOSIM statistic R: 0.03704 Significance: 0.300	ANOSIM statistic R: 0.179 Significance: **0.019***
Treatment trunks	ANOSIM statistic R: 0.3895 Significance: **0.001***	ANOSIM statistic R: 0.2432 Significance: **0.006***

Control trunks	ANOSIM statistic R: 0.149 Significance: **0.048***	ANOSIM statistic R: 0.1315 Significance: **0.079**

Table R.3: Sørensen index dissimilarity values created in the vegan package in R. A value of 0 indicates identical composition between samples in two crowns and a 1 indicates no overlap in composition. Values in blue increased in similarity (i.e. dissimilarity indices decreased) between the first and second observation; red values indicate decreased similarity.

Site #	Tree pair	Observation 1	Observation 2
Site 1	Middle & End	**0.714**	**0.429**
	Azteca & End	**1.000**	**0.800**
	Azteca & Middle	**1.000**	**0.600**
Site 2	Middle & End	**0.600**	**0.550**
	Azteca & End	**0.429**	**0.500**
	Azteca & Middle	**0.750**	**0.330**
Site 8	Middle & End	**0.778**	**0.500**
	Azteca & End	**0.556**	**0.667**
	Azteca & Middle	**1.000**	**0.600**
Site 9	Middle & End	**0.667**	**0.467**
	Azteca & End	**0.556**	**0.429**
	Azteca & Middle	**0.636**	**0.692**
Site 12	Middle & End	**0.692**	**0.538**
	Azteca & End	**0.636**	**0.600**
	Azteca & Middle	**0.333**	**0.455**
Site 14	Middle & End	**0.714**	**0.600**
	Azteca & End	**0.636**	**0.538**
	Azteca & Middle	**1.000**	**0.500**

Figure R.1: Boxplot of the mean number of species per crown in 1) the treatment group before connection, 2) the treatment group after connection, 3) the control group before connection and 4) the control group after connection. Error bars located at data points closest to but less than 1.5 times the interquartile range.
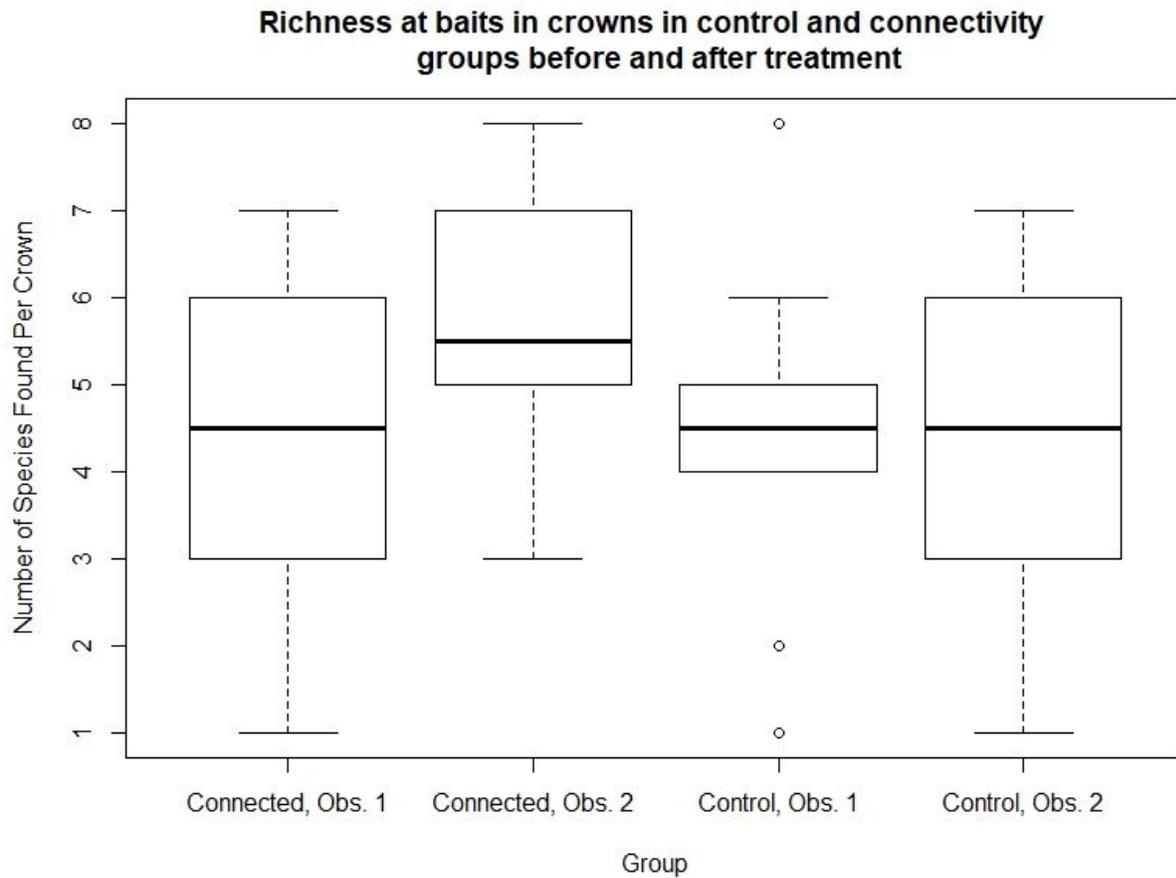
Figure R.2: Rarefaction curves created with sample-based incidence data from crowns in the control and treatment groups on the first and second observations.
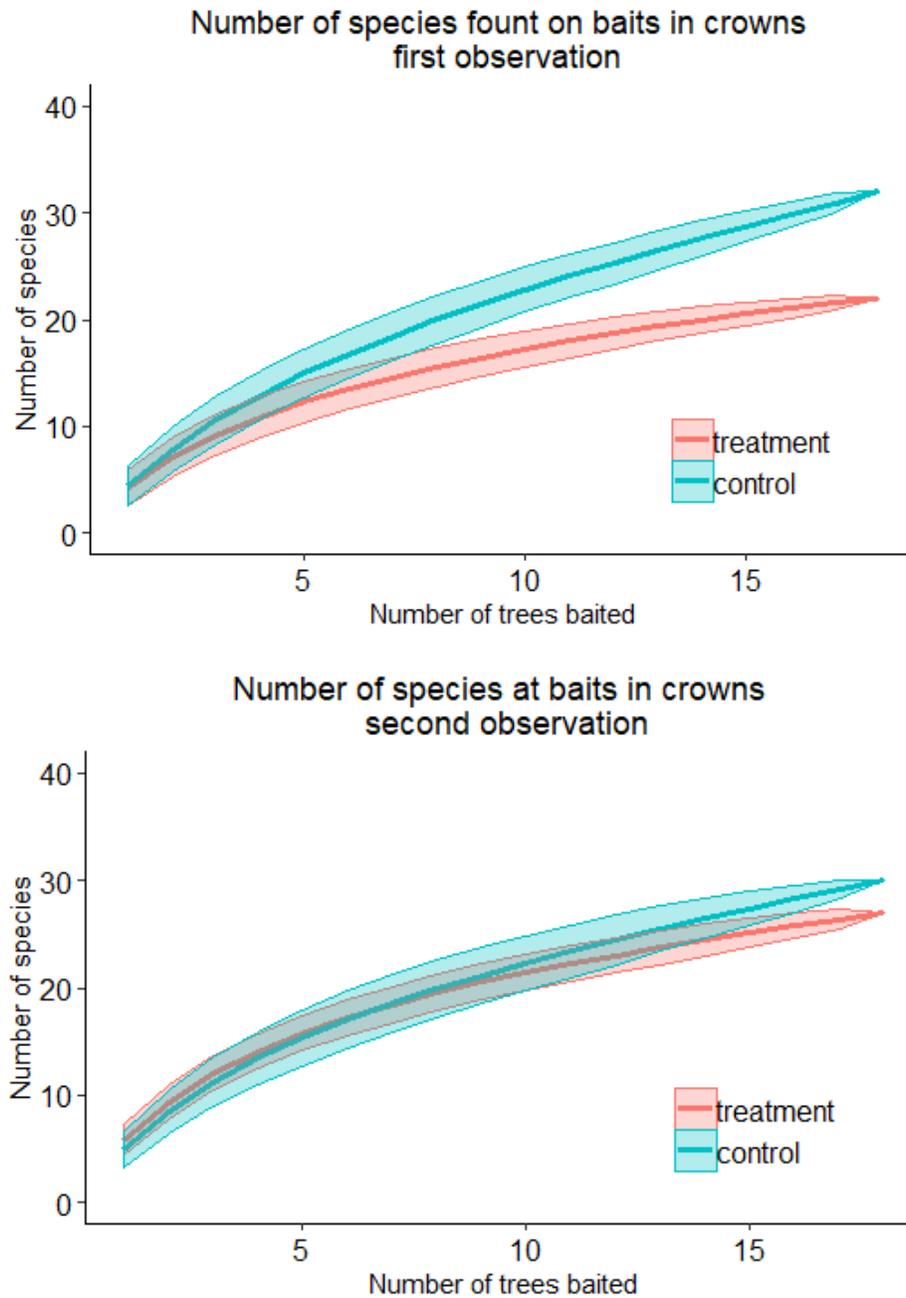
Fig. R. 3: A rarefaction curve showing species accumulation with data from baits on both trunks and crowns in all 3 observations.
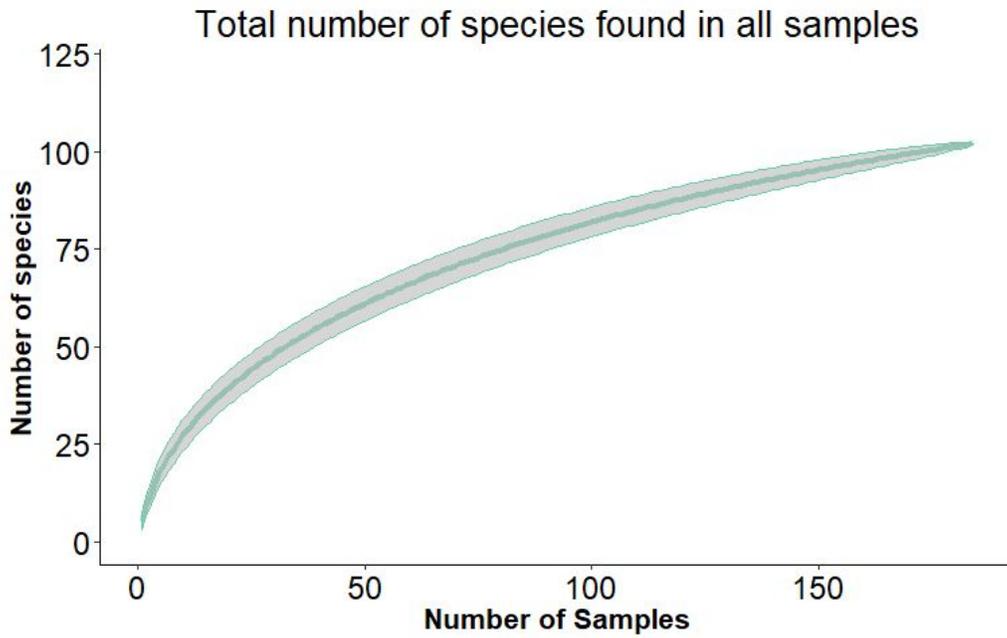

Figure R.4: Rarefaction curves created with sample-based incidence data from crowns and trunks from samples on all trees throughout the study.
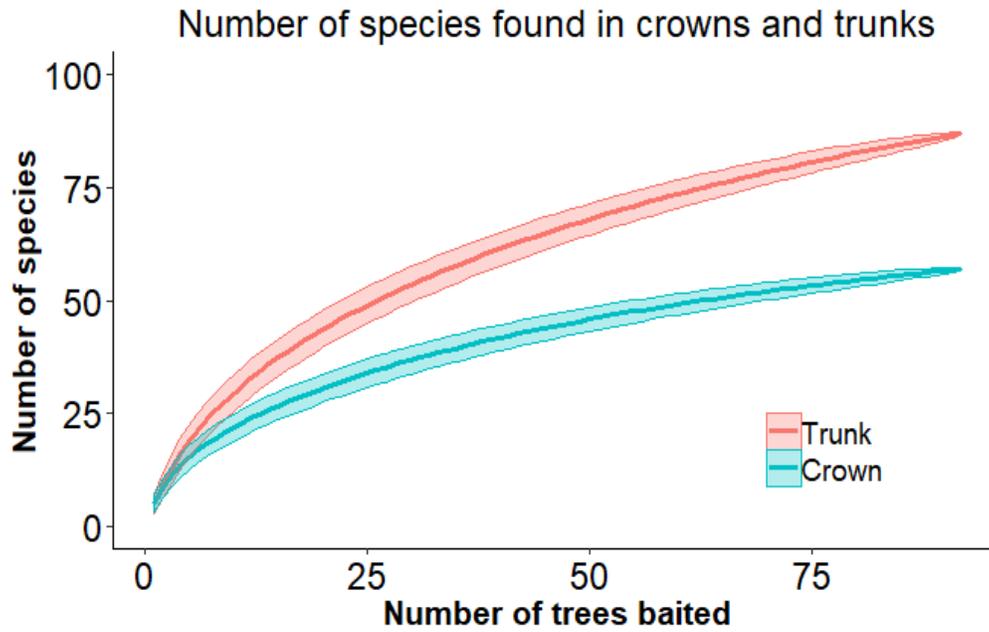
Figure R.5: Non-metric multi-dimensional scaling plots showing trees (labeled with ID numbers) grouped by position (A = tree with *Azteca* nest, M = middle and E = end).
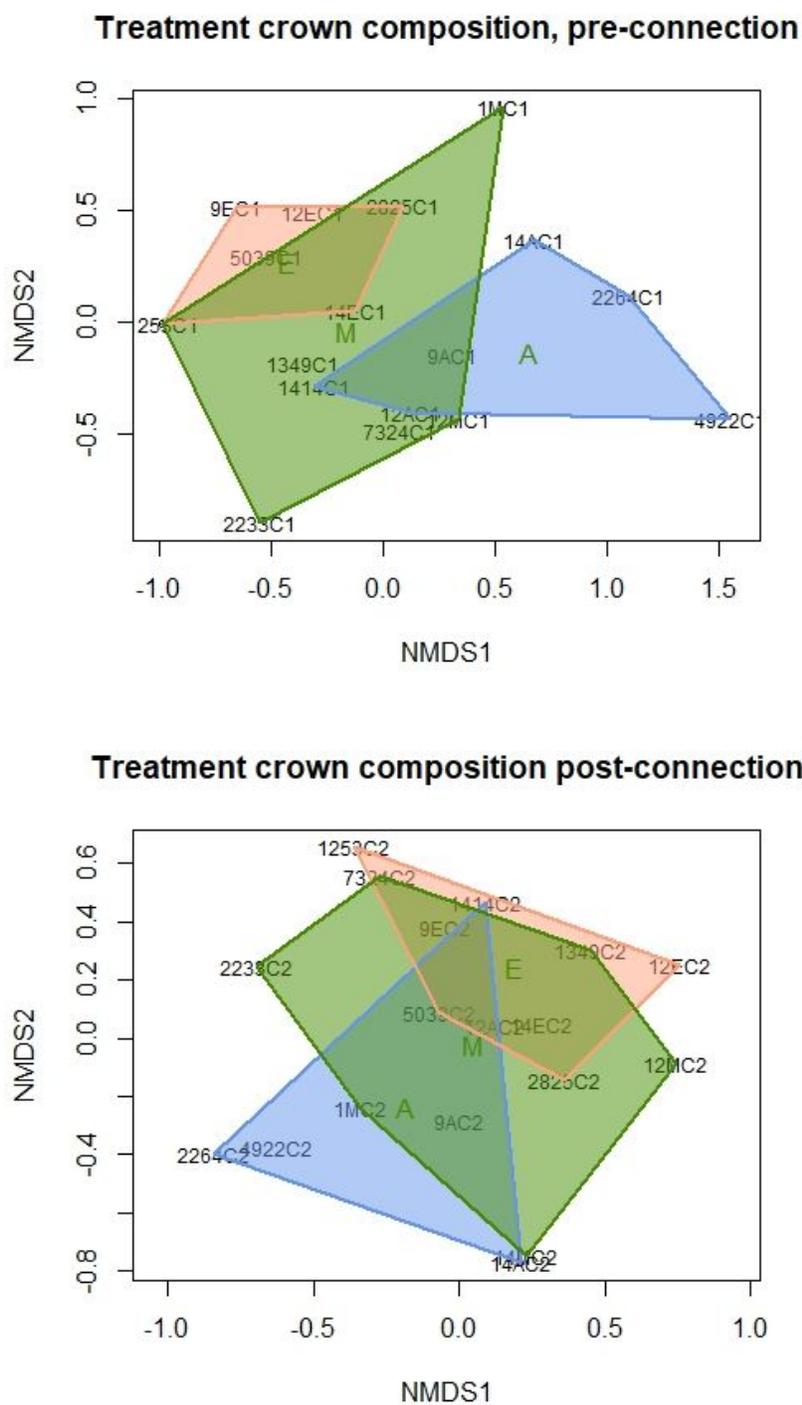

Treatment crown composition, pre-connection

Treatment crown composition post-connection

Fig. R.6: A lower triangle heat map illustrating co-occurrence values of individual species pairs and highlighting pairs that exhibit "positive" or "negative" associations.

Fig. R.7: Non-metric multidimensional scaling showing sampling location (T for trunk and C for crown followed by the tree identification number) arranged according to similarity of species found at that location on the first sampling. Trunk samples are found within the brown polygon while crown samples are located within the green polygon. Gold lines drawn from a centroid to each sample on an *Azteca* nest tree show clustering of samples collected on *Azteca* nest trees.

Figure R.8: Co-occurrence measured by C-score is indicated by the red line overlaid on a random distribution of co-occurrence rates (blue histogram). Inner dashed lines represent a 95% confidence interval for a one-tailed test while finer dotted lines indicate a 95% confidence interval for a two-tailed test for the distribution created with the null model. Co-occurrence rates on the trunks of trees with connected crowns are contrasted with rates on trunks in the control group on the second observation (above) and the first observation (below).
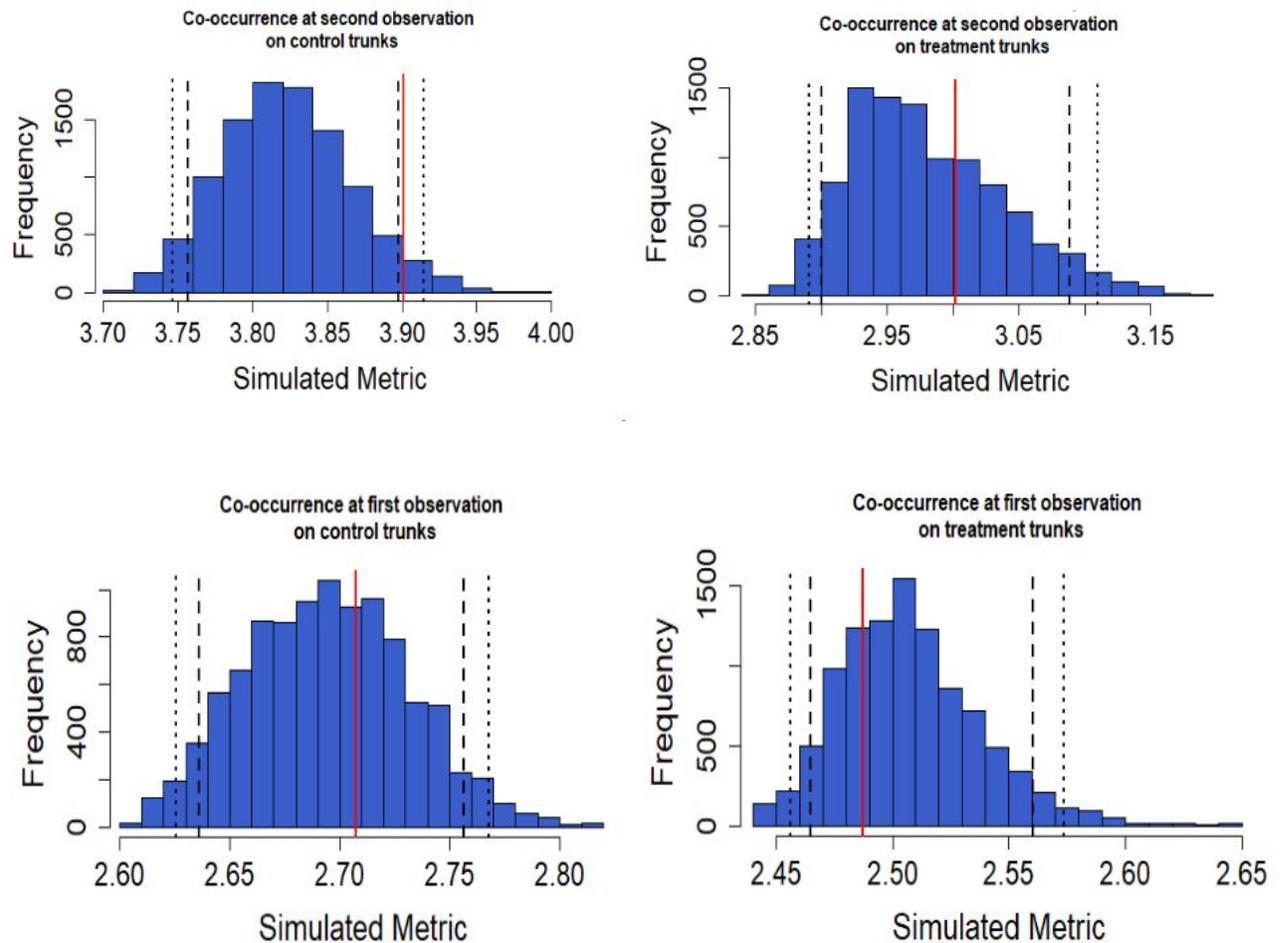
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