[Working title]: Evaluating the Role of Endophyte-Rich Leaves in Protecting Tropical Trees Against Generalist Herbivores and Pathogens

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# 1. Abstract

# 2. Keywords:

Atta colombica, Calonectria, foliar fungal endophytes, herbivory, pathogen, symbioses, tropical trees

# 3. Introduction

### 3.0.1 Hypothesis

If FEF improves leaf defenses against plant pests, then generalist herbivores and pathogens will remove or damage less leaf tissue from plants with treated high FEF levels relative to those with low FEF loads.

### 3.0.2 Predictions

Leaf-cutter ants will remove less plant material from leaves with higher FEF abundance and richness, but this result will be modulated by leaf traits related to defenses. Leaves with longer lifespans will be less attractive to leaf-cutter ants. Alternatively, low FEF diversity in those leaves may outweigh this selection factor. Leaves treated with high FEF levels will have a smaller area of pathogen damage compared to those treated with low FEF levels. Endophyte-mediated defenses against pathogens will be most important in short-lived leaves since long-lived leaves are expected to rely more on constitutive defenses (e.g., leaf toughness).

# 4. Materials and Methods

## 4.1 Field

Growth and host plant inoculation seven tropical tree species was conducted at the greenhouses in the Gamboa Research Station, Smithsonian Tropical Research institute, Republic of Panama. The species, *Theobroma cacao*, *Dypterix* sp., *Lacmellea panamensis*, *Apeiba membranacea*, *Heisteria concinna*, *Chrysophyllum caimito*, and *Cordia alliodora* were chosen due to their variance in leaf traits (J.Wright unpublished data) and the availability of seeds in January- April 2019. Seeds of tree species were collected from the forest floor and grown in the greenhouse. Seedlings were kept in a chamber made out of PVC and clear plastic to prevent inoculation from spore fall inside the greenhouse. NEEDS INFORMATION ON THE SOIL MIXTURE AND AUTOCLAVING PROTOCOL. Seedlings reached a minimum of 4 true leaves before endophyte inoculation. Then 10 individual plants of each species were exposed to 10 nights of spore fall to achieve a high endophyte load (E+) and 10 homologous plants were kept inside the greenhouse plastic chamber to maintain a low endophyte load (*E-*) (Fig. ? MAKE A DIRAGRAM?). Plants exposed to spore fall were placed near (~10 m) the forest edge at dusk (~18:OO hours) and returned to the greenhouse at dawn (~07:00 hours) (Bittleston et al., 2011).

### 4.1.1 Leaf trait measurements

Three mature leaves were haphazardly collected from each of the individual plants in each treatment (E+, E-) within 7-10 days after inoculation treatment. Anthocyanin (ACI) content and leaf thickness (LT) were measured while the leaf was still attached to the plant. We measured anthocyanin content with ACM-200plus (Opti-Sciences Inc. Hudson, New Hampshire, U.S.A.) on three haphazardly selected locations (working from the petiole out to the leaf tip) on the leaf surface of three haphazardly selected leaves for a total of nine measurements per plant (Tellez et al., 2022). To account for leaf thickness, the ACM-200 calculates an anthocyanin content index (ACI) value from the ratio of % transmittance at 931 nm/% transmittance at 525 nm (**opti-sciencesinc?**). On compound leaves (i.e., *Dypterix* sp.) we measured at three different leaflets. Leaf thickness (μm) was measured with a Mitutoyo 7327 Micrometer Gauge (Mitutoyo, Takatsu-ku, Kawasaki, Japan) at six different points on the leaf lamina; at the base, mid-leaf and tip on both sides of the mid-vein, taking care to avoid major and secondary veins. After ACI and leaf LT measurements were completed, we removed the leaves from their stems, placed them inside a plastic bag (i.e. ZiplocⓇ), place in an ice chest and moved them to the lab for further measurements. Leaf punch strength (LPS) was measured with an Imada DST-11a digital force gauge (Imada Inc., Northbrook, IL, United States) by conducting punch-and-die tests with a sharp-edged cylindrical steel punch (2.0 mm diameter) and a steel die with a sharp-edged aperture of small clearance (0.05 mm). The leaf punch measurements were taken at six diffrent points of the leaf by puncturing the lamina at the base, mid-leaf and tip on both sides of the mid-vein, avoiding minor leaf veins when possible (Tellez et al., 2022). Once LPS was measured, we used a 7 mm diameter punch hole to puncture disks for leaf mass per area (LMA) measurements. We collected three disks per leaf (see Supplementary material for details). The disk punches dried at 60 °C for 48-72 hours. before being weighed.

### 4.1.2 Leaf tissue preparation for molecular work

The selected leaves were also used to profile endophyte community composition, abundance, and richness via amplicon sequencing (Illumina MiSeq). The leaf tissue remaining after the leaf trait measurements had the main vein and margins excised so that only the lamina remained. The lamina was haphazardly cut into 2 x 2 mm segments, enough to obtain a total of 16, and surface sterilized by sequential rinsing in 95% ethanol (10 s), 0.5 NaOCl (2 mins) and 70% ethanol (2 mins), as per (Arnold et al., 2003; Higgins et al., 2014; Tellez et al., 2022). After, leaves were air-dried briefly under sterile conditions. Sixteen leaf segments per leaf, a total of forty-eight leaf segments per plant, were plated in 2% malt extract agar (MEA), sealed with Parafilm M (Bemis Company Inc., U.S.A.) and incubated at room temperature. The cultured leaf segments were used to estimate endophyte colonization of *E-* and *E+* leaves. The presence or absence of endophytic fungi in the leaf cuttings was assessed 7 days after plating. The remaining sterilized leaf lamina was preserved in sterile 15 mL tubes with ~ 10 mL CTAB solution (1 M Tris–HCl pH 8, 5 M NaCl, 0.5 M EDTA, and 20 g CTAB). Leaf tissue in CTAB solution was used for amplicon sequencing (described in detail below). All leaf tissue handling was performed in a biosafety cabinet with all surfaces sterilized by exposure to UV light for 30 minutes and cleaned sequentially in between samples with 95% ethanol, 0.5% NaOCl and 70% ethanol to prevent cross contamination.

## 4.2 Amplicon sequencing

Leaf tissue in CTAB solution was stored for 2 months at room temperature prior to being placed at -80 C for 3 months before extracting DNA. In preparation for DNA extraction, we decontaminated all instruments, materials, and surfaces with DNAway (Molecular BioProducts Inc., San Diego, CA, United States), 95% Ethanol, 0.5 % NaOCl, and 70 % Ethanol, and subsequently treated with UV light for 30 minutes in biosafety cabinet. We then transferred 0.2 – 0.3 g of leaf tissue into duplicate sterile 2mL tubes, resulting in 2 subsamples. Total genomic DNA from subsamples was extracted as described in U’Ren & Arnold (2017). In brief, we added two sterile 3.2 mm stainless steel beads to each tube and proceeded to lyophilize samples for 72 hours to fully remove CTAB content from tissue. After this period, we submerged the sample tubes in liquid nitrogen for 30s and proceeded to homogenize samples to a fine powder for 45 s in FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA). Afterwards, we repeated the decontamination procedure described before and used QIAGEN DNeasy 96 PowerPlant Pro-HTP Kit (U’Ren & Arnold, 2017) (QIAGEN, Valencia, CA, USA). After all genomic DNA was extracted, we pooled the subsamples for each individual sample before amplification. We used sterile equipment and pipettes with aerosol-resistant tips with filters in all steps before amplification. We followed a two-step amplification approach previously described by Sarmiento et al. (2017) and U´Ren & Arnold (2017). We used primers for the fungal ITSrDNA region, ITS1f (5’-CTTGGTCATTTAGAGGAAGTAA-3’) and ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) with modified universal consensus sequences CS1 and CS2 and 0–5 bp for phase-shifting. Every sample was amplified in two parallel reactions containing 1-2 µL of DNA template (U’Ren & Arnold, 2017; see also Tellez et al., 2022). We visualized PCR (PCR1) reactions with SYBR Green 1 (Invitrogen, Carlsbad, CA, USA) on 2% agarose gel (Oita et al., 2021). Based on the electrophoresis band intensity, we combined parallel PCR1 reactions and diluted 5 µL of amplicon product with molecular grade water to standardize to a concentration of 1:15 (Sarmiento et al., 2017 for details; Tellez et al., 2022). We included DNA extraction blanks and PCR1 negatives in this step. We used a separate set of sterile pipettes, tips, and equipment to reduce contamination. We used a designated PCR area to restrict contact with pre-PCR materials (Oita et al., 2021). We used 1 µL of PCR1 product from samples and negative control for a second PCR (PCR2) with barcode adapters (IBEST Genomics Resource Core, Moscow, ID, USA). Each PCR2 reaction (total 15 µL) contained 1X Phusion Flash High Fidelity PCR Master Mix, 0.075 µM of barcoded primers (forward and reverse pooled at a concentration of 2 µM) and 0.24mg/mL of BSA following Sarmiento (2017) and U’Ren & Arnold (2017). Before final pooling for sequencing, we purified the amplicons using Agencourt AMPure XP Beads (Beckman Coulter Inc, Brea, CA USA) to a ratio of 1:1 following the manufacturer’s instructions. The products were evaluated with Bio Analyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) (Tellez et al., 2022). We quantified the samples through University of Arizona Genetics Core, and subsequently diluted them to the same concentration to prevent over representation of samples with higher concentration, see (CITATION). Amplicons were normalized to 1 ng/µL, then pooled 2 µL of each for sequencing. No contamination was detected visually or by fluorometric analysis. To provide robust controls we combined 5 µL of each PCR1 negative and the DNA extraction blanks and sequenced them as samples. Ultimately, we combined samples into a single tube with 20 ng/µL of amplified DNA with barcoded adapters for sequencing on the Illumina MiSeq platform with Reagent Kit v3 (2 × 300 bp) following protocols from the IBEST Genomics Resource Core at the University of Idaho, USA. Again, we included the DNA extraction blanks and two PCR1 negatives and sequenced with samples. Sequencing yielded 3,778,081 total ITS1 reads.

### 4.2.1 Mock Communities

We processed and sequenced 12 mock communities following the methods described above. This allowed us to assess the quality of our NGS data set. We used two mock communities that consisted of PCR product from DNA extractions of 32 phylogenetically distinct fungi, representing lineages that are typically observed as endophytes: Ascomycota, Basidiomycota, Zygomycota and Chytridiomycota (Oita et al., 2021; see Daru et al., 2019 for details). In brief, we used six mock communities with equimolar concentrations of DNA from all 32 fungal taxa and another six mock communities with tiered concentrations of DNA from the same fungal taxa (Daru et al., 2019). Each mock community was sequenced five times (i.e., five replicates) (Oita et al., 2021). The read abundance from the equimolar and tiered communities was positively associated with the expected read number (with replicates as a random factor: R2Adj = 0.87, P = XXXX, see Supplementary Material). Using mock communities allowed us to evaluate the sequencing effectiveness in communities with known composition and structure (Bowman & Arnold, 2021). Henceforth, we used read abundance as a relevant proxy for biological OTU abundance (U’Ren et al., 2019).

### 4.2.2 Bioinformatic analyses

We used VSEARCH (v2.14.1) for *de novo* chimera detection, dereplication and sequence alignment. VSEARCH is an open-source alternative to USEARCH that uses an optimal global aligner (full dynamic programming Needleman-Wunsch), resulting in more accurate alignments and sensitivity (Rognes et al., 2016). For mock communities and experimental samples, we used forward reads (ITS1) for downstream bioinformatics analyses due to their high quality, rather than reverse reads (ITS4). Following Sarmiento et al. (2017), we concatenated all reads in a single file and used FastQC reports to assess Phred scores above 30 and determine the adequate length of truncation. We processed 892,713 of sequence reads from mock communities and 3,778,081 from experimental samples. We truncated mock community and experimental sample reads to a length of 250 bp with command fast\_trunclen and filtered them at a maximum expected error of 1.0 with command fast\_maxee. We then clustered unique sequence zero radius OTUs (that is, zOTUs; analogous to amplicon sequence variants (Callahan et al., 2016)), by using commands derep\_fulllength and minseqlength set at 2. Sequentially we denoised and removed chimeras from read sequences with commands cluster\_unoise, and uchime3\_denovo, respectively (see Supplementary YYY for details). Finally, we clustered zOTUs at a 95% sequence similarity with command usearch\_global and option id set at 0.95. After which, 3,035,960 sequence reads from experimental samples remained. Taxonomy was assigned with the Tree-Based Alignment Selector Toolkit [v2.2; Carbone et al. (2019)] by placing unknowns within the Pezizomycotina v2 reference tree (Carbone et al., 2017). ITS sequences were blasted against the UNITE database by the ribosomal database project (RDP) classifier. A total of 2147 OTUs hits were obtained and are composed of 68.6% Ascomycota, 26.8% Basidiomycota,<0.05% Chytridiomycota, <0.05% Glomeromycota, <0.05% Mortierellomycota, <0.05% Rozellomycota, 0.05% Kickxellomycota, and 4.2 % BLAST hit misses. Only OTUs representing Ascomycota were used for downstream statistical analyses since foliar endophyte communities in tropical trees are dominated by Ascomycota (Arnold & Lutzoni, 2007). For each OTU identified, we removed laboratory contaminants from experimental samples by substracting the average read count found in control samples from the DNA extraction and PCR steps. Our analysis of mock communities allowed use to identify and remove false OTUs from experimental samples, those with fewer than 10 reads, and remove 0.1% of the read relative abundance across all samples (Oita et al., 2021). Removed reads represent the frequency of reads classified as contamination in the mock communities relative to the expected read count. Three experimental samples from *Theobroma cacao* (=2) and *Apeiba membranacea* (=1) were removed from all analyses due to incomplete entries. After pruning taxa with zero reads from experimental samples, we identified 260 OTUs found exclusively in control (*E-*) plants (=78) and deemed them as artifacts resulting from the greenhouse conditions. Consequently, these were consistently eliminated from treatment (*E+*) plants across all species. We converted reads for each fungal OTU to proportions of total sequence abundance per sample to reduce differences in sampling effort, following previous studies (Weiss et al. (2017); McMurdie & Holmes (2014) ). We then removed singletons and obtained an average of 2,464,558 sequence reads in 529 Ascomycota OTUs across 156 experimental samples of 7 tree species. All analyses post taxonomic assignment were performed in R [v. 4.3.2; R Core Team (2023)] using the phyloseq package (McMurdie & Holmes, 2013) and custom scripts (see Supplementary Material).

### 4.2.3 Herbivore assays

A fresh fourth leaf was used in ant assays. To assess leaf-cutter ant damage, we introduced one detached leaf per plant per treatment to an actively foraging leaf-cutter ant colony for a two-hour assay. We presented leaf-cutter ant colonies with a choice of an E+ or an E- leaf on one disposable plastic plate next to an active nest trail. Carefully, we collected and placed debris from the trail leading up to the plate to lure ants into the plate. We initiated the ant assay as soon as one ant entered the plate and explored the leaf contents (for ~ 10-20 seconds). Every five minutes we took a digital photo of the choice arena until about 75% of the leaf content of one of the leaves was consumed. We used the digital photo at time zero and at the end of trial to quantify the leaf area removed using ImageJ [v1.52r; Schneider et al. (2012)]. Ant recruitment was estimated by counting individuals in the choice arena throughout trial event.

### 4.2.4 Pathogen assays

For the pathogen assays, we introduced an agar plug inoculated with hyphae of *Calonectria* sp. (*P+* treatment), and an agar plug without the pathogen (*P-* control) to similarly aged/sized leaves within 10-14 days after endophyte inoculations (Gilbert & Webb, 2007). Leaves with the *P+* or *P-* treatment were misted with sterile water two times a day (morning and afternoon) to maintain moisture. After four days, we removed the plugs and took digital photos to analyze leaf area damage using ImageJ [v1.52r; Schneider et al. (2012)].

### 4.2.5 Replication Statement

|  |  |  |
| --- | --- | --- |
| **Scale of inference** | **Scale at which the factor of interest is applied** | **Number of replicates at the appropriate scale** |
| Leaf functional traits | Species | Treatment = 2 (E-, E+), Tropical tree species = 7, Replicates per species = 5. Replicate leaves per plant = 3 |
| Genomic data | Species | Treatment = 2 (E-, E+), Tropical tree species = 7, Replicates per species = 5 |
| Herbivore and Pathogen trials | Species/individual | Treatment = 2 (E-, E+), Tropical tree species = 7, Replicates per species = 5, Replicates per plant = 1 |

### 4.2.6 Statistical Analyses

We explored how leaf functional traits and FEF correlated to herbivory and pathogen damage on leaves. We present the analyses for each tree species at the leaf and at the plant level. Leaf functional traits are presented at the leaf level, while FEF data was explored and is presented at the plant level. In analyses where leaf functional traits and FEF are combined we used averages of the leaf functional traits.

First, we compared the means of herbivory (%) and pathogen (%) damage, and leaf functional traits for each species and treatment groups using paired two-sided Student’s t-Test and analysis of variance (ANOVA) with the compare\_means and stat\_compare\_means functions from the ggpubr package in R (Kassambara, 2023), which wrap and extend the anova and t.test functions from the stats package (R Core Team, 2023).

Secondly, we calculated a Bray-Curtis dissimilarity matrix with our OTU relative abundance data and computed a distance based redundancy analysis (dbRDA) by applying the dbRDA function in the vegan package to our dissimilarity matrix (Oksanen et al., 2022). We computed forward model selection for dbRDA analysis With the ordistep function which selects terms based on *p* values (Blanchet et al., 2008; Oksanen et al., 2022). We started with our initial model containing only the intercept (dissimilarity\_matrix ~ 1) and setting the functions arguments to the following: scope = formula(*m*), where *m* is the formula with a defined range including leaf functional traits, tree species and treatment groups; Pin = 0.5, Pout = 0.05, trace = T, permutations = how(nperm = 999), steps = 50.The dbRDA is considered analogous to a permutational analysis of variance (PERMANOVA) with non-Euclidean distance (Anderson, 2017; **mcardle2001?**). Its corresponding visualizations appropriately illustrate underlying patterns of compositional differences (Anderson, 2017; Legendre & Anderson, 1999; **mcardle2001?**). Applied here to visualize associations between leaf functional traits and FEF communities in host tree species and treatment groups.

Thirdly, We arbitrarily designated percent leaf damage in herbivore assays as high (>70%), medium (31-69%) and low (<30%) and in pathogen assays as high (>30%) and low (<29%). These categories allowed us to explore the correlations between host tree species and treatment group combinations preferred by FEF OTUs. To achieve this we used the multipatt function from the indicspecies package in *R* (De Cáceres & Legendre, 2009). We calculated the the *point biserial correlation coefficient* for each OTU at all tree species and treatment group combinations by applying the multipatt function with arguments func= “r.g” and control = how(nperm=999) to our OTU abundance matrix (De Cáceres & Legendre, 2009) (CITE MORE). We adjusted *p* values to account false discovery rates in multiple comparison by using “BH” method (Benjamini & Hochberg, 1995) in the p.adjust function from the stats package (R Core Team, 2023). We then filtered the adjusted a *p* value with a cutoff of <0.05.

Fourthly, Principal Component Analysis (PCA) was used to reduce dimensions among covariates and reveal underlying interactions that could influence FEF abundance, diversity and community composition in host tree species. The PCA was computed using the prcomp function in R statistical software (R Core Team, 2023). A complete PCA was computed with variables ACI, LT, LPS, and LMA. We then proceeded to compute a PCA with the data from leaves of plants used in the herbivory (*n* = 210) and pathogen assays (*n* = 192). We then took from the herbivory and pathogen PCA the principal components that explained the most variance (PC1 and PC2) and regressed them to herbivory (%) and pathogen damage (%). We also regressed ACI, LT, LPS, LMA and Shannon’s Diversity Index to logit transformed herbivory (%) and pathogen damage (%). We used the logit function from the car package for logit transformation of variables and the lm function from the stat package for simple linear regressions (Fox & Weisberg, 2019; R Core Team, 2023).

Lastly, to test for our hypothesis and predictions, we used a general linear mixed model (GLM) with herbivory and pathogen damage percentage (logit transformed) as the response variable. To determine which fixed effects to include in the models we used the vif function from the car package in *R* to calculate the variance inflation factor for all explanatory variables (ACI, LT, LPS and LMA) (Fox & Weisberg, 2019; R Core Team, 2023). Complementary to this, we calculated Pearson’s coefficient for each leaf functional traits with by creating a correlation matrix and applying the cor function from the stats package to assess correlations among traits (R Core Team, 2023) (SUPPLEMENTARY FIGURE?). We opted to maintain explanatory variables pertaining to physical barriers (LT, LPS and LMA) and exclude ACI from subsequent general linear models (GLMs) due to high collinearity with LPS (*r* = 0.54) and LMA (*r* = 0.73). Every variable kept exhibits some degree of collinearity and this is well recorded in the literature (CITE HERE).

# 5. Results

Seedlings exposed to forest spore fall, *E+*, had a significantly higher proportion of leaf segments colonized by fungal endophytes across all species (data from cultures, Fig. S1). Using our molecular data set we saw that seedlings with *E+* treatment had a significantly higher FEF relative abundance (*p* <0.05) for all tree species when compared to the *E-* treatment with the exception of *C. cainito* and *H. concinna* (Fig. 1a). Despite these significant differences, there was a high degree of variability in endophyte relative abundance within each treatment type (Fig. 1).

We observed general differences in leaf functional traits among species (Table 2). For ACI and LPS we did not find significant differences between treatments (*E-* and *E+*) when we combined all host species (Fig. S2a and Fig. S4a respectively). For LT and LMA we saw statistically significant differences between *E-* and *E+* treatment groups when we combined all host species (Fig S3a and Fig. S5a, respectively). As predicted, we did observe statistically significant lower herbivory in the *E+* treatment compared to the *E-* treatment when we combined all host species (ADD STAT *p* <= 0.05) (Fig. S6a). We did not observe statistically significant differences in pathogen damage between *E-* and *E+* treatments when we combined all host species (Fig. S7a). Although, we did see significant differences between control and pathogen exposed leaves in the *E-* treatment group (ADD STAT TEST *p* < 0.0001) (Fig. S7a). As well as, control and and pathogen exposed leaves in the *E+* treatment group (ADD STAT TEST *p* < 0.01) (Fig. S7a).

The dbRDA analyses revealed that 6.34% of the variance is accounted for by the leaf functional traits, the constraining variables. The first axis (dbRDA1) explains 49% and the second axis (dbRDA2) explains 21.3% of the constrained variance (Fig. 2). We observe a high degree of overlap between the FEF communities (Fig. 2) indicating that the communities are similar in composition across host species and treatment groups (Fig. 2). We see tight clustering of FEF communities in *C. cainito* and *L. panamensis* although these are overlapped by *A. membranacea* pointing to a distinct subset composition of FEF OTUs within the latter (Fig. 2). All leaf functional traits significantly correlated with FEF community composition (Fig.2).

Multilevel pattern analysis revealed the OTUs significantly correlated with host trees species (Table S5). In summary, 72 out of 569 Ascomycota OTUs are significantly correlated to our host trees species (Table S5). Seedlings used in herbivory assays had 13 OTUs significantly correlated with high herbivory damage (>70%) and 3 and 1 OTUs significantly correlated with medium (31-69%) and low (<30%) herbivory damage, respectively (Table S6). Seedlings used in pathogen damage assays had 11 OTUs significantly correlated with high (>30%) pathogen damage (Table S7). Because the genomic data scale is at the plant level we cannot tease apart what OTUs are correlated with leaves treated with control and *Calonectria* sp. inoculated agar plugs treatments in the pathogen assays. We found 30 OTUs significantly correlated with *E+* treatment (Table S8).

The PCA revealed how leaf traits (ACI, LT, LPS and LMA) were related. We plotted leaf trait data according to tree species groups on the PCA axes to show how the variance in the complete data set is explained by PC1 (60%) and PC2 (27%) (Fig. 2a). We observed that ACI, LPS and LMA loadings tracked along PC1 towards more negative values, showing correlation among these traits (Fig. 2a). Traits LT and LPS were orthogonal to each other in Fig. 2a, indicative of low correlation. We note distinct grouping of species along PC1 such as *C. alliodora* in the direction of positive values of PC1 and *C. cainito* towards negative values. Along PC1 we see distinct and tight clustering according to species for all except *H. concinna* and *A. membranacea* which overlap with various other species. We note that LT loading tracks towards negative values along PC2 (Fig.2a). Clear species groupings are detected, such as *Dypterix* sp. located towards positive values and *L. panamensis* towards negative values of the PC2 axis. We note a similar relationship between the leaf traits with respect to PC1 and PC2 in individual seedlings used for herbivory versus pathogen damage trials (Fig. 2b-2c). The PCA of leaf traits from seedlings used in herbivory trials has a PC1 explaining 57.5% of the variance and a PC2 explaining 28% of the variance in the subset data (Fig. 2b). We saw an inversion of the LT loading in direction of positive values, as well as the main tree species clustered (i.e. *Dypteryx* sp. and *A. membranacea*) along PC2 (Fig. 2b) with respect to Fig. 2a. The PCA of leaf traits from seedling used in pathogen damage trials has a PC1 explaining 64% of the variance and a PC2 explaining 25% of the variance in the subset data (Fig. 2c). We detected similar relationships among leaf traits and PC axes in the pathogen damage subset data (Fig. 2c) when compared to the complete data set (Fig. 2a).

Simple linear regressions of herbivory (%) against PC1 and PC2 revealed a statistically significant positive relationship (*p* <0.001) (Fig. 4a and 3b). Even though we note large spread in the data (Fig. 4a and 3b), we see a statistically significant positive trend of herbivory plotted against PC1, where positive values represent greater values of ACI, LPS and LMA. Herbivory plotted against PC2 shows a statistically significant positive trend, where positive values represent greater LT (Fig. 4b). Percent pathogen damage plotted against PC1 revealed a statistically significant positive relationship (*p* = <0.001), in which positive values represent greater values of ACI, LPS and LMA (Fig. 4c). We did not see a statistically significant relationship (*p* = 0.223) between pathogen damage and PC2 (Fig. 4d).

**TALK ABOUT SIMPLE LINEAR REGRESSIONS OF SUPPLEMENTARY FIGURES?**

# 6. Discussion

# 7. References

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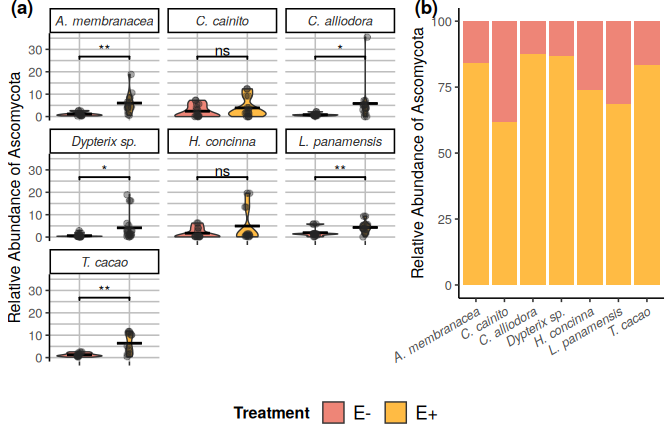
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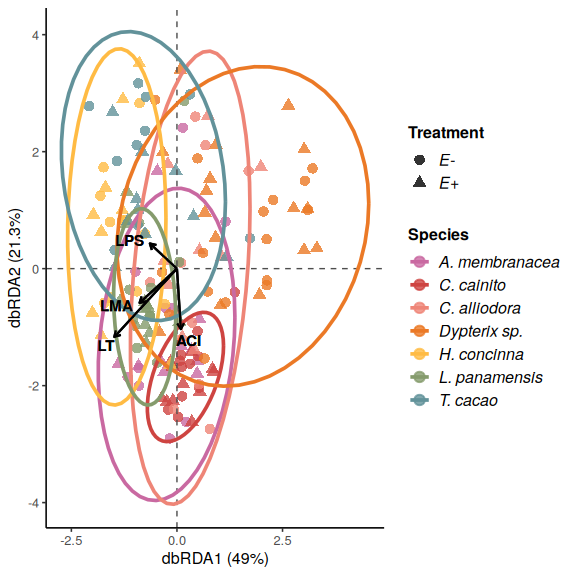
# 8. Figures

## 8.1 Figure 1



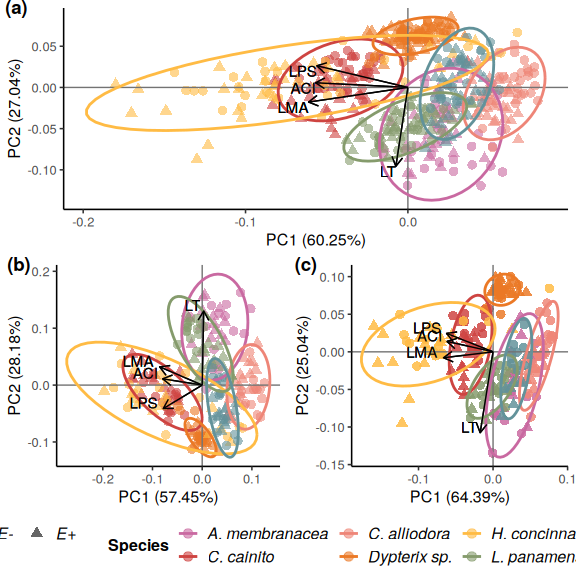
Relative abundance (RA) of Ascomycota OTUs of seven tree species used in the study. (a) Violin plots show individuals’ RA and and its distribution by species. The horizontal line within the violins represents the mean RA per species. (b) The RA of OTU’s by treatment withing each tree species. Pink filled violin plots represent low endophyte (*E-*) treatment and yellow filled represent high endophyte (*E+*) treatment. Relative abundance is the percentage of endophyte colonization within individuals of the same species. Significance levels are represented by asterisks [*p* = 0.05 (\*), *p* = 0.01 (\*\**), and* p = 0.001 (\*\*\*)].

## 8.2 Figure 2



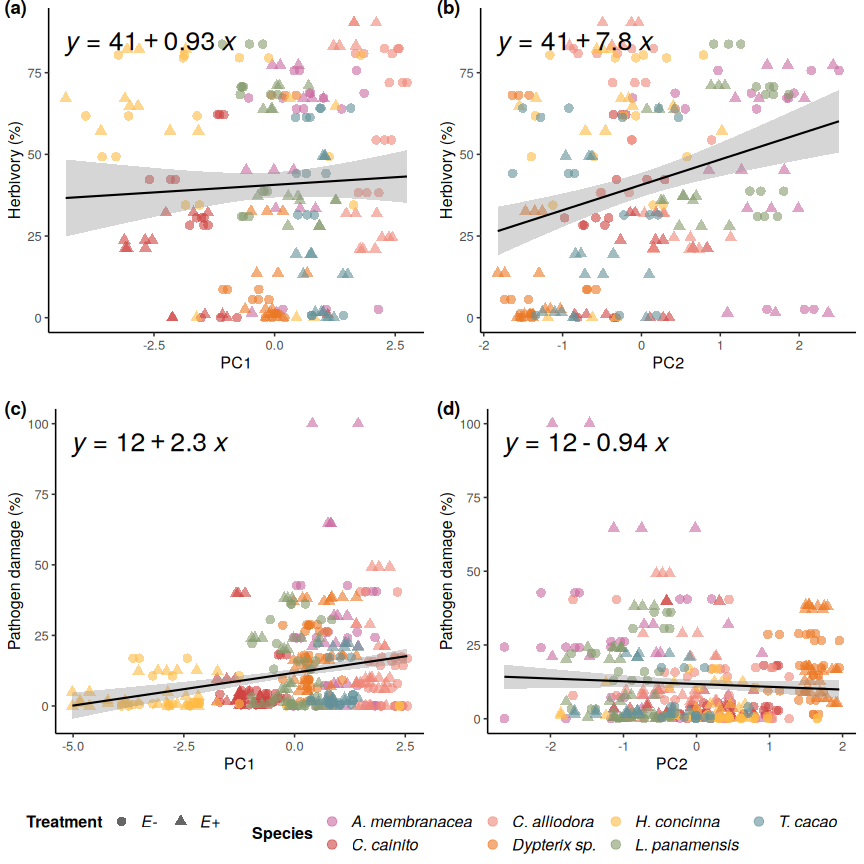
NO CAPTION YET

## 8.3 Figure 3



Leaf Functional traits are conserved within tree species regardless of endophyte load treatment. (a) Principal Component Analysis (PCA) of leaf functional traits from all tree species separated by *E-* and *E+* treatment. (b) PCA of leaf functional traits of plants solely used in ant herbivory assays. (c) PCA leaf functional traits of plants used solely in pathogen damage assays. Colors represent individual species. Circle and triangles represent low (*E-*) and high (*E+*) endophyte treatments, respectively. Colored ellipses correspond to tree species and represent 95% confidence intervals.

## 8.4 Figure 4



Simple linear regressions of herbivory and pathogen damage on PC1 and PC2 axes from PCAs of leaf traits for herbivory and pathogen damage assays. Linear regression of a) percent herbivory damage and PC1 axis (R2-adjusted= -0.0024, *p* = 0.447); b) percent herbivory damage and PC2 axis (R2-adjusted = 0.079, *p* = <0.001); c) percent pathogen damage and PC1 axis (R2-adjusted = 0.064, *p* = <0.001); and d) percent pathogen damage and PC2 axis (R2-adjusted = 0.0016, *p* = 0.207). Colors represent individual species. Circle and triangles represent *E-* and *E+* treatments, respectively.

# 9. Tables

## 9.1 Table 1

## 9.2 Table 2 : Summary statistics for the leaf functional traits

|  | ***A. membranacea, n = 831*** | ***C. alliodora, n = 1001*** | ***C. cainito, n = 1501*** | ***Dypterix sp., n = 2881*** | ***H. concinna, n = 1321*** | ***L. panamensis, n = 1851*** | ***T. cacao, n = 1761*** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| *Treatment* |  |  |  |  |  |  |  |
| E- (n = 570) | 47 | 54 | 75 | 144 | 66 | 95 | 89 |
| E+ (n = 544) | 36 | 46 | 75 | 144 | 66 | 90 | 87 |
| Anthocyanins (ACI) | 5.35 ± 1.06 | 3.47 ± 0.43 | 8.21 ± 1.41 | 6.34 ± 1.15 | 6.77 ± 2.86 | 5.91 ± 1.30 | 4.18 ± 0.77 |
| Leaf Thickness (LT) (µm) | 270 ± 45 | 207 ± 37 | 205 ± 30 | 148 ± 13 | 214 ± 42 | 245 ± 18 | 200 ± 43 |
| Leaf Punch Strength (LPS) (N mm/1) | 0.22 ± 0.05 | 0.21 ± 0.05 | 0.53 ± 0.09 | 0.43 ± 0.06 | 0.77 ± 0.23 | 0.33 ± 0.04 | 0.38 ± 0.06 |
| Leaf Mass per Area (LMA) (mg/mm) | 0.0011 ± 0.0002 | 0.0007 ± 0.0001 | 0.0015 ± 0.0002 | 0.0011 ± 0.0001 | 0.0017 ± 0.0004 | 0.0014 ± 0.0002 | 0.0009 ± 0.0001 |
| 1n; Mean ± SD | | | | | | | |

## 9.3 Table 3: Linear mixed effects models for predicting leaf herbivory and pathogenicity

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | logit herbivory | | | logit pathogenicity | | |
| Predictors | Estimates | CI | p | Estimates | CI | p |
| (Intercept) | -0.26 | -2.31 – 1.80 | 0.806 | -3.42 | -4.40 – -2.43 | **<0.001** |
| Thickness | -0.01 | -0.02 – -0.00 | **0.013** | 0.00 | -0.00 – 0.01 | 0.097 |
| LMA | 1741.22 | 768.82 – 2713.62 | **0.001** | 171.65 | -181.40 – 524.70 | 0.340 |
| E load [E+] | -0.78 | -1.11 – -0.45 | **<0.001** |  |  |  |
| Random Effects | | | | | | |
| σ2 | 3.34 | | | 2.16 | | |
| τ00 | 1.93 Species | | | 0.80 Treatment | | |
|  |  | | | 0.02 E\_load | | |
|  |  | | | 0.96 Species | | |
| ICC | 0.37 | | |  | | |
| N | 7 Species | | | 2 Treatment | | |
|  |  | | | 2 E\_load | | |
|  |  | | | 7 Species | | |
| Observations | 210 | | | 382 | | |
| Marginal R2 / Conditional R2 | 0.089 / 0.423 | | | 0.011 / NA | | |
| AIC | 745.559 | | | 1012.259 | | |
| AICc | 747.143 | | | 1013.248 | | |

# 10. Supplementary Materials

## 10.1 Table S1

| **Table S1: Student's t-Tests of mean anthocyanins (ACI)**  Pairwise comparisons of ACI between species. | | | | |
| --- | --- | --- | --- | --- |
|  | *p* - values | | | |
| Comparison Species*1* | p | p.adj | p.format | p.signif*2* |
| ***A. membranacea*** | | | | |
| *C. cainito* | **2.636 × 10^-16** | **4.500 × 10^-15** | **2.6e-16** | **\*\*\*\*** |
| *C. alliodora* | **3.713 × 10^-15** | **5.600 × 10^-14** | **3.7e-15** | **\*\*\*\*** |
| *Dypterix* sp. | **2.296 × 10^-6** | **1.600 × 10^-5** | **2.3e-06** | **\*\*\*\*** |
| *H. concinna* | **6.179 × 10^-6** | **3.700 × 10^-5** | **6.2e-06** | **\*\*\*\*** |
| *L. panamensis* | **1.538 × 10^-2** | **4.600 × 10^-2** | **0.01538** | **\*** |
| *T. cacao* | **9.137 × 10^-8** | **8.200 × 10^-7** | **9.1e-08** | **\*\*\*\*** |
| ***C. cainito*** | | | | |
| *C. alliodora* | **3.154 × 10^-23** | **6.300 × 10^-22** | **< 2e-16** | **\*\*\*\*** |
| *Dypterix* sp. | **4.559 × 10^-10** | **5.000 × 10^-9** | **4.6e-10** | **\*\*\*\*** |
| *H. concinna* | 5.309 × 10^-1 | 5.300 × 10^-1 | 0.53085 | ns |
| *L. panamensis* | **1.599 × 10^-11** | **2.200 × 10^-10** | **1.6e-11** | **\*\*\*\*** |
| *T. cacao* | **3.656 × 10^-22** | **6.900 × 10^-21** | **< 2e-16** | **\*\*\*\*** |
| ***C. alliodora*** | | | | |
| *Dypterix* sp. | **1.150 × 10^-26** | **2.400 × 10^-25** | **< 2e-16** | **\*\*\*\*** |
| *H. concinna* | **7.276 × 10^-11** | **9.500 × 10^-10** | **7.3e-11** | **\*\*\*\*** |
| *L. panamensis* | **2.486 × 10^-15** | **4.000 × 10^-14** | **2.5e-15** | **\*\*\*\*** |
| *T. cacao* | **1.428 × 10^-7** | **1.100 × 10^-6** | **1.4e-07** | **\*\*\*\*** |
| ***Dypterix* sp.** | | | | |
| *H. concinna* | **3.050 × 10^-3** | **1.200 × 10^-2** | **0.00305** | **\*\*** |
| *L. panamensis* | 6.646 × 10^-2 | 1.300 × 10^-1 | 0.06646 | ns |
| *T. cacao* | **3.807 × 10^-18** | **6.900 × 10^-17** | **< 2e-16** | **\*\*\*\*** |
| ***H. concinna*** | | | | |
| *L. panamensis* | **3.062 × 10^-4** | **1.500 × 10^-3** | **0.00031** | **\*\*\*** |
| *T. cacao* | **5.704 × 10^-9** | **5.700 × 10^-8** | **5.7e-09** | **\*\*\*\*** |
| ***L. panamensis*** | | | | |
| *T. cacao* | **3.583 × 10^-10** | **4.300 × 10^-9** | **3.6e-10** | **\*\*\*\*** |
| *1* *n* = 156 individuals | | | | |
| *2*Significance levels are represented by *ns* (not significant) and asterisks [*p* <= 0.05 (\*), *p <*= 0.01 (\*\*), *p <*= 0.001 (\*\*\*), and *p* < 0.0001 (\*\*\*\*)]. | | | | |

## 10.2 Table S2

| **Table S2: Student's t-Tests of mean leaf thickness (LT) (μm)**  Pairwise comparisons of LT between species. | | | | |
| --- | --- | --- | --- | --- |
|  | *p* - values | | | |
| Comparison Species*1* | p | p.adj | p.format | p.signif*2* |
| ***A. membranacea*** | | | | |
| *C. cainito* | **1.793 × 10^-8** | **2.300 × 10^-7** | **1.8e-08** | **\*\*\*\*** |
| *C. alliodora* | **6.857 × 10^-8** | **8.200 × 10^-7** | **6.9e-08** | **\*\*\*\*** |
| *Dypterix* sp. | **7.836 × 10^-18** | **1.500 × 10^-16** | **< 2e-16** | **\*\*\*\*** |
| *H. concinna* | **1.255 × 10^-5** | **1.000 × 10^-4** | **1.3e-05** | **\*\*\*\*** |
| *L. panamensis* | 4.986 × 10^-1 | 1.000 | 0.499 | ns |
| *T. cacao* | **1.604 × 10^-6** | **1.400 × 10^-5** | **1.6e-06** | **\*\*\*\*** |
| ***C. cainito*** | | | | |
| *C. alliodora* | 7.854 × 10^-1 | 1.000 | 0.785 | ns |
| *Dypterix* sp. | **2.605 × 10^-19** | **5.200 × 10^-18** | **< 2e-16** | **\*\*\*\*** |
| *H. concinna* | 6.876 × 10^-2 | 4.800 × 10^-1 | 0.069 | ns |
| *L. panamensis* | **1.382 × 10^-12** | **2.100 × 10^-11** | **1.4e-12** | **\*\*\*\*** |
| *T. cacao* | 4.765 × 10^-1 | 1.000 | 0.477 | ns |
| ***C. alliodora*** | | | | |
| *Dypterix* sp. | **8.662 × 10^-17** | **1.500 × 10^-15** | **< 2e-16** | **\*\*\*\*** |
| *H. concinna* | 1.347 × 10^-1 | 8.100 × 10^-1 | 0.135 | ns |
| *L. panamensis* | **1.161 × 10^-10** | **1.600 × 10^-9** | **1.2e-10** | **\*\*\*\*** |
| *T. cacao* | 6.481 × 10^-1 | 1.000 | 0.648 | ns |
| ***Dypterix* sp.** | | | | |
| *H. concinna* | **8.177 × 10^-17** | **1.500 × 10^-15** | **< 2e-16** | **\*\*\*\*** |
| *L. panamensis* | **8.008 × 10^-32** | **1.700 × 10^-30** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **4.639 × 10^-13** | **7.400 × 10^-12** | **4.6e-13** | **\*\*\*\*** |
| ***H. concinna*** | | | | |
| *L. panamensis* | **7.274 × 10^-7** | **7.300 × 10^-6** | **7.3e-07** | **\*\*\*\*** |
| *T. cacao* | 3.649 × 10^-1 | 1.000 | 0.365 | ns |
| ***L. panamensis*** | | | | |
| *T. cacao* | **1.707 × 10^-7** | **1.900 × 10^-6** | **1.7e-07** | **\*\*\*\*** |
| *1* *n* = 156 individuals | | | | |
| *2*Significance levels are represented by *ns* (not significant) and asterisks [*p* <= 0.05 (\*), *p <*= 0.01 (\*\*), *p <*= 0.001 (\*\*\*), and *p* < 0.0001 (\*\*\*\*)]. | | | | |

## 10.3 Table S3

| **Table S3: Student's t-Tests of mean leaf punch strength (LPS) (N mm-1)**  Pairwise comparisons of LPS between species. | | | | |
| --- | --- | --- | --- | --- |
|  | *p* - values | | | |
| Comparison Species*1* | p | p.adj | p.format | p.signif*2* |
| ***A. membranacea*** | | | | |
| *C. cainito* | **9.032 × 10^-36** | **1.600 × 10^-34** | **< 2e-16** | **\*\*\*\*** |
| *C. alliodora* | 3.180 × 10^-1 | 3.200 × 10^-1 | 0.32 | ns |
| *Dypterix* sp. | **3.538 × 10^-43** | **7.400 × 10^-42** | **< 2e-16** | **\*\*\*\*** |
| *H. concinna* | **7.548 × 10^-21** | **8.700 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| *L. panamensis* | **7.304 × 10^-26** | **1.200 × 10^-24** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **7.242 × 10^-21** | **8.700 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| ***C. cainito*** | | | | |
| *C. alliodora* | **3.873 × 10^-39** | **7.700 × 10^-38** | **< 2e-16** | **\*\*\*\*** |
| *Dypterix* sp. | **3.649 × 10^-16** | **2.200 × 10^-15** | **3.6e-16** | **\*\*\*\*** |
| *H. concinna* | **6.101 × 10^-12** | **2.400 × 10^-11** | **6.1e-12** | **\*\*\*\*** |
| *L. panamensis* | **3.975 × 10^-28** | **6.800 × 10^-27** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **7.651 × 10^-21** | **8.700 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| ***C. alliodora*** | | | | |
| *Dypterix* sp. | **1.738 × 10^-36** | **3.300 × 10^-35** | **< 2e-16** | **\*\*\*\*** |
| *H. concinna* | **1.267 × 10^-21** | **1.600 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| *L. panamensis* | **8.205 × 10^-21** | **8.700 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **1.371 × 10^-22** | **1.900 × 10^-21** | **< 2e-16** | **\*\*\*\*** |
| ***Dypterix* sp.** | | | | |
| *H. concinna* | **1.617 × 10^-15** | **8.100 × 10^-15** | **1.6e-15** | **\*\*\*\*** |
| *L. panamensis* | **7.768 × 10^-26** | **1.200 × 10^-24** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **3.965 × 10^-7** | **8.800 × 10^-7** | **4.0e-07** | **\*\*\*\*** |
| ***H. concinna*** | | | | |
| *L. panamensis* | **2.293 × 10^-18** | **1.800 × 10^-17** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **1.173 × 10^-17** | **8.200 × 10^-17** | **< 2e-16** | **\*\*\*\*** |
| ***L. panamensis*** | | | | |
| *T. cacao* | **2.949 × 10^-7** | **8.800 × 10^-7** | **2.9e-07** | **\*\*\*\*** |
| *1* *n* = 156 individuals | | | | |
| *2*Significance levels are represented by *ns* (not significant) and asterisks [*p* <= 0.05 (\*), *p <*= 0.01 (\*\*), *p <*= 0.001 (\*\*\*), and *p* < 0.0001 (\*\*\*\*)]. | | | | |

## 10.4 Table S4

| **Table S4:** **Student's t-Tests of mean leaf mass per area (LMA) (mg mm-2)**  Pairwise comparisons of LMA between species. | | | | |
| --- | --- | --- | --- | --- |
|  | *p* - values | | | |
| Comparison Species*1* | p | p.adj | p.format | p.signif*2* |
| ***A. membranacea*** | | | | |
| *C. cainito* | **9.032 × 10^-36** | **1.600 × 10^-34** | **< 2e-16** | **\*\*\*\*** |
| *C. alliodora* | 3.180 × 10^-1 | 3.200 × 10^-1 | 0.32 | ns |
| *Dypterix* sp. | **3.538 × 10^-43** | **7.400 × 10^-42** | **< 2e-16** | **\*\*\*\*** |
| *H. concinna* | **7.548 × 10^-21** | **8.700 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| *L. panamensis* | **7.304 × 10^-26** | **1.200 × 10^-24** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **7.242 × 10^-21** | **8.700 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| ***C. cainito*** | | | | |
| *C. alliodora* | **3.873 × 10^-39** | **7.700 × 10^-38** | **< 2e-16** | **\*\*\*\*** |
| *Dypterix* sp. | **3.649 × 10^-16** | **2.200 × 10^-15** | **3.6e-16** | **\*\*\*\*** |
| *H. concinna* | **6.101 × 10^-12** | **2.400 × 10^-11** | **6.1e-12** | **\*\*\*\*** |
| *L. panamensis* | **3.975 × 10^-28** | **6.800 × 10^-27** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **7.651 × 10^-21** | **8.700 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| ***C. alliodora*** | | | | |
| *Dypterix* sp. | **1.738 × 10^-36** | **3.300 × 10^-35** | **< 2e-16** | **\*\*\*\*** |
| *H. concinna* | **1.267 × 10^-21** | **1.600 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| *L. panamensis* | **8.205 × 10^-21** | **8.700 × 10^-20** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **1.371 × 10^-22** | **1.900 × 10^-21** | **< 2e-16** | **\*\*\*\*** |
| ***Dypterix* sp.** | | | | |
| *H. concinna* | **1.617 × 10^-15** | **8.100 × 10^-15** | **1.6e-15** | **\*\*\*\*** |
| *L. panamensis* | **7.768 × 10^-26** | **1.200 × 10^-24** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **3.965 × 10^-7** | **8.800 × 10^-7** | **4.0e-07** | **\*\*\*\*** |
| ***H. concinna*** | | | | |
| *L. panamensis* | **2.293 × 10^-18** | **1.800 × 10^-17** | **< 2e-16** | **\*\*\*\*** |
| *T. cacao* | **1.173 × 10^-17** | **8.200 × 10^-17** | **< 2e-16** | **\*\*\*\*** |
| ***L. panamensis*** | | | | |
| *T. cacao* | **2.949 × 10^-7** | **8.800 × 10^-7** | **2.9e-07** | **\*\*\*\*** |
| *1* *n* = 156 individuals | | | | |
| *2* Significance levels are represented by *ns* (not significant) and asterisks [*p* <= 0.05 (\*), *p <*= 0.01 (\*\*), *p <*= 0.001 (\*\*\*), and *p* < 0.0001 (\*\*\*\*)]. | | | | |

## 10.5 Table S5

| **Table S5: Taxonomy of OTUs significantly correlated OTUs with tree host species** | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  | Multilevel pattern analysis | | | |
| Kingdom | Phylum | Class | Order | Family | Genus | Species | OTU | Index | Stat | *p1* | *p*adj*2* |
| ***T. cacao*** | | | | | | | | | | | |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Mycosphaerellaceae | Coremiopassalora | *Coremiopassalora leptophlebae* | OTU 2 | 7 | 0.507 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Mycosphaerellaceae | Pseudocercospora | *Pseudocercospora sp* | OTU 5 | 7 | 0.531 | \*\* | 0.012 |
| Fungi | Ascomycota | Eurotiomycetes | Eurotiales | Trichocomaceae | Talaromyces | *Talaromyces sp* | OTU 3 | 7 | 0.469 | \*\* | 0.012 |
| Fungi | Ascomycota | unidentified | unidentified | unidentified | unidentified | *Ascomycota sp* | OTU 14 | 7 | 0.500 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Cladosporiaceae | Cladosporium | *Cladosporium sp* | OTU 20 | 7 | 0.382 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Mycosphaerellaceae | Zasmidium | *Zasmidium queenslandicum* | OTU 95 | 7 | 0.390 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Hypocreales\_fam\_Incertae\_sedis | Acremonium | *Acremonium sp* | OTU 84 | 7 | 0.430 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Dissoconiaceae | Ramichloridium | *Ramichloridium sp* | OTU 79 | 7 | 0.362 | \*\* | 0.019 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Mycosphaerellaceae | Mycosphaerella | *Mycosphaerella sp* | OTU 183 | 7 | 0.427 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Annulohypoxylon | *Annulohypoxylon urceolatum* | OTU 279 | 7 | 0.352 | \*\* | 0.019 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Mycosphaerellaceae | Zasmidium | *Zasmidium commune* | OTU 286 | 7 | 0.331 | \*\* | 0.041 |
| ***H. concinna*** | | | | | | | | | | | |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | *Fusarium sp* | OTU 7 | 5 | 0.330 | \*\* | 0.012 |
| Fungi | Ascomycota | Eurotiomycetes | Chaetothyriales | Herpotrichiellaceae | Exophiala | *Exophiala oligosperma* | OTU 21 | 5 | 0.220 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Clavicipitaceae | unidentified | *Clavicipitaceae sp* | OTU 53 | 5 | 0.281 | \*\* | 0.012 |
| Fungi | Ascomycota | Saccharomycetes | Saccharomycetales | Saccharomycetales\_fam\_Incertae\_sedis | Candida | *Candida parapsilosis* | OTU 67 | 5 | 0.272 | \*\* | 0.027 |
| Fungi | Ascomycota | Eurotiomycetes | Chaetothyriales | Trichomeriaceae | Bradymyces | *Bradymyces sp* | OTU 160 | 5 | 0.251 | \*\* | 0.047 |
| Fungi | Ascomycota | Eurotiomycetes | Chaetothyriales | Herpotrichiellaceae | Exophiala | *Exophiala oligosperma* | OTU 173 | 5 | 0.350 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Sordariales | Chaetomiaceae | unidentified | *Chaetomiaceae sp* | OTU 209 | 5 | 0.351 | \*\* | 0.019 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | Didymellaceae | Neodidymelliopsis | *Neodidymelliopsis sambuci* | OTU 596 | 5 | 0.351 | \*\* | 0.019 |
| ***Dypterix sp.*** | | | | | | | | | | | |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Dissoconiaceae | Uwebraunia | *Uwebraunia dekkeri* | OTU 12 | 4 | 0.418 | \*\* | 0.012 |
| Fungi | Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus | *Aspergillus sp* | OTU 122 | 4 | 0.257 | \*\* | 0.047 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Dissoconiaceae | Ramichloridium | *Ramichloridium punctatum* | OTU 151 | 4 | 0.378 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | unidentified | *Xylariaceae sp* | OTU 216 | 4 | 0.390 | \*\* | 0.019 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Schizothyriaceae | Zygophiala | *Zygophiala qianensis* | OTU 305 | 4 | 0.333 | \*\* | 0.034 |
| ***A. membranacea*** | | | | | | | | | | | |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | unidentified | unidentified | *Xylariales sp* | OTU 25 | 1 | 0.219 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Mycosphaerellaceae | Septoria | *Septoria sp* | OTU 26 | 1 | 0.366 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Xylaria | *Xylaria curta* | OTU 172 | 9 | 0.413 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | Pleosporaceae | Curvularia | *Curvularia sp* | OTU 120 | 1 | 0.352 | \*\* | 0.047 |
| ***C. alliodora*** | | | | | | | | | | | |
| Fungi | Ascomycota | Dothideomycetes | Botryosphaeriales | Phyllostictaceae | Phyllosticta | *Phyllosticta capitalensis* | OTU 32 | 19 | 0.362 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe longicolla* | OTU 31 | 3 | 0.531 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Glomerellaceae | Colletotrichum | *Colletotrichum gigasporum* | OTU 34 | 3 | 0.374 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Xylaria | *Xylaria sp* | OTU 62 | 3 | 0.516 | \*\* | 0.012 |
| Fungi | Ascomycota | unidentified | unidentified | unidentified | unidentified | *Ascomycota sp* | OTU 52 | 3 | 0.494 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe sp* | OTU 99 | 3 | 0.443 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Sordariales | Chaetomiaceae | Ovatospora | *Ovatospora brasiliensis* | OTU 61 | 3 | 0.374 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | Phaeosphaeriaceae | Setophoma | *Setophoma sp* | OTU 71 | 3 | 0.386 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Annulohypoxylon | *Annulohypoxylon stygium* | OTU 78 | 3 | 0.503 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Hypoxylon | *Hypoxylon sp* | OTU 101 | 3 | 0.483 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe sp* | OTU 223 | 3 | 0.415 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Xylaria | *Xylaria sp* | OTU 212 | 3 | 0.501 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Cladosporiaceae | Melomastia | *Melomastia sp* | OTU 117 | 3 | 0.429 | \*\* | 0.012 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | Morosphaeriaceae | Acrocalymma | *Acrocalymma sp* | OTU 169 | 3 | 0.434 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe sp* | OTU 512 | 3 | 0.461 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe melonis* | OTU 179 | 3 | 0.475 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariales\_fam\_Incertae\_sedis | Oxydothis | *Oxydothis garethjonesii* | OTU 94 | 57 | 0.366 | \*\* | 0.027 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | unidentified | *Xylariaceae sp* | OTU 106 | 3 | 0.455 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Hypoxylon | *Hypoxylon hypomiltum* | OTU 142 | 3 | 0.369 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Xylaria | *Xylaria sp* | OTU 262 | 3 | 0.399 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe sp* | OTU 327 | 3 | 0.480 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Hypoxylon | *Hypoxylon submonticulosum* | OTU 202 | 3 | 0.339 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Amphisphaeriaceae | Lepteutypa | *Lepteutypa sambuci* | OTU 210 | 3 | 0.410 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | unidentified | *Xylariaceae sp* | OTU 380 | 3 | 0.351 | \*\* | 0.019 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | unidentified | *Xylariaceae sp* | OTU 537 | 3 | 0.451 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Sordariales | Lasiosphaeriaceae | unidentified | *Lasiosphaeriaceae sp* | OTU 219 | 3 | 0.349 | \*\* | 0.019 |
| Fungi | Ascomycota | unidentified | unidentified | unidentified | unidentified | *Ascomycota sp* | OTU 192 | 3 | 0.395 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Lopadostoma | *Lopadostoma americanum* | OTU 936 | 3 | 0.417 | \*\* | 0.019 |
| Fungi | Ascomycota | Sordariomycetes | Sordariales | Sordariales\_fam\_Incertae\_sedis | Ramophialophora | *Ramophialophora sp* | OTU 362 | 3 | 0.330 | \*\* | 0.041 |
| Fungi | Ascomycota | unidentified | unidentified | unidentified | unidentified | *Ascomycota sp* | OTU 784 | 3 | 0.399 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | unidentified | unidentified | unidentified | *Sordariomycetes sp* | OTU 397 | 3 | 0.435 | \*\* | 0.019 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe fraxini-angustifoliae* | OTU 575 | 3 | 0.379 | \*\* | 0.019 |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Glomerellaceae | Colletotrichum | *Colletotrichum ignotum* | OTU 614 | 3 | 0.342 | \*\* | 0.034 |
| ***C. cainito*** | | | | | | | | | | | |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | unidentified | unidentified | *Xylariales sp* | OTU 58 | 2 | 0.362 | \*\* | 0.027 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Hypocreales\_fam\_Incertae\_sedis | Sarocladium | *Sarocladium gamsii* | OTU 75 | 18 | 0.335 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Valsaceae | Phomopsis | *Phomopsis sp* | OTU 86 | 2 | 0.323 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Sordariales | Cephalothecaceae | Phialemonium | *Phialemonium dimorphosporum* | OTU 139 | 2 | 0.334 | \*\* | 0.019 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Hypocreales\_fam\_Incertae\_sedis | Acremonium | *Acremonium hennebertii* | OTU 145 | 2 | 0.406 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | unidentified | unidentified | *Xylariales sp* | OTU 128 | 50 | 0.341 | \*\* | 0.041 |
| Fungi | Ascomycota | Arthoniomycetes | Lichenostigmatales | Phaeococcomycetaceae | Phaeococcomyces | *Phaeococcomyces rothmanniae* | OTU 197 | 2 | 0.352 | \*\* | 0.012 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe sp* | OTU 761 | 2 | 0.418 | \*\* | 0.012 |
| ***L. panamensis*** | | | | | | | | | | | |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariales\_fam\_Incertae\_sedis | Oxydothis | *Oxydothis sp* | OTU 221 | 6 | 0.335 | \*\* | 0.027 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | unidentified | unidentified | *Pleosporales sp* | OTU 232 | 6 | 0.336 | \*\* | 0.027 |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Glomerellales\_fam\_Incertae\_sedis | Malaysiasca | *Malaysiasca phaii* | OTU 735 | 6 | 0.309 | \*\* | 0.019 |
| *1* Significance levels are represented by *ns* (not significant) and asterisks [*p* <= 0.05 (\*), *p <*= 0.01 (\*\*), *p <*= 0.001 (\*\*\*), and *p* < 0.0001 (\*\*\*\*)]. | | | | | | | | | | | |
| *2*Benjamini & Hochberg method adjustment for multiple comparisons | | | | | | | | | | | |

## 10.6 Table S6

| **Table S6: Taxonomy of significantly correlated OTUs with *Atta colombica* herbivory levels** | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  | Multilevel pattern analysis | | | |
| Kingdom | Phylum | Class | Order | Family | Genus | Species | OTU | Index | Stat | *p2* | *p*adj*3* |
| **Medium** | | | | | | | | | | | |
| Fungi | Ascomycota | Eurotiomycetes | Chaetothyriales | Cyphellophoraceae | Cyphellophora | *Cyphellophora oxyspora* | OTU 19 | 3 | 0.293 | \* | 1.000 |
| Fungi | Ascomycota | unidentified | unidentified | unidentified | unidentified | *Ascomycota sp* | OTU 153 | 3 | 0.307 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | unidentified | unidentified | *Hypocreales sp* | OTU 682 | 3 | 0.307 | \* | 1.000 |
| **High** | | | | | | | | | | | |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Xylaria | *Xylaria sp* | OTU 62 | 1 | 0.315 | \* | 1.000 |
| Fungi | Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus | *Aspergillus terreus* | OTU 55 | 1 | 0.232 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Xylaria | *Xylaria sp* | OTU 212 | 1 | 0.343 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | unidentified | *Xylariaceae sp* | OTU 106 | 1 | 0.306 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Microascales | unidentified | unidentified | *Microascales sp* | OTU 608 | 1 | 0.268 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Cordycipitaceae | Beauveria | *Beauveria sp* | OTU 204 | 1 | 0.283 | \* | 1.000 |
| Fungi | Ascomycota | unidentified | unidentified | unidentified | unidentified | *Ascomycota sp* | OTU 784 | 1 | 0.258 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Sordariales | unidentified | unidentified | *Sordariales sp* | OTU 437 | 1 | 0.300 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | unidentified | unidentified | unidentified | *Sordariomycetes sp* | OTU 492 | 1 | 0.257 | \* | 1.000 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | Lentitheciaceae | Poaceascoma | *Poaceascoma sp* | OTU 644 | 1 | 0.280 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Microascales | unidentified | unidentified | *Microascales sp* | OTU 1043 | 1 | 0.301 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Microascales | Halosphaeriaceae | unidentified | *Halosphaeriaceae sp* | OTU 1053 | 1 | 0.245 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariales\_fam\_Incertae\_sedis | Phialemoniopsis | *Phialemoniopsis sp* | OTU 1067 | 1 | 0.303 | \* | 1.000 |
| **Low** | | | | | | | | | | | |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Hypocreales\_fam\_Incertae\_sedis | Acremonium | *Acremonium hennebertii* | OTU 100 | 2 | 0.323 | \*\* | 1.000 |
| *1*High = >70% leaf area damage, Medium = 31-69% leaf area damage, Low = <30% leaf area damage | | | | | | | | | | | |
| *2*Significance levels are represented by *ns* (not significant) and asterisks [*p* <= 0.05 (\*), *p <*= 0.01 (\*\*), *p <*= 0.001 (\*\*\*), and *p* < 0.0001 (\*\*\*\*)]. | | | | | | | | | | | |
| *3*Benjamini & Hochberg method adjustment for multiple comparisons | | | | | | | | | | | |

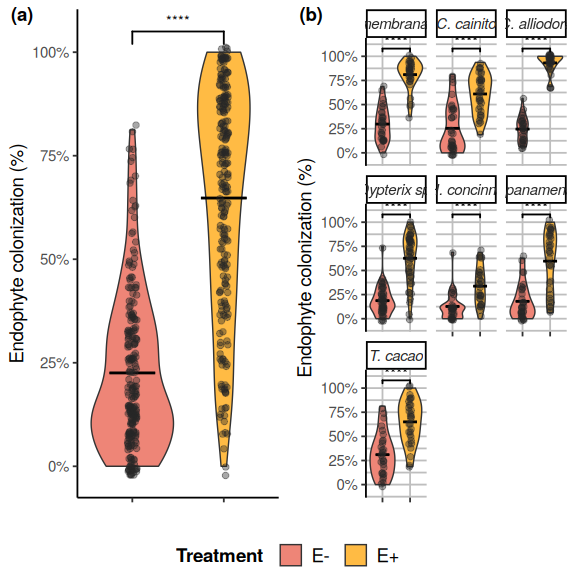
## 10.7 Table S7

| **Table S7: Taxonomy of significantly correlated OTUs with *Calonectria* sp. pathogen damage levels** | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  | Multilevel pattern analysis | | | |
| Kingdom | Phylum | Class | Order | Family | Genus | Species | OTU | Index | Stat | *p2* | *p*adj*3* |
| **High** | | | | | | | | | | | |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Glomerellaceae | Colletotrichum | *Colletotrichum fructicola* | OTU 1 | 1 | 0.222 | \* | 1.000 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Dissoconiaceae | Ramichloridium | *Ramichloridium apiculatum* | OTU 18 | 1 | 0.239 | \* | 1.000 |
| Fungi | Ascomycota | Dothideomycetes | Botryosphaeriales | Phyllostictaceae | Phyllosticta | *Phyllosticta capitalensis* | OTU 32 | 1 | 0.229 | ns | 1.000 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | unidentified | unidentified | *Pleosporales sp* | OTU 70 | 1 | 0.226 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | unidentified | unidentified | unidentified | *Sordariomycetes sp* | OTU 114 | 1 | 0.187 | \* | 1.000 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | Leptosphaeriaceae | Leptosphaeria | *Leptosphaeria modesta* | OTU 108 | 1 | 0.236 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Chaetosphaeriales | Chaetosphaeriaceae | unidentified | *Chaetosphaeriaceae sp* | OTU 177 | 1 | 0.204 | ns | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Hypoxylon | *Hypoxylon sp* | OTU 244 | 1 | 0.205 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | unidentified | unidentified | *Hypocreales sp* | OTU 196 | 1 | 0.227 | ns | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Sordariales | Lasiosphaeriaceae | unidentified | *Lasiosphaeriaceae sp* | OTU 258 | 1 | 0.225 | \* | 1.000 |
| Fungi | Ascomycota | Sordariomycetes | Phomatosporales | Phomatosporaceae | Phomatospora | *Phomatospora sp* | OTU 637 | 1 | 0.209 | \* | 1.000 |
| *1*High = >30% leaf area damage, Low = <29% leaf area damage | | | | | | | | | | | |
| *2*Significance levels are represented by *ns* (not significant) and asterisks [*p* <= 0.05 (\*), *p <*= 0.01 (\*\*), *p <*= 0.001 (\*\*\*), and *p* < 0.0001 (\*\*\*\*)]. | | | | | | | | | | | |
| *3*Benjamini & Hochberg method adjustment for multiple comparisons | | | | | | | | | | | |

## 10.8 Table S8

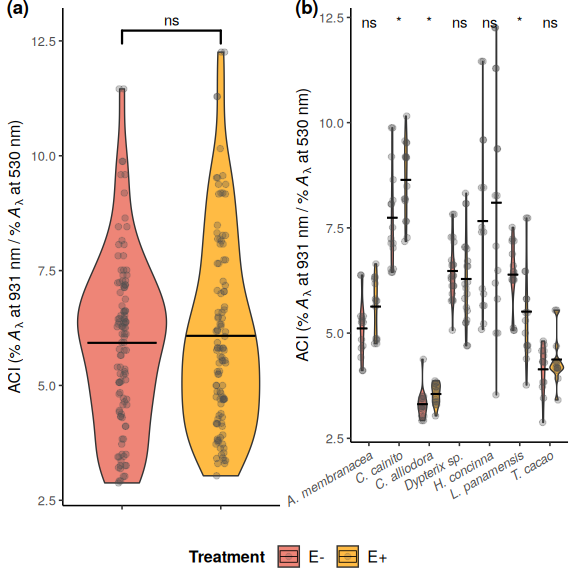
| **Table S8: Taxonomy of significantly correlated OTUs with FEF inoculation levels** | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  | Multilevel pattern analysis | | | |
| Kingdom | Phylum | Class | Order | Family | Genus | Species | OTU | Index | Stat | *p1* | *p*adj*2* |
| **E+** | | | | | | | | | | | |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Glomerellaceae | Colletotrichum | *Colletotrichum fructicola* | OTU 1 | 2 | 0.110 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Sporocadaceae | Neopestalotiopsis | *Neopestalotiopsis sp* | OTU 10 | 2 | 0.196 | \*\* | 0.022 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Dissoconiaceae | Uwebraunia | *Uwebraunia dekkeri* | OTU 12 | 2 | 0.202 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | unidentified | unidentified | unidentified | *Sordariomycetes sp* | OTU 36 | 2 | 0.415 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | unidentified | unidentified | *Hypocreales sp* | OTU 39 | 2 | 0.301 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe longicolla* | OTU 31 | 2 | 0.208 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Plectosphaerellaceae | Wallrothiella | *Wallrothiella subiculosa* | OTU 35 | 2 | 0.271 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Glomerellaceae | Colletotrichum | *Colletotrichum gigasporum* | OTU 34 | 2 | 0.273 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | unidentified | unidentified | unidentified | *Sordariomycetes sp* | OTU 42 | 2 | 0.416 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Sporocadaceae | Pseudopestalotiopsis | *Pseudopestalotiopsis sp* | OTU 46 | 2 | 0.192 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Hypoceales | Amplistromataceae | Amplistroma | *Amplistroma erinaceum* | OTU 60 | 2 | 0.319 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | Xylaria | *Xylaria sp* | OTU 62 | 2 | 0.236 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Hypocreales\_fam\_Incertae\_sedis | Acremonium | *Acremonium sp* | OTU 49 | 2 | 0.174 | \*\* | 0.022 |
| Fungi | Ascomycota | unidentified | unidentified | unidentified | unidentified | *Ascomycota sp* | OTU 52 | 2 | 0.289 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe sp* | OTU 99 | 2 | 0.210 | \*\* | 0.037 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Cladosporiaceae | Cladosporium | *Cladosporium sp* | OTU 132 | 2 | 0.196 | \*\* | 0.037 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Hypocreales\_fam\_Incertae\_sedis | Acremonium | *Acremonium hennebertii* | OTU 77 | 2 | 0.270 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe sp* | OTU 223 | 2 | 0.179 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Hypocreales | Hypocreales\_fam\_Incertae\_sedis | Acremonium | *Acremonium hennebertii* | OTU 100 | 2 | 0.262 | \*\* | 0.022 |
| Fungi | Ascomycota | Dothideomycetes | Capnodiales | Cladosporiaceae | Melomastia | *Melomastia sp* | OTU 117 | 2 | 0.167 | \*\* | 0.037 |
| Fungi | Ascomycota | Sordariomycetes | unidentified | unidentified | unidentified | *Sordariomycetes sp* | OTU 92 | 2 | 0.293 | \*\* | 0.022 |
| Fungi | Ascomycota | Dothideomycetes | Pleosporales | Leptosphaeriaceae | Leptosphaeria | *Leptosphaeria modesta* | OTU 108 | 2 | 0.282 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Diaporthales | Diaporthaceae | Diaporthe | *Diaporthe melonis* | OTU 179 | 2 | 0.223 | \*\* | 0.037 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariales\_fam\_Incertae\_sedis | Oxydothis | *Oxydothis garethjonesii* | OTU 94 | 2 | 0.300 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Xylariales | Xylariaceae | unidentified | *Xylariaceae sp* | OTU 106 | 2 | 0.256 | \*\* | 0.022 |
| Fungi | Ascomycota | Eurotiomycetes | Chaetothyriales | unidentified | unidentified | *Chaetothyriales sp* | OTU 126 | 2 | 0.308 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Plectosphaerellaceae | unidentified | *Plectosphaerellaceae sp* | OTU 146 | 2 | 0.130 | \*\* | 0.037 |
| Fungi | Ascomycota | Sordariomycetes | Sordariomycetes\_ord\_Incertae\_sedis | Sordariomycetes\_fam\_Incertae\_sedis | Distoseptispora | *Distoseptispora sp* | OTU 148 | 2 | 0.288 | \*\* | 0.022 |
| Fungi | Ascomycota | Eurotiomycetes | Chaetothyriales | Herpotrichiellaceae | Phialophora | *Phialophora geniculata* | OTU 278 | 2 | 0.301 | \*\* | 0.022 |
| Fungi | Ascomycota | Sordariomycetes | Glomerellales | Plectosphaerellaceae | Plectosphaerella | *Plectosphaerella cucumerina* | OTU 390 | 2 | 0.197 | \*\* | 0.022 |
| Fungi | Ascomycota | Dothideomycetes | unidentified | unidentified | unidentified | *Dothideomycetes sp* | OTU 201 | 2 | 0.269 | \*\* | 0.022 |
| *1*Significance levels are represented by *ns* (not significant) and asterisks [*p* <= 0.05 (\*), *p <*= 0.01 (\*\*), *p <*= 0.001 (\*\*\*), and *p* < 0.0001 (\*\*\*\*)]. | | | | | | | | | | | |
| *2*Benjamini & Hochberg method adjustment for multiple comparisons | | | | | | | | | | | |

## 10.9 Figure S1a- S1b



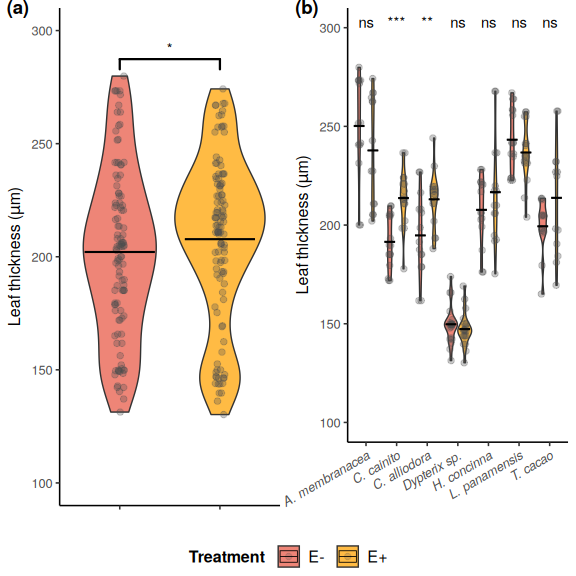
Foliar endophytic fungi (FEF) colonization of seven tropical tree species in malt extract agar (MEA 2%). a) Comparison of mean percent colonization of leaves by FEF measured 7 days after placing leaf pieces on plates. Statistical significance was calculated with a Student’s t-Test. Violin plots show the distribution of colonization values for all tree species within treatment groups (*E-* and *E+*). b) Comparison of mean percent colonization of leaves by FEF measured 7 days after culture. Violin plots show the distribution of percent colonization values for each species per treatment group. Pink filled violins represent low FEF group (*E-*) and yellow filled violins represent high FEF group (*E+*). Significance levels are represented by ns (not significant) and asterisks [p <= 0.05 (\*), p <= 0.01 (\*\*), p <= 0.001 (\*\*\*), and p < 0.0001 (\*\*\*\*)].

## 10.10 Figure S2a-S2b



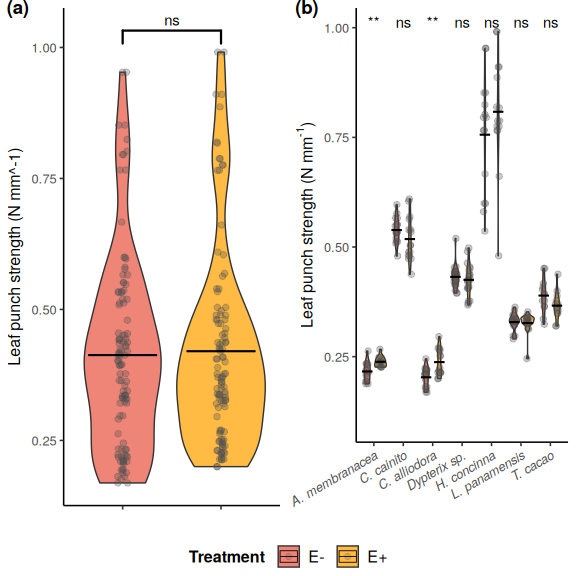
Distributions of values and means of anthocyanin content (ACI) in treatment groups (*E-* and *E+*) and tree species. a) Comparison of ACI means between treatment groups across individuals of all species. Statistical significance was calculated using a two-sided Student’s t-Test. b) Comparison of ACI means between treatment types of each species. Statistical significance was calculated with an analysis of variance (ANOVA). Pink filled violins represent low FEF group (*E-*) and yellow filed violins represent high FEF group (*E+*). Significance levels are represented by ns (not significant) and asterisks [p <= 0.05 (\*), p <= 0.01 (\*\*), p <= 0.001 (\*\*\*), and p < 0.0001 (\*\*\*\*)]..

## 10.11 Figure S3a-S3b



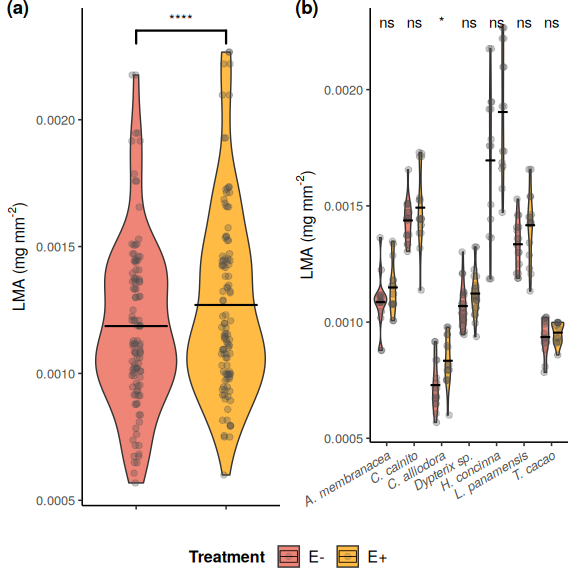
Distributions of values and means of leaf thickness (LT) (μg) in treatment groups (*E-* and *E+*) and tree species. a) Comparison of LT means between treatment groups across individuals of all species. Statistical significance was calculated using a two-sided Student’s t-Test. b) Comparison of LT means between treatment types of each species. Statistical significance was calculated with an analysis of variance (ANOVA). Pink filled violins represent low FEF group (*E-*) and yellow filed violins represent high FEF group (*E+*). Significance levels are represented by ns (not significant) and asterisks [p <= 0.05 (\*), p <= 0.01 (\*\*), p <= 0.001 (\*\*\*), and p < 0.0001 (\*\*\*\*)].

## 10.12 Figure S4a-S4b



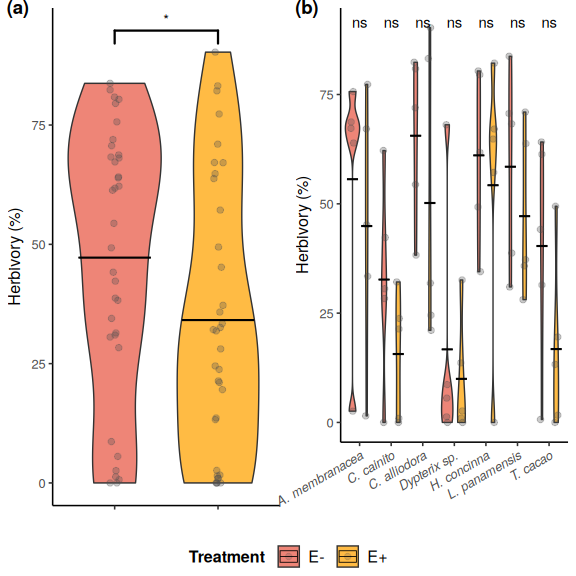
Distributions of values and means of leaf punch stength (LPS) (N mm^-1) in treatment groups (*E-* and *E+*) and tree species. a) Comparison of LPS means between treatment groups across individuals of all species. Statistical significance was calculated using a two-sided Student’s t-Test. b) Comparison of LPS means between treatment types of each species. Statistical significance was calculated with an analysis of variance (ANOVA). Pink filled violins represent low FEF group (*E-*) and yellow filed violins represent high FEF group (*E+*). Significance levels are represented by ns (not significant) and asterisks [p <= 0.05 (\*), p <= 0.01 (\*\*), p <= 0.001 (\*\*\*), and p < 0.0001 (\*\*\*\*)].

## 10.13 Figure S5a-S5b



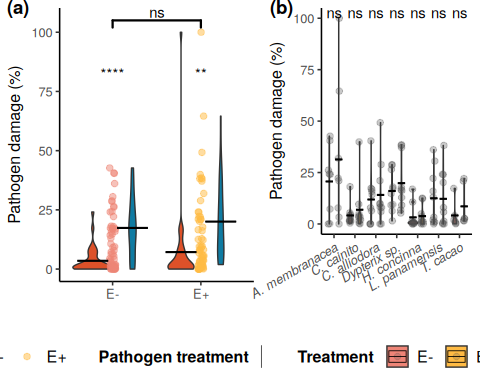
Distributions of values and means of leaf mass per area (LMA) (mg mm^2) in treatment groups (*E-* and *E+*) and tree species. a) Comparison of LMA means between treatment groups across individuals of all species. Statistical significance was calculated using a two-sided Student’s t-Test. b) Comparison of LMA means between treatment types of each species. Statistical significance was calculated with an analysis of variance (ANOVA). Pink filled violins represent low FEF group (*E-*) and yellow filed violins represent high FEF group (*E+*). Significance levels are represented by ns (not significant) and asterisks [p <= 0.05 (\*), p <= 0.01 (\*\*), p <= 0.001 (\*\*\*), and p < 0.0001 (\*\*\*\*)].

## 10.14 Figure S6a-S6b



Distributions of values and means of herbivory (%) damage caused by *Atta colombica* in treatment groups (*E-* and *E+*) and tree species. a) Comparison of herbivory (%) means between treatment groups across individuals of all species. Statistical significance was calculated using a two-sided Student’s t-Test. b) Comparison of herbivory (%) means between treatment types of each species. Statistical significance was calculated with an analysis of variance (ANOVA). Pink filled violins represent low FEF group (*E-*) and yellow filed violins represent high FEF group (*E+*). Significance levels are represented by *ns* (not significant) and asterisks [*p* = 0.05 (\*), *p* = 0.01 (\**), and* p\* = 0.001 (\*\*\*)].Significance levels are represented by ns (not significant) and asterisks [p <= 0.05 (\*), p <= 0.01 (\*\*), p <= 0.001 (\*\*\*), and p < 0.0001 (\*\*\*\*)].

## 10.15 Figure S7a-S7b



NO CAPTION YET

MIGHT SPLIT into two figures

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