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Host affinity of endophytic fungi and the potential for reciprocal interactions involving host secondary chemistry

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PREMISE: Interactions between fungal endophytes and their host plants present useful systems for identifying important factors affecting assembly of host-associated microbiomes. Here we investigated the role of secondary chemistry in mediating host affinity of asymptomatic foliar endophytic fungi using *Psychotria* spp. and *Theobroma cacao* (cacao) as hosts.

METHODS: First, we surveyed endophytic communities in *Psychotria* species in a natural common garden using culture-based methods. Then we compared differences in endophytic community composition with differences in foliar secondary chemistry in the same host species, determined by liquid chromatography–tandem mass spectrometry. Finally, we tested how inoculation with live and heat-killed endophytes affected the cacao chemical profile.

RESULTS: Despite sharing a common environment and source pool for endophyte spores, different *Psychotria* host species harbored strikingly different endophytic communities that reflected intrinsic differences in their leaf chemical profiles. In *T. cacao*, inoculation with live and heat-killed endophytes produced distinct cacao chemical profiles not found in uninoculated plants or pure fungal cultures, suggesting that endophytes, like pathogens, induce changes in secondary chemical profiles of their host plant.

CONCLUSIONS: Collectively our results suggest at least two potential processes: (1) Plant secondary chemistry influences assembly and composition of fungal endophytic communities, and (2) host colonization by endophytes subsequently induces changes in the host chemical landscape. We propose a series of testable predictions based on the possibility that reciprocal chemical interactions are a general property of plant–endophyte interactions.

KEY WORDS Community assembly; metabolomics; microbiome; *Psychotria*; species specificity; *Theobroma cacao*.

Fungal endophytes associated with leaves are ubiquitous across plants and influence the ecological trajectories of their hosts (U'Ren et al., 2019). Unlike pathogens, endophytes are primarily defined by their lack of visible symptoms associated with internal infection of plant tissues (Carroll, 1988; Wilson, 1995; Rodriguez et al., 2009). The presence of certain endophytes or the composition of endophytic communities can have distinct outcomes for the health of their host. Some fungal endophytes have been shown to benefit

host plants by facilitating nutrient acquisition (Hiruma et al., 2016; Christian et al., 2019) and promoting abiotic stress tolerance (Bae et al., 2009; Yamaji et al., 2016). Further, endophytic fungal communities have been shown to reduce pathogen and herbivore damage in a wide range of host plants (Arnold et al., 2003; Estrada et al., 2013; Mejía et al., 2014; Cosme et al., 2016; Christian et al., 2017). For example, despite being closely related to pathogenic congeners, *Colletotrichum tropicale* is a defensive endophyte common in *Theobroma cacao* (cacao tree) that can reduce pathogen damage in its host's tissues (Arnold et al., 2003; Rojas et al., 2010; Mejía et al., 2014; Christian et al., 2017). On the other hand, endophytes can also be costly to plants, and some taxa or isolates have been shown to reduce photosynthetic rate (Mejía et al., 2014) and facilitate disease (Adame-Álvarez et al., 2014; Busby et al., 2016). Due to their taxonomic and functional diversity, as well as their tractability as experimental systems, fungal endophytes provide useful insights for understanding plant–fungal interactions and host-associated microbiomes in general (Christian et al., 2015).

In most host plant species, endophytic fungi are horizontally transmitted as spores, which represent a subset of an extremely diverse pool of potential colonizers in the environment. The diversity of potential colonizers is important both ecologically and evolutionarily because different symbionts can have dramatically different effects on any given host (e.g., mutualistic or pathogenic) (Herre et al., 1999). While many individual species that comprise and dominate endophytic communities are generalists (Vincent et al., 2016; Wang et al., 2019), communities of endophytes often have some degree of affinity to particular host species (Gange et al., 2007; Wearn et al., 2012; Vincent et al., 2016; Dastogeer et al., 2018; Wang et al., 2019; but see Higgins et al., 2014). Degree of host affinity of endophytic communities can be influenced by the relative abundance of generalists (Vincent et al., 2016), or alternatively, the presence or absence of rare members of the microbiome (Vincent et al., 2016; Apigo and Oono, 2018). The assembly of the endophytic community in a given host can subsequently have important outcomes for host health. For example, close proximity of cacao seedlings to leaf litter from healthy cacao adults promoted assembly of stereotypical cacao-specific endophytic communities. These host-specific communities were dominated by the well-known defensive endophyte *C. tropicale* and suppressed pathogen damage in host tissues (Christian et al., 2017).

Nevertheless, many studies have demonstrated a large degree of unexplained variation in endophytic community composition (Eschen et al., 2010; Christian et al., 2016; Giauque and Hawkes, 2016; Whitaker et al., 2018), so a major goal in the field is to identify what unexplored factors could be contributing to the distribution of fungal endophytes among hosts (U'Ren et al., 2019). These factors could be intrinsic to hosts (e.g., host chemistry, leaf traits: Van Bael et al., 2017), extrinsic to hosts (e.g., climate and geography: Zimmerman and Vitousek, 2012; U'Ren et al., 2012); dispersal of spores: Christian et al., 2017), or simply stochastic variation in community assembly from a diverse source pool. To correctly identify host intrinsic factors, experiments are needed that compare endophytic communities among host species that vary in their intrinsic characteristics in common gardens that control for local environment and spore source.

Chemical differences among hosts have been hypothesized as a potential mechanism underlying observed levels of host specificity and activity of endophytic communities (Arnold et al., 2003; Herre et al., 2007). In tropical trees, within-species variation in plant secondary chemistry (including variation among young and old leaves [Brenes-Arguedas et al., 2006]) is usually small compared to among-species differences (Sedio et al., 2017). However, it is unclear which chemicals are produced by hosts or by endophytes (Soliman et al., 2013; Vallet et al., 2018) and which are inducible from interactions between the two (Mejía et al., 2008; Hartley et al., 2015). These chemicals have the potential to exhibit a range of ecologically important effects, and while they could have either beneficial or detrimental effects on hosts, they are most often investigated in the context of mounting defensive responses. For example, particular endophytic species or communities can have direct antagonistic effects on pathogens or can trigger plant defense reactions in ways that lead to a higher tolerance to pathogens (Zamioudis and Pieterse, 2012; Mejía et al., 2014; Hardoim et al., 2015; Hartley et al., 2015; Fister et al., 2016). However, despite recent progress, the diversity of endophytic effects on host secondary chemistry beyond the induction of defensive pathways are still understudied and therefore poorly understood, despite the wide array of potential consequences endophytes can have for plant hosts. Manipulative experiments using tractable systems are needed to understand how endophytes induce changes in host secondary chemistry.

A major challenge in the areas of microbiome ecology and plant–fungal interactions is connecting processes and outcomes of community assembly to functional effects in the host (e.g., Christian et al., 2017). Here we combine results from three experiments conducted first using a common garden of five chemically distinct species from the genus *Psychotria* (Rubiaceae), and then using *Theobroma cacao* (Malvaceae) to illustrate interactions between host chemistry and endophytic colonization. *Psychotria* is an abundant, species-rich genus in tropical habitats for which extensive phylogenetic information has been collected (Sedio et al., 2012). This genus further exhibits extreme chemical diversity (Sedio et al., 2017). The diversity in secondary chemistry observed among species reflects strikingly different patterns of host use by herbivores and other natural enemies and has been proposed as a mechanism promoting the coexistence of co-occurring plant species (Sedio et al., 2017). Individuals of different *Psychotria* species often grow within 1–2 m of each other. These naturally occurring "common gardens" provide the opportunity to separate the effects of abiotic and biotic characteristics of a site from intrinsic host plant effects on endophytic communities. On the other hand, cacao is a genetically well-characterized crop plant (Argout et al., 2011) that provides great experimental tractability and serves as a model system for studying the details of the genetic and physiological effects of different endophytic fungi (Arnold et al., 2003; Mejía et al., 2014; Christian et al., 2019). Therefore, ecological and chemical studies of hosts and their associated endophytic communities can be analyzed in a detailed comparative framework. Together, *Psychotria* and *T. cacao* present complementary comparative and experimental advantages for generating and testing hypotheses concerning plant–endophyte interactions.

First, we identify differences in endophytic community composition among *Psychotria* species growing in a natural common garden. Then we show that these differences correspond to differences in host chemical profiles. These results are consistent with previous suggestions that host chemistry can influence which endophyte species successfully colonize and proliferate across different host species. Finally, using *Theobroma cacao*, we experimentally demonstrate that the presence of endophytes can also induce subsequent changes in host secondary chemistry. By considering these

experiments in tandem, we are able to generate a series of testable predictions based on our hypothesis that reciprocal chemical interactions are a general property of plant–endophyte interactions.

MATERIALS AND METHODS

Study system

All experiments took place in Panama, using five species from the genus *Psychotria* (Rubiaceae), and *Theobroma cacao* (Malvaceae). Plants in the genus *Psychotria* are small understory trees in tropical forests and one of the largest genera of flowering plants worldwide, with approximately 1850 species (Taylor, 1996). This genus is very common and diverse in Panama; on Barro Colorado Island alone, there are 22 species and an estimated 10 million stems on the 15.9 km^2 island (Sedio et al., 2012). *Theobroma cacao* is an understory tree species native to the New World tropics and, as the source of chocolate, is an economically important fruit tree crop (Argout et al., 2011).

Experiment 1: Species-specificity of *Psychotria* leaf endophytes

One mature leaf of *P. gracilenta*, *P. capitata*, *P. acuminata*, and *P. marginata* was obtained from each of six host individuals growing in a plot in Gamboa, Panama in March 2018. All sampled individuals in the plot occurred within 5 m of a central point. Although this was a natural common garden (i.e., species were not planted in an array), species were homogeneously spatially distributed within the plot, and individuals were separated by an average of 1–2 m. Selection of leaves was standardized for age based on leaf thickness and color. Leaf area was measured, and then leaves were rinsed with tap water and cut into 2 × 2 mm tissue fragments. Tissue fragments from the center of the leaf were selected (Cannon and Simmons, 2002), and surface-sterilized as follows to remove any microbial colonizers attached to the leaf surface: tissue fragments were submerged and agitated in 70% v/v ethanol for 3 min, 0.525% v/v sodium hypochlorite for 2 min, and then sterile water for 1 min. Sixteen pieces from each leaf were plated on 2% w/v potato dextrose agar (PDA) and left to grow. Plates were checked daily, and hyphae emerging from eight randomly predetermined pieces for each leaf were subcultured onto individual plates. If multiple fungi were emerging from one tissue fragment, both were subcultured onto individual plates.

Pure isolates were identified using morphological characteristics by an expert fungal taxonomist/systematist (E. I. Rojas), and isolate morphotype was cross-checked with reference isolates previously sequenced using the ITS barcode from our group's previous projects to assign taxa. Reference cultures of all morphotypes, including those for which no taxon could be assigned, are maintained as vouchers in sterile water at the Smithsonian Tropical Research Institute in Panama. All analyses were performed using R v. 3.5.2 (R Core Team, 2014). To test the effect of host species on fungal community composition, permutational multivariate analysis of variance using distance matrices was used with the Bray–Curtis dissimilarity index (PERMANOVA) (vegan package, function adonis). Pairwise comparisons of Bray–Curtis dissimilarity percentages among host species based on the contribution of each morphospecies to average between-group dissimilarity were also calculated (Vegan package, function simper). The effect of host species and leaf area on the Shannon diversity index and richness (number of morphospecies per host) was determined using ANOVA (i.e., Shannon Diversity Index ~ Host Species + Leaf Area; Richness ~ Host Species + Leaf Area).

Experiment 2: Metabolomic differences among *Psychotria* spp.

We examined metabolomic differences among the four species of *Psychotria* used in Experiment 1 (*P. gracilenta*, *P. capitata*, *P. acuminata*, and *P. marginata*) and a fifth species of *Psychotria*, *P. horizontalis*. *Psychotria* leaf tissues were analyzed using a Bruker maXis Impact quadrupole-time-of-flight mass spectrometer (Bruker Daltonics, Billerica, Massachusetts, USA); otherwise methods were identical to those described by Sedio et al. (2017, 2018a, 2018b) and also applied to *Theobroma cacao* and *Colletotrichum* (below). Briefly, three individuals of each focal species of *Psychotria* were sampled on Barro Colorado Island, Panama, between June and October 2010. Sampled leaves were between 50% and 90% fully expanded, but not yet lignified. Fresh leaf tissue was flash-frozen in liquid nitrogen and stored at −80°C. Samples were extracted using a 90:10 methanol–water (pH 5) solvent, which is appropriate for small organic molecules ranging widely in polarity; the mild acidity aids in the extraction of alkaloids. Extracts were analyzed using high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (UHPLC-ESI-MS/MS; Sedio et al., 2017, 2018a). Each extract was analyzed twice, and the mean ion abundance for each compound was calculated to represent each individual tree.

In contrast with the data analysis methods described by Sedio et al. (2017, 2018a, 2018b), we used feature-based molecular networking to take advantage of a broader range of LC and MS variables to group spectra into features that reflect unique molecular structures based on LC retention time, parent mass (MS1), and fragmentation patterns (MS2) using MZmine 2 (Pluskal et al., 2010). We generated molecular networks that quantify the structural similarity of compounds using the Global Natural Products Social (GNPS) Molecular Networking platform (Wang et al., 2016). The GNPS method calculates the structural similarity of every pair of molecules as the cosine of the angle between the vectors that represent their MS2 spectra and typically employs a lower threshold of cos = 0.7 for a pair of compounds to retain a link in the resulting network. For every pair of samples, we calculated the chemical structural-compositional similarity (CSCS) metric of (Sedio et al., 2017), which quantifies the structural similarity of compounds in two samples, weighted by their abundance. Commonly used similarity indices, such as Bray–Curtis, account for shared compounds, but ignore the structural similarity of compounds that are not shared between samples. For illustration, consider that compounds *x* and *y* are structurally similar. Species *A* contains compound *x* but not *y*, and species *B* contains *y* but not *x*. In this example, compounds *x* and *y* contribute zero to Bray–Curtis similarity, but make a positive contribution to CSCS because they are structurally similar.

Finally, to visualize intra-and interspecific chemical variation, we plotted all 15 individuals representing five species in two dimensions using non-metric multidimensional scaling (NMDS) and the MASS package in R. Although it does not account for structurally similar compounds, we used PERMANOVA with the Bray–Curtis dissimilarity index (vegan package, function adonis) on the raw chemical data as an additional test of whether conspecific individuals were more chemically similar than heterospecifics.

Experiment 3: Response of the cacao metabolome to endophytic fungi

Theobroma cacao seeds were collected in commercial plantations in Bocas del Toro Province, Panama. Seeds were surface-sterilized in 0.525% v/v sodium hypochlorite for 3 min and rinsed twice with sterile water (Arnold et al., 2003; Mejía et al., 2014). Seeds were planted in trays containing a 2:1 sterile mixture of clay-rich soil from Barro Colorado Island and river sand from the Chagres River, both in Panama. One-month-old cacao plants were transplanted into individual pots containing 600 mL of this same substrate and watered without wetting aerial tissues so as to avoid fungal colonization (Mejía et al., 2014). Seed germination and seedling growth took place in Percival growth chambers (Percival Scientific, Perry, Iowa, USA) at 70% humidity, 12 h light at 27°C /12 h dark at 25°C.

The beneficial endophyte *Colletotrichum tropicale* that had been isolated previously from *T. cacao* was cultured on 2% w/v malt extract agar (MEA). After 10 d, 10 mL of sterile water was added to the culture, which was then scraped to suspend hyphae and spores and poured into sterile liquid medium containing 500 mL of 1.5% molasses yeast broth (Mejía et al., 2008). The liquid culture was shaken at 125 rpm at room temperature for 7 d to produce mycelia and another 7 d to facilitate sporulation. *Colletotrichum tropicale* spores were filtered to separate them from mycelia, concentrated, and resuspended in sterile water with 0.01% v/v Tween 20. For inoculation experiments, the spore suspension was adjusted to approximately 2×10^6 spores/mL (Mejía et al., 2008).

A spray bottle was used to treat cacao plants with a suspension of viable *C. tropicale* spores ($N = 4$) or nonviable *C. tropicale* spores ($N = 4$). The suspension of nonviable spores was created by heat-shocking the suspension in an autoclave at 121°C and 15 psi for 15 min. Nonviable spores were plated on 2% MEA to verify nonviability. An additional nine plants that were not inoculated with spores or with the sterile water and Tween medium were used as a control group. All plants were placed in the forest in Gamboa, Panama for 72 h, covered with a suspended plastic tarp to protect them from rain. Two leaves were then collected from each plant, rinsed in tap water, and cut into 32 2 × 2 mm pieces, before they were surface-sterilized as described above (see Experiment 1). Sixteen tissue fragments from each leaf were plated on 2% MEA. Emerging hyphae were subcultured onto fresh plates, and pure cultures were identified by E. I. Rojas. A mixed effects model was used to test how treatment group affected percentage colonization by fungi per leaf, with plant included as a random effect.

Secondary chemistry of leaves from the same cacao plants used for endophyte isolation and the *C. tropicale* fungal culture used to inoculate them were analyzed using the methods described by Sedio et al. (2017, 2018a, 2018b). Briefly, samples were extracted using a 90:10 methanol–water (pH 5) solvent as previously described for the *Psychotria* samples. Extracts were analyzed using UHPLC-ESI-MS/MS (Sedio et al., 2017, 2018a). Note that in contrast to *Psychotria* (above), *T. cacao* and *C. tropicale* were analyzed using a Bruker micrOTOF-Q III quadrupole-time-of-flight mass spectrometer (Bruker Daltonics). Spectra were grouped into features that reflect unique molecular structures as described above (Experiment 2). Structures were predicted using SIRIUS (Böcker et al., 2009), and molecular networks that quantify the structural similarity of compounds were generated using the Global Natural Products Social (GNPS) Molecular Networking platform (Wang et al., 2016). The CSCS metric was calculated for every pair of samples. Finally, we used a permutation test to evaluate the significance of differences between within-treatment metabolomic similarity and between-treatment metabolomic similarity, following the method of Sedio et al. (2017). If the observed difference was > 95% of the distribution of all possible differences, the treatment effect was significant.

RESULTS

Experiment 1: Species-specificity of *Psychotria* leaf endophytes

Forty-three fungal morphospecies were generated from 200 isolates (Appendix S1). The number of morphospecies per leaf sample ranged from 2 to 10, with an average of 5.3 morphospecies per leaf sample. The two most common fungal genera were *Colletotrichum* and *Xylaria*. Endophytic community structure was strongly affected by host species ($F_{3, 18} = 3.6178$, $R^2 = 0.37616$, $P = 0.001$) (Fig. 1). Average between-group Bray–Curtis dissimilarity was the highest between *P. gracilenta* and *P. capitata* (86.0% dissimilar) and the lowest between *P. gracilenta* and *P. acuminata* (60.5% dissimilar) (Appendix S2). Host species did not have a significant effect on Shannon diversity ($F_{3,17} = 0.9312$, $P = 0.4470$) or richness ($F_{3,17} = 0.3960$, $P = 0.7576$) of fungal communities (Appendix S2), nor did leaf area (diversity: $F_{1, 17} = 0.2064$, $P = 0.6553$; richness: $F_{1, 17} = 0.0112$, $P = 0.9170$).

Experiment 2: Metabolomic differences among *Psychotria* spp.

A total of 1401 structurally unique metabolites were detected by LC-MS/MS, ranging in mass from 131.1071 to 1521.043 Daltons (Da). The five species of *Psychotria* (*P. horizontalis* and the four species used in Experiment 1) varied in their secondary metabolite

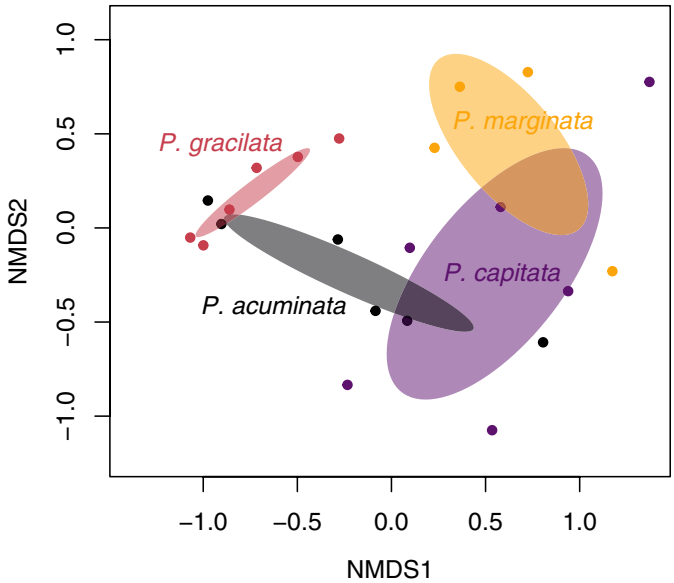

FIGURE 1. Endophytic community composition differed among four species of *Psychotria* in Experiment 1 (PERMANOVA using the Bray–Curtis dissimilarity index: $F_{3, 18} = 3.6178$, $R^2 = 0.37616$, $P = 0.001$). Shaded circles represent 95% confidence intervals.

composition (Fig. 2A; Appendix S3), whereas conspecific individuals were chemically similar to one another (Fig. 2B; PERMANOVA $F_{1,4} = 10.41, P = 0.001$).

Experiment 3: Response of the cacao metabolome to endophytic fungi

A total of 1763 structurally unique compounds were detected by LC-MS/MS, ranging in mass from 135.119 to 1471.404 Da. Molecular formulas were predicted for a total of 1410 compounds using SIRIUS (Böcker et al., 2009).

Inoculation of *T. cacao* leaves with *C. tropicale* spores resulted in a measurable effect on the metabolome of the leaf whether the inoculum consisted of viable or heat-killed, nonviable spores. Uninoculated *T. cacao* differed significantly from those inoculated with *C. tropicale* (Table 1, Fig. 3A) and from those inoculated with heat-killed, nonviable *C. tropicale* spores (Table 1, Fig. 3B). *Theobroma cacao* leaves inoculated with live *C. tropicale* spores showed little difference from *T. cacao* leaves inoculated with heat-killed, nonviable spores (Table 1, Fig. 3C). In contrast, leaves of *T. cacao* inoculated with viable *C. tropicale* spores differed significantly from the inoculum itself,

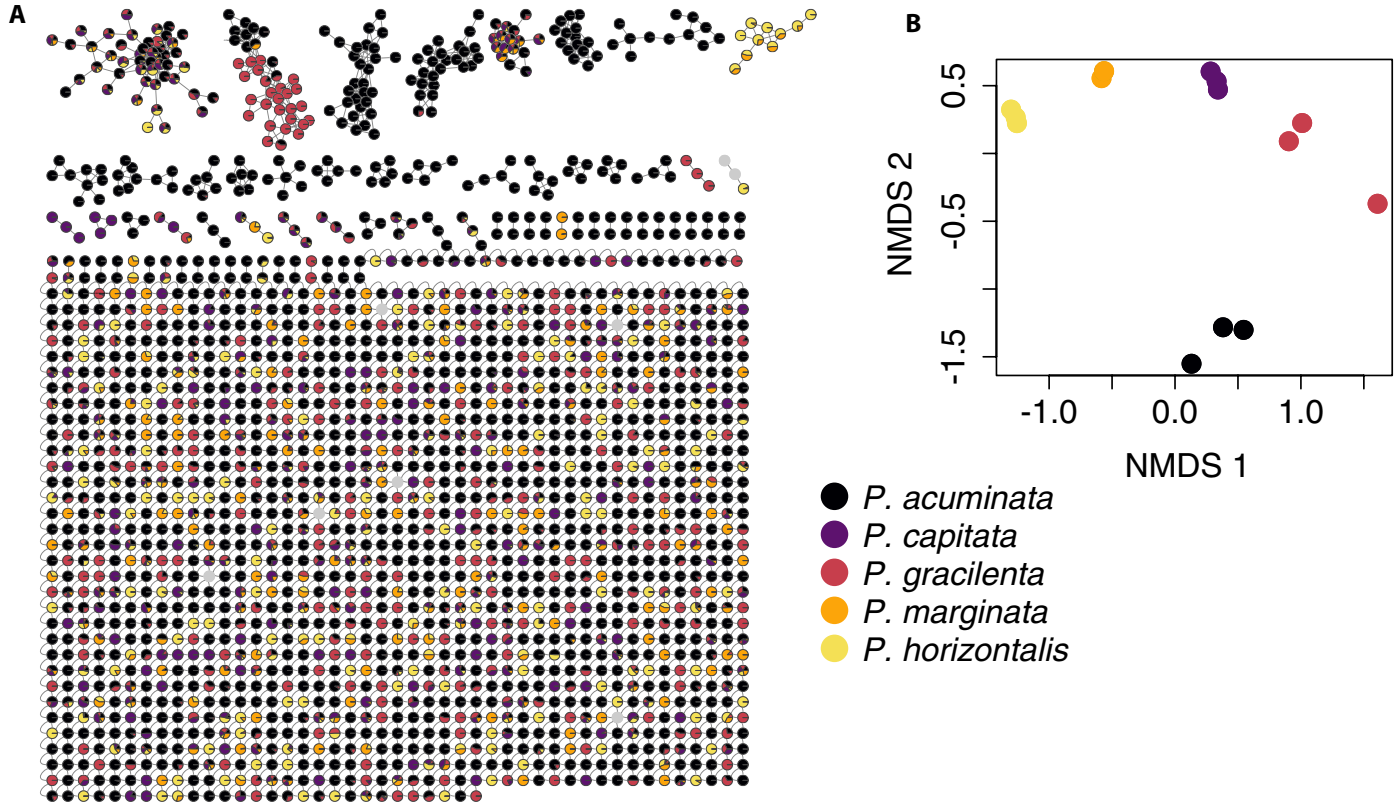

FIGURE 2. Chemical composition and similarity indicate interspecific chemical variation among the five species of *Psychotria* used in Experiment 2. (A) A molecular network indicates the structural similarity of 1401 unique compounds detected in five species of *Psychotria* linked by a cosine similarity score of ≥0.7. Nodes represent compounds with unique molecular structures, as inferred by mass, fragmentation pattern, and liquid chromatography retention time; links between nodes indicate molecular structural similarity between compounds. Larger networks represent clusters of many structurally similar compounds. Pie charts indicate the relative abundance of each compound in the five species. (B) Nonmetric multidimensional scaling axes reflect the chemical similarity of three individuals of each of five *Psychotria* species from Barro Colorado Island, Panama (PERMANOVA $F = 10.41$, $P = 0.001$).

TABLE 1. The effect of inoculation treatment on chemical structural compositional similarity (CSCS) in *Theobroma cacao* (Experiment 3). A permutation test was used to evaluate the null hypothesis that similarity was equal within versus between treatment categories. All treatments refer to young leaves of *T. cacao* with the exception of the *Colletotrichum tropicale* liquid spore culture. Treatments were endophyte-free leaves, leaves inoculated with *C. tropicale* spores, leaves inoculated with nonviable, heat-killed *C. tropicale* spores, and *C. tropicale* grown in liquid culture.

Comparison	CSCS within treatment	CSCS between treatments	Difference in CSCS	*P*
Endophyte-free vs. Live *C. tropicale* inoculation	0.937	0.857	0.080	0.043
Endophyte-free vs. Heat-killed *C. tropicale* inoculation	0.935	0.867	0.067	0.029
Live *C. tropicale* inoculation vs. Heat-killed *C. tropicale* inoculation	0.924	0.922	0.002	0.429
Live *C. tropicale* inoculation vs. *C. tropicale* liquid culture	0.875	0.500	0.375	0.029

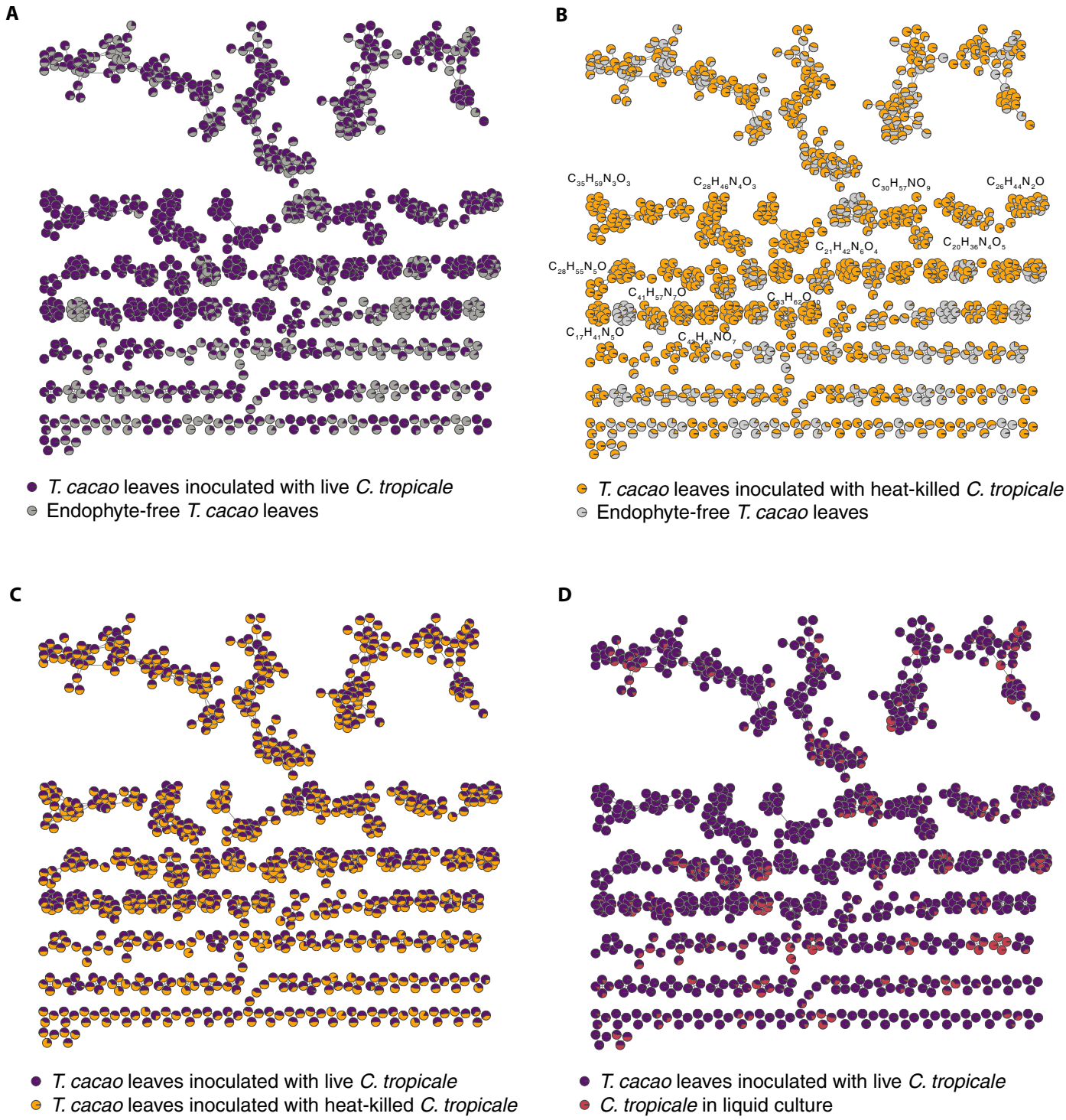

FIGURE 3. Inoculation treatment significantly affected relative abundance of chemical compounds in *Theobroma cacao* in Experiment 3. Included in the molecular networks are 1763 compounds in clusters of ≥3 compounds linked by a cosine similarity score of ≥ 0.7. Nodes represent compounds with unique molecular structures, as inferred by mass, fragmentation pattern, and liquid chromatography retention time; links between nodes indicate molecular structural similarity between compounds. (A) Comparison between endophyte-free *T. cacao* leaves and those inoculated with the endophyte *Colletototrichum tropicale*. (B) Comparison between endophyte-free *T. cacao* leaves and those inoculated with heat-killed, nonviable *C. tropicale* spores. (C) Comparison between *T. cacao* leaves inoculated with live *C. tropicale* spores and those inoculated with heat-killed, nonviable *C. tropicale* spores. (D) Comparison between *T. cacao* leaves inoculated with live *C. tropicale* spores and *C. tropicale* growing in liquid culture. The molecular formulas of selected compounds that appear to be induced in *T. cacao* leaves inoculated with heat-killed, nonviable *C. tropicale* spores are shown in panel B. These results suggest that the primary effect of endophyte inoculation is to induce the expression of secondary metabolites endogenous to the *T. cacao* host plant.

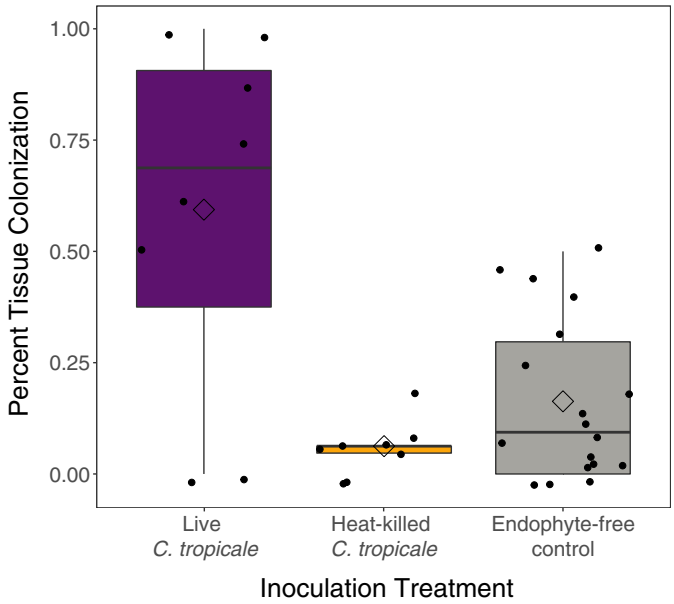

FIGURE 4. Isolation frequency of endophytic fungi from *Theobroma cacao* differed among inoculation treatments in Experiment 3. More fungi were isolated from plants inoculated with live *Colletotorichum tropicale* spores than plants inoculated with heat-killed spores or control plants ($\chi^2 = 14.254$, df = 2, $P < 0.001$). All fungi reisolated from plants previously inoculated with live *C. tropicale* spores were identified as *C. tropicale,* whereas more diverse assemblages were isolated from the other two treatment groups. Boxes show first and third quartiles with the median as a heavy line, the mean as a diamond, and whiskers extend to 1.5 times the interquartile range.

C. tropicale grown in liquid culture in isolation from its host plant (Table 1, Fig. 3D).

After 72 h of exposure to natural fungal spore sources in the field, *T. cacao* individuals inoculated with viable *C. tropicale* spores were still colonized exclusively by *C. tropicale*, which was reisolated from 59.4% of sampled plant tissue. No other fungi were isolated from these plants. Plants that were treated with heat-killed *C. tropicale* and control plants had more types of fungi isolated from their tissues (5 and 7 morphospecies, respectively). However, overall isolation rate was much lower in these two treatment groups ($\chi^2 = 14.254$, df = 2, $P < 0.001$), with 6.3% colonization of plants treated with heat-killed *C. tropicale* spores before field exposure and 16.3% colonization of uninoculated control plants (Fig. 4).

DISCUSSION

Overall, our results suggest that host plants and components of their aboveground microbial communities potentially exert effects on one another, mediated by host secondary chemistry. First, across four sympatric *Psychotria* species we observed striking differences between the dominant members of their leaf endophytic communities, suggesting strong differential host affinity. Second, we showed that the composition of leaf endophytic communities in *Psychotria* corresponds with differences among them in host secondary chemistry, supporting suggestions from previous studies that host secondary chemistry can at least partially explain differential host affinity of endophytic fungi (Arnold et al., 2003). Finally, using experimental inoculations with live and heat-killed endophytes in *T. cacao,* we demonstrated the presence of endophytic spores affects some components of host secondary chemistry. Taken together, we hypothesize that reciprocal interactions or feedbacks occur between fungal endophytes and the hosts that they colonize, via secondary chemistry expressed in host tissues. Specifically, we hypothesize that (1) host chemistry affects the endophytes that colonize and/or proliferate in host tissues, and (2) colonization by these fungi then alters the chemistry of their host, potentially in ways that promote both fungal and host fitness.

We observed clear differential host affinity of endophytic communities, with roughly 38% of variance in endophytic community composition explained by host identity in Experiment 1. We also found that differential host affinity corresponded with differences in chemical profiles among these hosts. We speculate that specific compounds that account for these striking differences in chemical profiles differentially promote or inhibit different individual endophytic fungal species that constitute these communities (Arnold et al., 2003). Although within the genus *Psychotria*, *P. gracilenta*, *P. capitata*, and *P. acuminata* are closely related to one another (Sedio et al., 2012), they are chemically distinct and this is reflected in the differences between their associated endophytic communities (Figs. 1,2). While some studies have found evidence for species specificity of fungal endophytic communities in plant leaves (Wearn et al., 2012; Del Olmo-Ruiz and Arnold, 2017), others have not (Higgins et al., 2014). Our results suggest that evidence of host specificity in endophytic communities in some studies (but not others) could be due in part to the magnitude of differences in hosts' chemistry that differentially promotes dominant members of the fungal microbiome (Arnold et al., 2003).

In addition to the evidence that host secondary chemistry can potentially influence endophytic community composition, we also found evidence that endophytes can affect the chemical profiles of their hosts. We found that secondary chemical profiles of *T. cacao* leaves inoculated with both viable and heat-killed, nonviable *C. tropicale* were very similar and consistently and significantly differed from those of *C. tropicale* grown in liquid culture and those of untreated leaves (Fig. 3). Given that the live spore culture did not produce the chemicals that differentiated treated and untreated plants, we interpret this observation as indicating that plants produce a stereotypic set of chemicals as a response to *C. tropicale*, whether or not the endophyte is viable. Colonization by the live endophytic inoculum is more representative of how plants would encounter endophytes in nature. However, the heat-killed treatment lends insight into the mechanism by which plants respond to endophyte presence, which is likely host recognition of distinctive proteins on *C. tropicale* cell wall surfaces or components of the spore wall that are released with either heat-killing or germination. However, detailed tests are required to determine whether endophyte chemical production differs when in isolation versus when it is in the host plant, and the degree to which endophyte-derived compounds contribute to the overall secondary chemical profile of hosts. Additionally, experimental evidence is needed to exclude the possibility that similar metabolomic responses in the live and heat-killed *C. tropicale* treatments were simply due to exposure to the inoculum medium (sterile water and 0.01% Tween 20) without any endophyte present. Ultimately, it appears that host secondary chemistry results from both host-produced and endophyte-induced or endophyte-produced compounds. These results are consistent with previous research showing that both beneficial (Zamioudis

and Pieterse, 2012; Hartley et al., 2015) and pathogenic colonizers (Zamioudis and Pieterse, 2012) can alter expression of plant defensive chemistry.

There were strong differences in the endophytes that were recovered from the inoculated cacao plants after 72 h of spore exposure in the field. Plants that were previously inoculated with live *C. tropicale* spores were unsurprisingly dominated by *C. tropicale* (~60% of tissue colonized, with 100% of those isolates identified as *C. tropicale*). In sharp contrast, control plants that were not inoculated with endophytic fungi had a lower isolation rate (16.3%) than the plants that were inundated with live *C. tropicale* spores (Fig. 4), and the diversity of reisolated fungi was much higher. Interestingly, plants that were treated with heat-killed *C. tropicale* spores had very low colonization by fungi (6.3%) even after 3 d of field exposure (Fig. 4). With more exposure time in the field, plants from all three treatment groups would undoubtedly be colonized by more endophytic fungi. Nevertheless, our results suggest that early exposure to *C. tropicale*, whether or not it is viable, inhibits other fungi from subsequently colonizing plant tissue over short time scales. The likely mechanism is modification of plant chemistry and/or activation of host defensive pathways (e.g., upregulation of lignin and cellulose deposition) (Mejía et al., 2014). This result is consistent with previous work showing that exposure of endophyte-free cacao seedlings to leaf litter derived from healthy cacao adults enriched the cacao microbiome with *C. tropicale* (Christian et al., 2017). Those plants with more *C. tropicale* were then not only more resistant to pathogens, but also experienced an overall decrease in the abundance and diversity of other nonpathogenic fungal colonizers.

The upregulation of host defenses in response to heat-killed propagules is analogous to vaccinations in humans and other animals and has clear implications for disease and pest management. For example, treatment of *T. cacao* or other crop plants with nonviable spores of natural endophytes may induce the production of endogenous chemical defenses. Importantly, host defenses against pathogens that are mediated by endophytes could be enhanced without the metabolic costs associated with sustaining a standing microbial community (Mejía et al., 2014), the logistical difficulty of maintaining an engineered microbial community in nature (Busby et al., 2017), or the risk that a beneficial endophyte in one crop species might be a serious pathogen in another.

Together, the *Psychotria* and *T. cacao* systems offer a number of strengths and are useful systems for future work understanding host–microbe interactions. The *Psychotria* system offers a chemically and phylogenetically diverse group of organisms that often co-occur in nature (Sedio et al., 2012). Additionally, *Psychotria* can be propagated clonally, which will lend itself to experimental manipulation of endophytic communities against tightly controlled host genetic backgrounds. On the other hand, *T. cacao* provides an extremely tractable system for studying the physiological and genetic effects of a variety of endophytes and pathogens on hosts (Arnold et al., 2003; Herre et al., 2007; Rojas et al., 2010; Mejía et al., 2014) with genetic and genomic tools, including a sequenced genome (Argout et al., 2011). It has a well-characterized dominant endophyte (*C. tropicale*) that has clear, documented physiological and ecological effects on its host (Arnold et al., 2003; Mejía et al., 2014; Christian et al., 2017, 2019). We also have a growing understanding of how entire endophytic communities assemble in *T. cacao*, both as a function of spore source (Christian et al., 2017) and within-host interactions (Mejía et al., 2008).

CONCLUSIONS

By controlling for extrinsic environmental conditions, we found that closely related species of *Psychotria* that differ chemically also harbor different endophytic communities. This is consistent with previous suggestions that intrinsic factors, such as host chemistry, may play a role in structuring endophytic communities (Arnold et al., 2003), but also raises the question of how plastic host intrinsic characteristics are. In the experimentally tractable *T. cacao* system, we found that endophytic colonization can strongly influence expressed host chemistry. Combining these experiments, we hypothesize a feedback between plants and their fungal colonizers, in which host chemistry affects relative success of some endophytes within a community, and the colonization by endophytes also reciprocally affects the chemistry of its host in ways that are ultimately beneficial for both host and endophyte. Future research should explicitly test for a positive chemical feedback between different species of healthy host plants and the dominant members of their microbiota, in which endophytic colonization changes host chemistry in ways that both benefit the host and give the endophyte a competitive advantage against other colonizers.

Of course, synthesizing across multiple systems and experiments to understand host–endophyte interactions leaves us with many unanswered questions. Outstanding questions that will advance our understanding of these complex interactions include:

- To what extent are chemical differences among host plants attributable to the host or driven by microbial colonizers?
- What are the mechanisms or filters by which hosts influence which microbes colonize their tissues and which ones proliferate?
- To what extent does intrinsic host chemistry drive assembly of host-specific and/or relatively more beneficial endophytic communities?
- Do endophytes manipulate host chemistry in ways that benefit themselves and inhibit competitors (i.e., other endophytes as well as pathogens)?
- To what extent does spatial or temporal variation in host chemistry correlate with variation in endophytic community composition?

Future studies using in vivo inoculations of endophytic fungi into plant tissues will be informative in answering these questions. Particularly important will be studies focusing on young leaves, which are the most susceptible to colonization by endophytes, as well as to attack by pathogens (Coley and Kursar, 1996). Previous work has suggested that many chemical traits in young leaves are the result of selection by pathogens (Coley and Kursar, 1996), but our work suggests the potential caveat that chemicals previously interpreted as direct pathogen inhibitors may also be indirectly benefitting hosts by promoting certain beneficial endophytes. In vitro experiments will also be useful moving forward. For example, researchers could infuse agar plates with the dominant chemicals

identified from different host species to specifically test the hypothesis that those chemicals effectively promote growth of the dominant endophytes isolated from those same plants and inhibit the growth of rare species. Endophytic fungi are not only taxonomically and functionally diverse but also tractable and easy to manipulate. As we move forward, they will continue to provide a useful model system for understanding more general host–microbe interactions, especially as interdisciplinary teams combine fields such as community ecology, biochemistry, and plant pathology.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design of the experiments and collected data. S.P. and A.W. collected data for Experiment 1. B.E.S. collected data for Experiment 2. X.F.-B., L.A.R.-C., B.E.S., L.C.M., and E.A.H. designed Experiment 3. X.F.-B. executed Experiment 3 and curated all data. B.E.S. conducted all chemical analyses. E.I.R. identified fungi with assistance from L.C.M. N.C. and B.E.S. analyzed and interpreted data, and E.A.H. and J.W.S. provided assistance in interpreting data. N.C., B.E.S., E.A.H., and J.W.S. drafted the manuscript, and all authors revised it. All authors provided their final approval of the manuscript and will be accountable for all aspects of the work.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Fungal taxa isolated in Experiment 1 and their relative abundances across different host species.

APPENDIX S2. Overall Bray–Curtis dissimilarity, Shannon diversity index, and species richness in Experiment 1.

APPENDIX S3. Chemical structural-compositional similarity for five species of *Psychotria* in Experiment 2.

LITERATURE CITED

Adame-Álvarez, R.-M., J. Mendiola-Soto, and M. Heil. 2014. Order of arrival shifts endophyte–pathogen interactions in bean from resistance induction to disease facilitation. *FEMS Microbiology Letters* 355: 100–107.

Apigo, A., and R. Oono. 2018. Dimensions of host specificity in foliar fungal endophytes. *In* A. M. Pirttilä and A. C. Frank [eds.], Endophytes of forest trees, 15–42. Springer International, Basel, Switzerland.

Argout, X., J. Salse, J.-M. Aury, M. J. Guiltinan, G. Droc, J. Gouzy, M. Allegre, et al. 2011. The genome of *Theobroma cacao*. *Nature Genetics* 43: 101–108.

Arnold, A., L. Mejía, D. Kyllo, E. I. Rojas, Z. Maynard, N. Robbins, and E. A. Herre. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences, USA* 100: 15649–15654.

Bae, H., R. C. Sicher, M. S. Kim, S.-H. Kim, M. D. Strem, R. L. Melnick, and B. A. Bailey. 2009. The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *Journal of Experimental Botany* 60: 3279–3295.

Van Bael, S., C. Estrada, and A. E. Arnold. 2017. Foliar endophyte communities and leaf traits in tropical trees. *In* J. Dighton and J. F. White [eds.], The fungal community: its organization and role in the ecosystem, 4th ed., 65–78. CRC Taylor & Francis, Boca Raton, FL, USA.

Böcker, S., M. C. Letzel, Z. Lipták, and A. Pervukhin. 2009. SIRIUS: decomposing isotope patterns for metabolite identification. *Bioinformatics* 25: 218–224.

Brenes-Arguedas, T., M. W. Horton, P. D. Coley, J. Lokvam, R. A. Waddell, B. E. Meizoso-O'Meara, andT. A. Kursar. 2006. Contrasting mechanisms of secondary metabolite accumulation during leaf development in two tropical tree species with different leaf expansion strategies. *Oecologia* 14: 91–100.

Busby, P. E., K. G. Peay, and G. Newcombe. 2016. Common foliar fungi of *Populus trichocarpa* modify *Melampsora* rust disease severity. *New Phytologist* 209: 1681–1692.

Busby, P. E., C. Soman, M. R. Wagner, M. L. Friesen, J. Kremer, A. Bennett, M. Morsy, et al. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biology* 15: e2001793.

Cannon, P. F., and C. M. Simmons. 2002. Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. *Mycologia* 94: 210–220.

Carroll, G. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* 69: 2–9.

Christian, N., B. K. Whitaker, and K. Clay. 2015. Microbiomes: unifying animal and plant systems through the lens of community ecology theory. *Frontiers in Microbiology* 6: 1–15.

Christian, N., C. Sullivan, N. D. Visser, and K. Clay. 2016. Plant host and geographic location drive endophyte community composition in the face of perturbation. *Microbial Ecology* 72: 621–632.

Christian, N., E. A. Herre, L. C. Mejia, and K. Clay. 2017. Exposure to the leaf litter microbiome of healthy adults protects seedlings from pathogen damage. *Proceedings of the Royal Society, B, Biological Sciences* 284: 20170641.

Christian, N., E. A. Herre, and K. Clay. 2019. Foliar endophytic fungi alter patterns of nitrogen uptake and distribution in *Theobroma cacao*. *New Phytologist* 222: 1573–1583.

Coley, P. D., and T. A. Kursar. 1996. Anti-herbivore defenses of young tropical leaves: physiological constraints and ecological tradeoffs. *In* S. Mulkey, R. Chazdon, and A. Smith [eds.], Tropical forest plant ecophysiology, 305–336. Chapman and Hall, New York.

Cosme, M., J. Lu, M. Erb, M. J. Stout, P. Franken, and S. Wurst. 2016. A fungal endophyte helps plants to tolerate root herbivory through changes in gibberellin and jasmonate signaling. *New Phytologist* 211: 1065–1076.

Dastogeer, K. M. G., H. Li, K. Sivasithamparam, M. G. K. Jones, and S. J. Wylie. 2018. Host specificity of endophytic mycobiota of wild *Nicotiana* plants from arid regions of northern Australia. *Microbial Ecology* 75: 74–87.

Del Olmo-Ruiz, M., and A. E. Arnold. 2017. Community structure of fern-affiliated endophytes in three neotropical forests. *Journal of Tropical Ecology* 33: 60–73.

Eschen, R., S. Hunt, C. Mykura, A. C. Gange, and B. C. Sutton. 2010. The foliar endophytic fungal community composition in *Cirsium arvense* is affected

by mycorrhizal colonization and soil nutrient content. *Fungal Biology* 114: 991–998.

Estrada, C., W. T. Wcislo, and S. A. Van Bael. 2013. Symbiotic fungi alter plant chemistry that discourages leaf-cutting ants. *New Phytologist* 198: 241–251.

Fister, A. S., L. C. Mejia, Y. Zhang, E. A. Herre, S. N. Maximova, and M. J. Guiltinan. 2016. *Theobroma cacao* L. pathogenesis-related gene tandem array members show diverse expression dynamics in response to pathogen colonization. *BMC Genomics* 17: 363.

Gange, A. C., S. Dey, A. F. Currie, and B. C. Sutton. 2007. Site- and species-specific differences in endophyte occurrence in two herbaceous plants. *Journal of Ecology* 95: 614–622.

Giauque, H., and C. V. Hawkes. 2016. Historical and current climate drive spatial and temporal patterns in fungal endophyte diversity. *Fungal Ecology* 20: 108–114.

Hardoim, P. R., L. S. van Overbeek, G. Berg, A. M. Pirttilä, S. Compant, A. Campisano, M. Döring, and A. Sessitsch. 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews* 79: 293–320.

Hartley, S. E., R. Eschen, J. M. Horwood, A. C. Gange, E. M. Hill, and R. Holloway. 2015. Infection by a foliar endophyte elicits novel arabidopside-based plant defence reactions in its host, *Cirsium arvense*. *New Phytologist* 205: 816–827.

Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* 14: 49–53.

Herre, E. A., L. C. Mejía, D. A. Kyllo, E. Rojas, Z. Maynard, A. Butler, and S. A. Van Bael. 2007. Ecological implications of anti-pathogen effects of tropical fungal endophytes and mycorrhizae. *Ecology* 88: 550–558.

Higgins, K. L., A. E. Arnold, P. D. Coley, and T. A. Kursar. 2014. Communities of fungal endophytes in tropical forest grasses: highly diverse host- and habitat generalists characterized by strong spatial structure. *Fungal Ecology* 8: 1–11.

Hiruma, K., N. Gerlach, S. Sacristán, R. T. Nakano, S. Hacquard, B. Kracher, U. Neumann, et al. 2016. Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. *Cell* 165: 464–474.

Mejía, L. C., E. I. Rojas, Z. Maynard, S. A. Van Bael, A. E. Arnold, P. Hebbar, G. J. Samuels, et al. 2008. Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. *Biological Control* 46: 4–14.

Mejía, L. C., E. A. Herre, J. P. Sparks, K. Winter, M. García, S. A. Van Bael, J. Stitt, et al. 2014. Pervasive effects of an endophytic fungus on host genetic and phenotypic expression in a tropical tree. *Frontiers in Microbiology* 5: 1–15.

Pluskal, T., S. Castillo, A. Villar-Briones, and M. Orešič. 2010. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* 11: 395.

R Core Team. 2014. R: a language and environment for statistical computing. v.3.1.2. R Foundation for Statistical Computing, Vienna, Austria.

Rodriguez, R. J., J. F. White, A. E. Arnold, and R. S. Redman. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* 182: 314–330.

Rojas, E. I., S. A. Rehner, G. J. Samuels, S. A. Van Bael, E. A. Herre, P. Cannon, R. Chen, et al. 2010. *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. *Mycologia* 102: 1318–1338.

Sedio, B. E., S. J. Wright, and C. W. Dick. 2012. Trait evolution and the coexistence of a species swarm in the tropical forest understorey. *Journal of Ecology* 100: 1183–1193.

Sedio, B. E., J. C. Rojas Echeverri, C. A. Boya P., and S. J. Wright. 2017. Sources of variation in foliar secondary chemistry in a tropical forest tree community. *Ecology* 98: 616–623.

Sedio, B. E., C. A., Boya P., and J. C., Rojas Echeverri. 2018a. A protocol for high-throughput, untargeted forest community metabolomics using mass spectrometry molecular networks. *Applications in Plant Sciences* 6: e1033.

Sedio, B. E., J. D. Parker, S. M. McMahon, and S. J. Wright. 2018b. Comparative foliar metabolomics of a tropical and a temperate forest community. *Ecology* 99: 2647–2653.

Soliman, S. S. M., C. P. Trobacher, R. Tsao, J. S. Greenwood, and M. N. Raizada. 2013. A fungal endophyte induces transcription of genes encoding a redundant fungicide pathway in its host plant. *BMC Plant Biology* 13: 93.

Taylor, C. M. 1996. Overview of the Psychotriae (Rubiaceae) in the neotropics. *Opera Botanica Belgica* 7: 261–270.

U'Ren, J. M., F. Lutzoni, J. Miadlikowska, A. D. Laetsch, and A. E. Arnold. 2012. Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *American Journal of Botany* 99: 898–914.

U'Ren, J. M., F. Lutzoni, J. Miadlikowska, N. B. Zimmerman, I. Carbone, G. May, and A. E. Arnold. 2019. Host availability drives distributions of fungal endophytes in the imperilled boreal realm. *Nature Ecology & Evolution* 3: 1430–1437.

Vallet, M., M. Strittmatter, P. Murúa, S. Lacoste, J. Dupont, C. Hubas, G. Genta-Jouve, et al. 2018. Chemically-mediated interactions between macroalgae, their fungal endophytes, and protistan pathogens. *Frontiers in Microbiology* 9: 1–13.

Vincent, J. B., G. D. Weiblen, and G. May. 2016. Host associations and beta diversity of fungal endophyte communities in New Guinea rainforest trees. *Molecular Ecology* 25: 825–841.

Wang, M., J. J. Carver, V. V. Phelan, L. M. Sanchez, N. Garg, Y. Peng, D. D. Nguyen, et al. 2016. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature Biotechnology* 34: 828.

Wang, L., L. Ren, C. Li, C. Gao, X. Liu, M. Wang, and Y. Luo. 2019. Effects of endophytic fungi diversity in different coniferous species on the colonization of *Sirex noctilio* (Hymenoptera: Siricidae). *Scientific Reports* 9: 5077.

Wearn, J. A., B. C. Sutton, N. J. Morley, and A. C. Gange. 2012. Species and organ specificity of fungal endophytes in herbaceous grassland plants. *Journal of Ecology* 100: 1085–1092.

Whitaker, B. K., H. L. Reynolds, and K. Clay. 2018. Foliar fungal endophyte communities are structured by environment but not host ecotype in *Panicum virgatum* (switchgrass). *Ecology* 99: 2703–2711.

Wilson, D. 1995. Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos* 73: 274–276.

Yamaji, K., Y. Watanabe, H. Masuya, A. Shigeto, H. Yui, and T. Haruma. 2016. Root fungal endophytes enhance heavy-metal stress tolerance of *Clethra barbinervis* growing naturally at mining sites via growth enhancement, promotion of nutrient uptake and decrease of heavy-metal concentration. *PLoS One* 11: e0169089.

Zamioudis, C., and C. M. J. Pieterse. 2012. Modulation of host immunity by beneficial microbes. *Molecular Plant-Microbe Interactions* 25: 139–150.

Zimmerman, N. B., and P. M. Vitousek. 2012. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. *Proceedings of the National Academy of Sciences, USA* 109: 13022–13027.