Received: 23 November 2021 | Revised: 1 March 2022 | Accepted: 18 March 2022
DOI: 10.1002/ecy.3762

ARTICLE

ECOLOGY
ECOLOGICAL SOCIETY OF AMERICA

# The chemical ecology of tropical forest diversity: Environmental variation, chemical similarity, herbivory, and richness

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**Funding information**
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 401673/2012-0; Fundação Amazônia Paraense de Amparo à Pesquisa, Grant/Award Numbers: 2014/23417-7, 2014/50316-7; National Science Foundation, Grant/Award Numbers: DEB 1442103, NSF DEB 135538

**Handling Editor:** Erik E. Sotka

[Corrections added on 30 July 2022, after first online publication: Author name has been corrected to Gerardo Cebrian-Torrejon in this version.]

## Abstract

Species richness in tropical forests is correlated with other dimensions of diversity, including the diversity of plant–herbivore interactions and the phytochemical diversity that influences those interactions. Understanding the complexity of plant chemistry and the importance of phytochemical diversity for plant–insect interactions and overall forest richness has been enhanced significantly by the application of metabolomics to natural systems. The present work used proton nuclear magnetic resonance spectroscopy ($^1$H-NMR) profiling of crude leaf extracts to study phytochemical similarity and diversity among *Piper* plants growing naturally in the Atlantic Rainforest of Brazil. Spectral profile similarity and chemical diversity were quantified to examine the relationship between metrics of phytochemical diversity, specialist and generalist herbivory, and understory plant richness. Herbivory increased with understory species richness, while generalist herbivory increased and specialist herbivory decreased with the diversity of *Piper* leaf material available. Specialist herbivory increased when conspecific host plants were more spectroscopically dissimilar. Spectral similarity was lower among individuals of common species, and they were also more spectrally diverse, indicating phytochemical diversity is beneficial to plants. Canopy openness and soil nutrients also influenced chemistry and herbivory. The complex relationships uncovered in this study add information to our growing understanding of the importance of phytochemical diversity for plant–insect interactions and tropical plant species richness.

**KEYWORDS**
herbivore, light, metabolomics, nutrients, phytochemical similarity, plant–insect interactions, tropical forest richness

# INTRODUCTION

Climate, edaphic factors, and their stability over time contribute to the large-scale patterns of high plant richness and beta diversity in tropical ecosystems (Fine et al., 2010; Gentry, 1988; Mittelbach et al., 2007; Pennington et al., 2004). Alpha diversity is also extremely high for tropical forest plants, and this local-scale diversity is maintained in part by antagonistic interactions with insect herbivores (Alvarez-Loayza & Terborgh, 2011; Bagchi et al., 2014; Clark & Clark, 1984; Massad et al., 2013, 2015; Sullivan, 2003) and soil organisms (Mangan et al., 2010; Swamy & Terborgh, 2010). For example, the Janzen-Connell hypothesis posits that herbivores may exert density-dependent effects on plant mortality by feeding preferentially on seeds or plants occurring in dense patches of conspecifics, causing mortality and thereby opening space on the forest floor for the recruitment of additional species (Connell, 1971; Janzen, 1970). This hypothesis has inspired years of study (reviewed by Carson et al., 2008; Comita et al., 2014; Song et al., 2021), but not all data support its predictions (Hyatt et al., 2003; Brenes-Arguedas, 2012; Song et al., 2021). This may be because, as Janzen himself noted, herbivores do not perceive their would-be host plants as taxonomic units but rather as chemical entities that may be nutritious or deterrent, depending on their chemistry (Janzen, 1978). To examine the relationships between herbivores and high plant diversity, our study focused on specialist and generalist herbivory and their response to plant taxonomic and chemical diversity within small-scale plots in tropical forests.

## Phytochemical diversity and plant–insect interactions

The recent application of metabolomics to studies of plant secondary metabolites has demonstrated that plant chemistry varies considerably between and within species and that this variation is ecologically important (Coley et al., 2018; Endara et al., 2015, 2018; Kessler & Kalske, 2018; Moore et al., 2014; Peters et al., 2018; Richards et al., 2015; Salazar et al., 2016a, 2016b, 2018). For example, phytochemical diversity is a strong predictor of herbivory in tropical forests (Massad et al., 2017; Richards et al., 2015; Salazar et al., 2016a) and can be negatively (Salazar et al., 2018) or positively (Richards et al., 2015) correlated with herbivore community richness. Specialists exert considerable selective pressure on the phytochemical complexity of host plants, which affects both local plant diversity and the evolution of plants and insects (Ehrlich & Raven, 1964; Endara et al., 2015, 2018). In the diverse plant genus *Inga*, a chemogram of species generated using metabolomics was a better match with the phylogeny of specialist herbivores than the phylogeny of *Inga* itself, demonstrating the importance of plant–insect interactions in shaping plant chemistry and the evolution of specialist herbivores (Endara et al., 2018). In general, relationships between herbivory and phytochemistry are complex, and uncovering further patterns will contribute to emerging theory on the role of plant chemistry and herbivory in the maintenance of tropical forest diversity.

## Herbivory and plant richness

Specialist herbivores were the original focus of the Janzen-Connell hypothesis, and specialist feeding is hypothesized to increase with the density of conspecific stems (Carson et al., 2008; Connell, 1971; Janzen, 1970). In seedling carpets, specialist damage does lead to negative density dependence (Alvarez-Loayza & Terborgh, 2011), but few studies have actually measured specialist and generalist herbivory separately. Research examining diet breadth supports the hypothesis that specialist and generalist feeding is mediated by different plant traits and that both specialist and generalist herbivores can have community-wide effects on plant diversity (Dyer et al., 2010; Massad et al., 2017; Salazar et al., 2016a). Modeling further suggests that generalists may have positive effects on richness where species are spatially aggregated or where generalists are selective in their feeding (Sedio & Ostling, 2013). Tests of Janzen-Connell effects commonly quantify plant density and herbivory relationships by focusing on the number of stems of a given plant species in a community (Comita et al., 2014), but folivorous insects are likely attracted to patches with ample leaf area, regardless of the number of individuals. The diversity of leaf area in a patch may therefore be a more informative variable than the number of stems when exploring relationships between plant density and herbivory.

## Abiotic effects on herbivory

Herbivory is also affected by abiotic variables, both directly and indirectly, and variations in light and mineral nutrients exert substantial effect sizes on plant chemistry and herbivory. Indirect relationships are often mediated by plant defenses. For example, under conditions of high soil nutrients and low light, *Piper* amide production is optimized, leading to reduced generalist herbivory (Dyer et al., 2004). Many other studies also demonstrated the effects of light on plant defense and herbivory (Agrell et al., 2004; Karolewski et al., 2013; Nichols-Orians, 1991; Norghauer et al., 2008). On a larger scale, plant investments in defense and the resulting herbivory vary across soil types in the Amazon (Fine et al., 2006). Examining associations between abiotic variables and phytochemical diversity is therefore likely to yield a more complete picture of factors that influence herbivory and the role of herbivores in diversifying plant communities.

## Questions and hypotheses

This work addresses the determinants of tropical diversity by examining phytochemical diversity and herbivory and their effects on plant diversity. Focusing on the genus *Piper* and its associated herbivores, we quantified canopy openness, soil nutrients, resource availability (defined as *Piper* leaf area), plant species richness, metabolomic similarity and diversity, and specialist and generalist herbivory in 10-m-diameter plots in tropical forest understories. We then conducted targeted analyses of our most abundant species, present in multiple sites, to explore how specific chemical features contribute to measures of metabolic diversity and subsequent herbivory. The research was designed to address the following questions and hypotheses:

1. How is phytochemical similarity associated with specialist and generalist herbivory?

   *Generalist herbivory may be higher where chemistry among neighboring plants is more similar, whereas specialist herbivory may be less affected by phytochemical similarity.*

2. How does resource availability affect herbivory?

   *Specialist herbivory may be higher in forest patches where there is greater leaf area of preferred host plants, while generalist herbivory may be less affected by host plant leaf area.*

3. How does species richness affect herbivory?

   *Generalist herbivory may increase with greater species richness outside the genus* Piper, *and specialist herbivory may increase with* Piper *richness.*

4. Are environmental factors, such as light and soil nutrients, predictors of herbivory?

   *Herbivory may increase with enhanced light and soil nutrient availability* via *direct effects and indirect effects on phytochemistry.*

5. Are phytochemical diversity and similarity related to the abundance of conspecifics?

   *Phytochemical diversity of a given species may be higher and the chemical similarity of conspecifics may be lower where those species are more abundant.*

# METHODS

## Focal genus

*Piper* is both abundant and diverse in neotropical forest understories (Callejas-Posada, 2020; Dyer & Palmer, 2004; Sardi et al., 2018). The genus produces a wide range of secondary metabolites, including phenolics, neolignans, and amides (Dyer & Palmer, 2004; Kato & Furlan, 2007), and patterns of generalist and specialist herbivory have been well documented within the genus (Dyer et al., 2010; Salazar et al., 2013; Salazar & Marquis, 2012). Specialist and generalist herbivory is defined by diet breadth at the genus level, so specialists may be oligophagous within *Piper*.

## Field methods

Tropical understory plant communities were studied across four subtropical humid (Köppen-Geiger climate classification; Peel et al., 2007) Atlantic Rainforest remnants in southeastern Brazil (Appendix S1: Table S1). Twenty-one plots measuring 10 m in diameter were established at least 10 m from any forest edge, including trails and streams. Plots were centered on a randomly selected *Piper* individual, and all *Piper* individuals within the plots were marked and identified to species or morphospecies based on leaf and stem morphology. All leaves within reach were exhaustively searched for herbivores. *Piper* hosts many insect herbivores, including *Eois* (Geometridae) caterpillars that specialize on *Piper* but may feed on multiple species within the genus, Curculionidae and Chrysomelidae that specialize on the genus, other Lepidoptera that may be specialists (e.g., *Memphis* (Nymphalidae) or generalists (e.g., Erebidae), and Orthoptera generalists (Dyer et al., 2010; caterpillars.org; the authors personal observation, 1998-2022). Percentage herbivory was visually determined on all *Piper* individuals, and specialist damage was distinguished from generalist damage as accurately as possible. In general, damage originating at leaf margins was classified as generalist herbivory; damage in the center of leaves characteristic of specialist chrysomelid and curculionid beetles and characteristic "windows" created by *Eois* were categorized as specialist herbivory (Dyer et al., 2010). The number of leaves on each plant was recorded, and 10 leaves per species per plot were measured to determine the leaf area of each species in a plot. Non-*Piper* morphospecies with leaves between 50 cm and 2 m above ground were counted in each plot to determine understory richness; morphospecies were differentiated based on leaf and stem morphology. Canopy openness was measured in the center of the plot using a convex spherical crown densitometer (Forestry Suppliers, Model A). Three soil samples from the top 20 cm of soil were randomly collected from each plot and analyzed for N, C, Ca, K, Mg, and P in the Analytical Center at the University of São Paulo using standard protocols (http://ca.iq.usp.br/novo/). C and N were measured as percentages; Ca, K, Mg, Na, and P were quantified as milligrams per kilogram of soil. The sum of bases (Ca, K, Mg, and Na) was tested in analyses (Wigley et al., 2016).

Forty-one species of *Piper* were found across our plots. *Piper gaudichaudianum* Kunth was the most abundant species; 426 of 1354 *Piper* individuals in our data set were *P. gaudichaudianum*. The species was also widespread, being found in 11 of the plots and 3 of the field sites. *P. gaudichaudianum* was therefore selected as the focal species for targeted chemical analyses described in what follows. Individuals identified as *P. gaudichaudianum* may also be closely related species in the Radula clade, which are very difficult to distinguish, even with fertile specimens (E.J. Tepe, personal observation, 2014-2022).

## Chemical analysis

Immature and mature leaves with and without herbivory were collected from each *Piper* individual whenever possible. Detailed methods on extractions and proton nuclear magnetic resonance spectroscopy ($^1$H-NMR) data collection are in Appendix S1.

## Data analysis

The downfield region of the NMR spectra (δ 5.0–12.0) was used to calculate Morisita similarity index (MSI) values describing the similarity of spectral profiles for each *Piper* morphospecies in a plot (referred to as spectral similarity). The downfield region is less crowded and includes resonances of functional groups known to be important for deterring herbivory (Richards et al., 2015). To calculate the MSI, the sizes of NMR summed-area chemical shift bins were treated analogously to abundance data, and each bin's chemical shift was analogous to a species. Calculations were performed using SPADE (Chao et al., 2008). Spectral diversity within individual plants was calculated as Simpson's index (Simpson's index of species equivalents; Jost, 2006) based on the same downfield NMR peaks and their intensities.

Spectral similarity and diversity were included as endogenous variables in structural equation models (SEM) to examine hypothesized causal relationships between (a) *Piper* species richness, (b) understory species richness (outside of the genus *Piper*), and (c) specialist and generalist herbivory. Predictor variables tested in both models were the (i) leaf area of the individual plant and (ii) total leaf area of a given *Piper* species in a plot (these variables were considered a better measure of resource availability than the number of individual plants in a plot because some plots had many small plants), (iii) resource diversity calculated as Simpson's index of leaf area (based on leaf area per species rather than species abundance; this variable describes the diversity of resources available to herbivores), (iv) canopy openness, and (v) soil nutrient content. Diversity indices were calculated as species equivalents or true diversities (Jost, 2006). Models were based on a priori hypotheses, and nonsignificant associations were sequentially removed from models to arrive at the best fit model. All variables were transformed to *z*-scores for scale. Models were run with the **`lavaan`** package in R (Rosseel, 2012). Bayesian posterior probabilities were calculated for the relationships in the structural equation models with the **`blavaan`** package (Merkle & Rosseel, 2016) and are presented with the marginal log-likelihood and posterior predictive *p*-values (PPP values).

We utilized Bayesian models to test hypotheses of how different measures of phytochemical diversity may be affected by the abundance of conspecifics based on the ratio of the number of individuals of a given species in a plot to the total number of individuals in a plot. For analyses of chemical diversity, individuals were used as replicates, and study site and species were included as random factors. To test for differences in spectral similarity, species in a plot were used as replicates, and the random effect was location. Models were run with 4 chains and 4 cores with 2000 iterations, a warmup of 1000 iterations, and 4000 post-warm-up samples with a maximum tree depth of 20 in the **brms** package using RStan (Bürkner, 2017).

We also examined the effect size of herbivory based on quartiles of spectral similarity. Spectral similarity was calculated as described earlier, based on individuals of the same species in a plot. The effect size of herbivory was calculated by comparing the deviation of herbivory on an individual plant in a given quartile of spectral similarity from the overall data set using Hedges' g in the **effsize** package (Torchiano, 2019). These analyses were conducted for all species combined and for *P. gaudichaudianum* separately.

$^1$H-NMR data from *P. gaudichaudianum* were analyzed with a network approach using the **WGCNA** (Weighted Gene Co-expression Network Analysis) package in R (Langfelder & Horvath, 2008). This analysis clusters groups with covarying chemical shifts. Clusters represent protons associated with specific compounds or similar structural features (Richards et al., 2018). The data were evaluated to determine the appropriate power threshold for a scale-free topology, which in this case was the power of three. Eight modules were identified using the blockwise Modules function (merge cut height of 0.25 and minimum module size of 5). These modules were investigated to identify the specific compounds/features that each module represented. The eigenvalues of the modules for each sample were then analyzed with structural equation modeling to investigate how specific groups of compounds contribute to phytochemical similarity and effects on herbivory. Site-level environmental characteristics (canopy openness, slope, soil C, N, K, Na, Ca, Mg, and P) were represented as loadings from a factor analysis. All analyses were performed in R version 3.4.2 (R Core Team, 2021).

# RESULTS

In total, 1354 *Piper* individuals representing 42 species were examined across all plots. *Piper* abundance ranged from 3 to 222 individuals per plot with a mean of 62 individuals. Two plots had over 200 individuals; most of those plants were seedlings. The most species rich plot contained eight species and the least diverse plot had two species. On average, plots had 4.3 ± 0.4 SE species. Generalist herbivory averaged 5.9% ± 0.2 SE across individuals; specialist herbivory averaged 4.1% ± 0.2 SE.

The best-fit SEM examining the effects of *Piper* species richness on herbivory showed generalist herbivory decreased with increasing *Piper* species richness; specialist herbivory was not affected by *Piper* richness. Specialist herbivory declined when co-occurring conspecifics were more spectroscopically similar, but profile similarity did not affect generalist herbivory. Spectral diversity was not included in the best-fit model. The diversity of leaf material available led to increased generalist herbivory and decreased specialist herbivory. Canopy openness led to decreases in the diversity of leaf area and *Piper* richness overall. Plants were more spectroscopically similar under high light. Specialist herbivory increased with canopy openness, and generalist herbivory decreased with the sum of bases present in the soil. Increases in soil N and the sum of bases were associated with decreases in the diversity of *Piper* leaf area and *Piper* richness. Spectral similarity increased in soils rich in cations (Figure 1; $\chi^2 = 10.2$, df $= 9$, $p = 0.34$, Bayesian marginal log-likelihood $= -13{,}750.42$, PPP $= 0.46$).

The best-fit SEM assessing the effects of understory plant species richness on herbivory showed both specialist and generalist herbivory on *Piper* were higher in more species-rich patches of forest (Figure 2). Specialist herbivory decreased when spectral similarity within species was greater. Spectral diversity was not part of the best-fit model. Specialist damage also decreased as the diversity of available leaf material increased, whereas generalist damage increased where leaf area diversity was higher. Increases in soil cations were associated with a decrease in the diversity of leaf area and an increase in spectral similarity. Canopy openness led to increased specialist herbivory; it also decreased leaf area diversity while increasing overall understory species richness and spectral similarity (Figure 2; $\chi^2 = 13.6$, df $= 11$, $p = 0.26$, Bayesian marginal log-likelihood $= -13{,}922.49$, PPP $= 0.41$).

Species-level spectral similarity among co-occurring conspecifics decreased slightly with the relative abundance of a species in a plot, so neighboring conspecifics of common species were less spectroscopically similar. Spectral diversity at the level of an individual (calculated as Simpson's index) increased where species were more abundant, meaning common species were also more chemically diverse. Patterns were similar when only reproductive sized individuals were tested (Table 1). The effect size of specialist herbivory was lower in plots where there was more spectral similarity between all conspecifics and between individuals of *P. gaudichaudianum* in particular, meaning there was less than average specialist damage on individuals from plots with greater intraspecific spectral similarity (Figure 3).

WGCNA network analysis of *P. gaudichaudianum* resulted in the identification of seven modules

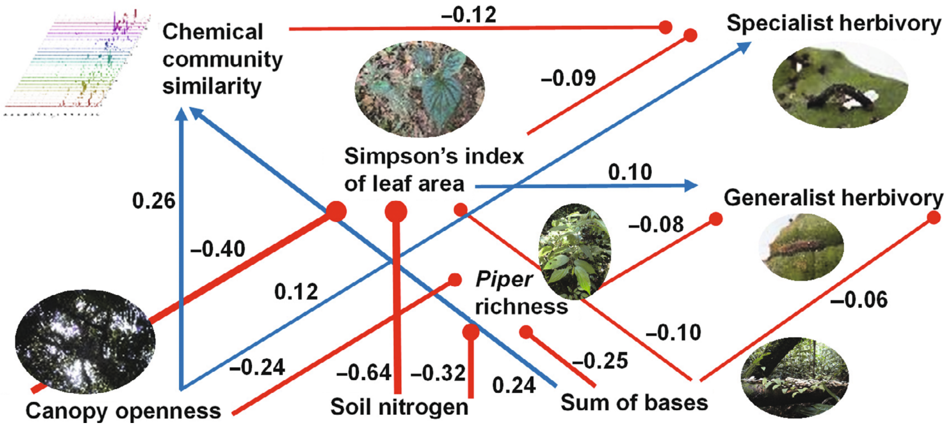

**FIGURE 1** Bayesian structural equation model showing relationships between *Piper* species richness, spectral similarity, herbivory, leaf area diversity, and other ecological data. Blue arrows indicate positive causal pathways, and red circles indicate negative causal pathways. Values are mean Bayesian posterior probability estimates; none of the standard deviations around the estimates included zero. Correlations are not drawn, but they include relationships between canopy openness and soil N (−0.24), canopy openness and the sum of bases in the soil (0.26), Simpson's index of leaf area and spectral similarity (−0.10), Simpson's index of leaf area and *Piper* richness (0.32), and specialist and generalist herbivory (−0.08).

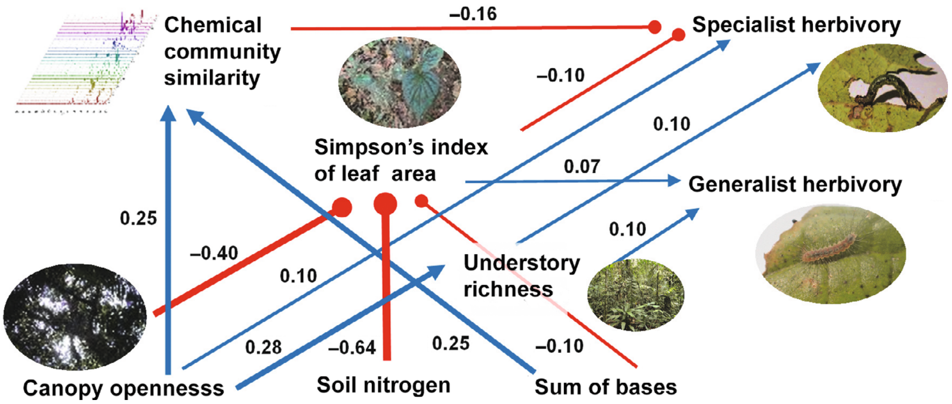

**FIGURE 2** Bayesian structural equation model showing relationships between non-*Piper* understory species richness and herbivory, spectral similarity, leaf area diversity, soil quality, and canopy openness. Symbols and values are the same as in Figure 1; none of the standard deviations around the probability estimates cross zero. Correlations are not drawn, but they include relationships between canopy openness and soil N (−0.24), canopy openness and the sum of bases in the soil (0.26), Simpson's index of leaf area and spectral similarity (−0.10), spectral similarity and understory richness (0.31), and specialist and generalist herbivory (−0.09).

**TABLE 1** Results of Bayesian analyses of chemical similarity and diversity as related to the abundance of conspecific *Piper* in a plot.

| Modeled predictors | PP | SDPD | 95% CI | ESS | Rhat |
|---|---|---|---|---|---|
| Chemical similarity | | | | | |
| RSA all individuals[a] | −0.08 | 0.08 | −0.23 to 0.08 | 3235 | 1.00 |
| RSA reproductive sized[a] | −0.09 | 0.09 | −0.28 to 0.10 | 2658 | 1.00 |
| Chemical diversity | | | | | |
| RSA all individuals[a] | 0.53 | 0.48 | −0.41 to 1.47 | 4179 | 1.00 |
| RSA reproductive sized | 0.68 | 0.73 | −0.74 to 2.10 | 3469 | 1.00 |

Abbreviations: CI, confidence interval; ESS, effective sample size; PP, posterior probability; RSA, relative species abundance; SDPD, standard deviation of posterior distribution; Rhat, potential scale reduction statistic (values close to 1.00 indicate model chains converged).

[a] Posterior probabilities equal to or larger than SDPD.

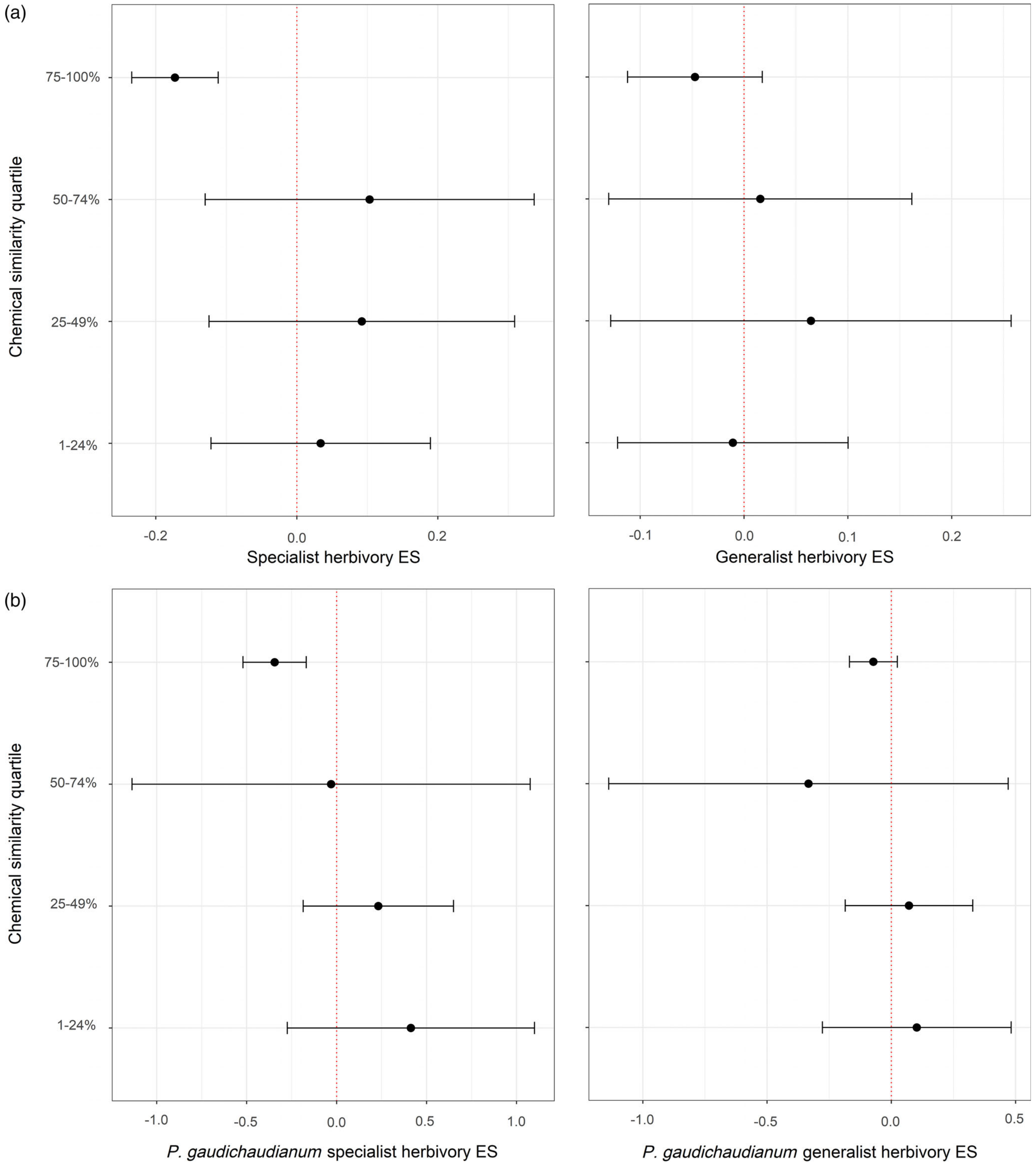

**FIGURE 3** Spectral similarity quartiles and effect sizes of specialist and generalist herbivory on (a) all individuals and (b) *Piper gaudichaudianum*. Error bars are 95% confidence interval.

representing sets of co-occurring and covarying peaks (Richards et al., 2018; Appendix S2: Table S1; Figures S1 and S2). Modules are described in Appendix S2. In the best-fit SEM describing the contributions of chemical modules to the spectral similarity and herbivory of *P. gaudichaudianum*, the taboganate module led to increased specialist herbivory (PE $= 7.49$, $p < 0.001$). Modules representing taboganates and phenylpropanoids decreased spectral similarity, and both specialist and generalist herbivory were lower where spectral similarity was higher (Appendix S2: Figure S3; $\chi^2 = 15.887$, df = 16, $p = 0.46$). The factor analysis of environmental data identified three factors that accounted for 79% of the variation (Appendix S2: Table S2). Taboganates were higher in low-light environments and decreased with soil nutrients. Chemical similarity increased with soil nutrients (Appendix S2: Figure S3).

# DISCUSSION

Herbivory in terrestrial ecosystems is a consequence of multiple dimensions of plant diversity, and in this study herbivory was affected by both species richness and phytochemical diversity. Overall, our data support the hypothesis that specialists and generalists respond differently to phytochemical profiles within small-scale communities. Generalists are less affected by spectral similarity, whereas specialist herbivory increased in plots with greater chemical dissimilarity. Specialists target particular species and may benefit from feeding on chemically diverse individuals, which are found in dissimilar host-plant populations (a simple regression of our data showed chemically diverse individuals were most often in chemically dissimilar communities; $F_{1,774} = 40.0$, $r^2 = 0.05$, $p < 0.001$). It is possible for specialists to exhibit oviposition and feeding preferences for individuals with higher chemical diversity in what may be chemically dissimilar populations, whereas generalists do well in more chemically similar communities (Wetzel & Whitehead, 2020). Recent work on Costa Rican *Piper* communities shows the chemistry of co-occurring species is more divergent than would be expected by chance (Salazar et al., 2016b), further supporting the hypothesis that chemical diversity limits herbivory, particularly by generalists. The importance of chemical diversity has also been documented for other genera. One of the defining characteristics of the diverse neotropical genus *Inga*, for example, is the divergence of defenses among closely related species (Kursar et al., 2009). A similar pattern has been documented among species in the genus *Vachellia* in Africa (Hattas et al., 2011).

## Phytochemical diversity and plant–insect interactions

Common species were individually more chemically diverse and formed less chemically similar populations than rare congeners. It is possible that rare species appeared more chemically similar because fewer individuals were available for analysis, and variation was undetected due to sampling. However, a potential benefit of high intraspecific chemical variation is to reduce the sharing of herbivores between neighboring conspecifics (Becerra, 2015; Coley et al., 2018; Coley & Kursar, 2014; Endara et al., 2015). Common species may therefore achieve greater abundance through increased chemical diversity and high intraspecific variation, making it difficult for herbivores to utilize all individuals in a population (Glassmire et al., 2019). These relationships require further study because in Costa Rica a negative relationship between *Piper* abundance and phytochemical diversity has been documented (Richards et al., 2015).

We found that the results from the most abundant, broadly distributed species, *P. gaudichaudianum*, supported the broader patterns at the community level. *P. gaudichaudianum* plants in chemically similar plots had reduced specialist and generalist herbivory. The chemical modules containing the major compounds of adult leaves, gaudichaudianic acid and prenylated benzoic acids, did not contribute significantly to variation in spectral similarity. Rather, the unique chemical signatures of taboganates and phenylpropanoids decreased chemical similarity. The methyl taboganates and chromene are biosynthetically related to prenylated benzoic acids. They have previously been reported in the roots of *P. gaudichaudianum* but not in leaves (Gaia et al., 2014, 2021; Ramos et al., 2009). These compounds are known antifungals (Terreaux et al., 1998) and are sequestered by root-feeding weevil larvae (Ramos et al., 2009). Specialist herbivores may therefore benefit from consuming these compounds.

Overall, relationships between spectral similarity and specialist and generalist herbivory were complex, and our results showed specialist herbivory decreased with population-level spectral similarity. This finding contradicts Langeheim and Stubblebine's hypothesis that variation in defense is adaptive for recruitment (Langeheim & Stubblebine, 1983). It is possible, however, that we would have found different relationships between phytochemical similarity and herbivory if we had focused on differences between seedlings and reproductive plants. Chemical data were not collected for many of the youngest seedlings in our data set because of their small size, but future work should follow chemical changes in cohorts of seedlings as they develop. A study with seedlings in reforestation found a positive correlation between chemical richness and herbivory but a negative correlation between chemical richness and pathogen damage (Chuluma, 2022). Work with other tropical species has documented a negative relationship between herbivory and population-level chemical diversity, indicating chemical defense diversity affords plants greater protection from herbivores (Bravo-Monzón et al., 2014) and increased survival (Sanchez-Hidalgo et al., 1999). Interestingly, patterns related to the compositional and structural similarity of the broad range of intrafoliar compounds documented here contrast with results from the same plots but focused on volatile compositional chemical similarity (Massad et al., 2017). Volatile similarity increased, rather than decreased, generalist herbivory. The chemical similarity of intrafoliar and volatile compounds likely operates in different ways—dissimilarity among volatiles can complicate host searching, particularly for generalists, while nonvolatile phytochemical dissimilarity in a community may not present a major barrier to generalists or may actually benefit them through dietary mixing (Singer et al., 2004).

## Herbivory and plant richness

The diversity of available resources (Simpson's index of *Piper* leaf area) increased generalist damage and decreased specialist damage, potentially because specialists are evolved to seek out particular hosts. The concept of using the diversity of leaf area as a predictor of herbivory builds on the resource concentration hypothesis and associational susceptibility (Barbosa et al., 2009; Root, 1973). Defining diversity based on leaf area, rather than individual plants, quantifies plant diversity from an herbivore's point of view. The presence of potential food (leaf area) may be more important than the number of individual shoots, especially when there are numerous seedlings in a habitat. In fact, the pattern for shoot diversity was different—both specialist and generalist herbivory decreased with *Piper* species richness. A study from another forest similarly found specialist herbivory decreased with *Piper* shoot diversity, but generalists responded in the opposite direction (Salazar et al., 2013). Analyzing diversity from the perspective of herbivores in terms of chemistry and resources may help ecologists better understand the complex relationships that structure tropical forest richness.

## Connecting abiotic conditions, taxonomic richness, phytochemical diversity, and herbivory

Consistent with a large body of literature demonstrating changes in phytochemistry in response to environmental variation (Koricheva et al., 1998; Massad & Dyer, 2010), abiotic factors determined relationships with herbivory. For example, canopy openness reduced generalist but increased specialist feeding. In Costa Rica, specialist and generalist herbivory on *Piper* increases with canopy openness (Salazar et al., 2013), and herbivory increases in treefall gaps in general (Piper et al., 2018). Greater light levels and increased soil nutrients may have allowed for increased production of defenses (Abdala-Roberts et al., 2014; Bryant et al., 1983; Dyer et al., 2004; Massad et al., 2012), which could have limited generalist feeding in our study. Intraspecific spectral similarity was higher under conditions of greater canopy openness and more soil cations. Generalist herbivory was in turn higher where chemical similarity was lower and, as noted, where canopies were more closed.

Relationships between species' relative abundance and chemical similarity showed that abundant species are less chemically similar, potentially because herbivores limit the establishment of similar seedlings. This result, in conjunction with the finding that specialist herbivory is higher in plots where individuals are more chemically dissimilar, suggests chemical dissimilarity is advantageous for species' establishment. Furthermore, individuals with greater chemical diversity often belonged to common species. These two components of phytochemistry, similarity at the community level and diversity at the individual level, should both be explored to understand plant–herbivore interactions in an ecosystem; generalist and specialist herbivory should also be disentangled as much as possible. *P. gaudichaudianum* was the most abundant and chemically diverse species in this study, and dissimilarity at the population level reduced herbivory by both specialist and generalists in this species.

In conclusion, multiple aspects of diversity affect community parameters. We are learning that phytochemical diversity and the similarity of chemical profiles are important functional traits that affect plant fitness, species interactions, and community assemblages. By looking more deeply into the effects of variable plant chemistry on herbivore communities, we will improve our understanding of the factors that govern tropical forest community structure.

## ACKNOWLEDGMENTS

We would like to thank the dedicated students who assisted with fieldwork as well as the support staff of the reserves where we worked. We appreciate Angela Smilanich for her assistance in organizing an International Research Experiences for Undergraduates (REU) student (National Science Foundation [NSF] Department of Environmental Biology [DEB] 135538). We are also grateful to the staff of the Analytical Center facility at Universidade de São Paulo (USP) for processing our soil samples. This work was made possible by the support of grants from the National Council for Scientific and Technological Development (CNPq) (401673/2012-0), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; São Paulo Research Foundation) (2014/23417-7 and 2014/50316-7), the National Science

Foundation (NSF) (DEB 1442103 and EN-2133818), and the Hitchcock Center for Chemical-Ecology fund.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data (Massad et al., 2022) are available from the Environmental Data Initiative at https://doi.org/10.6073/pasta/12ef61e0ca951d92aed76db57aa8bb7b.

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## REFERENCES

Abdala-Roberts, L., X. Moreira, J. C. Cervera, and V. Parra-Tabla. 2014. “Light Availability Influences Growth-Defense Trade-Offs in Big-Leaf Mahogany (Swietenia Macrophylla King).” *Biotropica* 46: 591–7.

Agrell, J., P. Anderson, W. Oleszek, A. Stochmal, and C. Agrell. 2004. “Combined Effects of Elevated CO2 and Herbivore Damage on Alfalfa and Cotton.” *Journal of Chemical Ecology* 30: 2309–24.

Alvarez-Loayza, P., and J. Terborgh. 2011. “Fates of Seedling Carpets in an Amazonian Floodplain Forest: Intra-Cohort Competition or Attack by Enemies?” *Journal of Ecology* 99: 1045–54.

Bagchi, R., R. E. Gallery, S. Gripenberg, S. J. Gurr, L. Narayan, C. E. Addis, R. P. Freckleton, and O. T. Lewis. 2014. “Pathogens and Insect Herbivores Drive Rainforest Plant Diversity and Composition.” *Nature* 506: 85–8.

Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. “Associational Resistance and Associational Susceptibility: Having Right or Wrong Neighbors.” *Annual Review of Ecology Evolution and Systematics* 40: 1–20.

Becerra, J. X. 2015. “On the Factors that Promote the Diversity of Herbivorous Insects and Plants in Tropical Forests.” *Proceedings of the National Academy of Sciences of the United States of America* 112: 6098–103.

Bravo-Monzón, A. E., E. Ríos-Vásquez, G. Delgado-Lamas, and F. J. Espinosa-García. 2014. “Chemical Diversity among Populations of Mikania Micrantha: Geographic Mosaic Structure and Herbivory.” *Oecologia* 174: 195–203.

Brenes-Arguedas, T. 2012. “Leaf Damage and Density-Dependent Effects on Six Inga Species in a Neotropical Forest.” *Revista De Biologia Tropical* 60: 1503–12.

Bryant, J. P., F. S. Chapin, and D. R. Klein. 1983. “Carbon/Nutrient Balance of Boreal Plants in Relation to Vertebrate Herbivory.” *Oikos* 40: 357–68.

Bürkner, P. C. 2017. “brms: An R Package for Bayesian Multilevel Models Using Stan.” *Journal of Statistical Software* 80(1): 1–28.

Callejas-Posada, R. 2020. “Piperaceae.” In *Flora Mesoamericana*, Vol 2 (pt. 2), edited by G. Davidse, C. Ulloa Ulloa, H. M. Hernández Macías, and S. Knapp, 1–590. St. Louis, MO: Missouri Botanical Garden Press i–xix.

Carson, W. P., J. T. Anderson, E. G. Leigh, Jr., and S. A. Schnitzer. 2008. “Challenges Associated with Testing and Falsifying the Janzen-Connell Hypothesis: A Review and Critique.” In *Tropical Forest Community Ecology*, edited by W. P. Carson and S. A. Schnitzer, 210–41. Malden, MA: Blackwell Publishing.

Chao, A., L. Jost, S. C. Chiang, Y.-H. Jiang, and R. L. Chazdon. 2008. “A Two-Stage Probabilistic Approach to Multiple-Community Similarity Indices.” *Biometrics* 64: 1178–86.

Chuluma, M. T. E. 2022. *Efeito de defesas de plantas na herbivoria por insectos e crescimento das mudas de espécies nativas no reflorestamento da Serra da Gorongosa*. Master’s Thesis. Goinha: Consórcio de BioEducação, Parque Nacional da Gorongosa.

Clark, D. A., and D. B. Clark. 1984. “Spacing Dynamics of a Tropical Rain Forest Tree: Evaluation of the Janzen-Connell Model.” *The American Naturalist* 124: 769–88.

Coley, P. D., M.-J. Endara, and T. A. Kursar. 2018. “Consequences of Interspecific Variation in Defenses and Herbivore Host Choice for the Ecology and Evolution of Inga, a Speciose Rainforest Tree.” *Oecologia* 187: 361–76.

Coley, P. D., and T. A. Kursar. 2014. “On Tropical Forests and their Pests.” *Science* 343: 35–6.

Comita, L. S., S. A. Queenborough, S. J. Murphy, J. L. Eck, K. Xu, M. Krishnadas, N. Beckman, and Y. Zhu. 2014. “Testing Predictions of the Janzen–Connell Hypothesis: A Meta-Analysis of Experimental Evidence for Distance- and Density-Dependent Seed and Seedling Survival.” *Journal of Ecology* 102: 845–56.

Connell, J. 1971. “On the Role of Natural Enemies in Preventing Competitive Exclusion in some Marine Animals and in Rain Forest Trees.” In *Dynamics of Populations*, edited by P. J. den Boer and G. Gradwell, 298–312. Wageningen: Pudoc.

Dyer, L. A., D. K. Letourneau, C. D. Dodson, M. A. Tobler, J. O. Stireman, and A. Hsu. 2004. “Ecological Causes and Consequences of Variation in Defensive Chemistry of a Neotropical Shrub.” *Ecology* 85: 2795–803.

Dyer, L. A., and A. D. N. Palmer. 2004. *Piper: A Model Genus for Studies of Phytochemistry, Ecology, and Evolution*. Boston, MA: Springer US.

Dyer, L. A., D. K. Letourneau, G. V. Chavarria, and D. S. Amoretti. 2010. “Herbivores on a Dominant Understory Shrub Increase Local Plant Diversity in Rain Forest Communities.” *Ecology* 91: 3707–18.

Endara, M.-J., A. Weinhold, J. E. Cox, N. L. Wiggins, P. D. Coley, and T. A. Kursar. 2015. “Divergent Evolution in Antiherbivore Defences within Species Complexes at a Single Amazonian Site.” *Journal of Ecology* 103: 1107–18.

Endara, M.-J., J. A. Nicholls, P. D. Coley, D. L. Forrister, G. C. Younkin, K. G. Dexter, C. A. Kidner, R. T. Pennington, G. N. Stone, and T. A. Kursar. 2018. "Tracking of Host Defenses and Phylogeny during the Radiation of Neotropical Inga-Feeding Sawflies (Hymenoptera; Argidae)." *Frontiers in Plant Science* 9: 1237.

Ehrlich, P., and R. Raven. 1964. "Butterflies and Plants – A Study in Coevolution." *Evolution* 18: 586–608.

Fine, P. V. A., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I. Sääksjärvi, J. C. Schultz, and P. D. Coley. 2006. "The Growth–Defense Trade-off and Habitat Specialization by Plants in Amazonian Forests." *Ecology* 87: S150–62.

Fine, P. V. A., R. García-Villacorta, N. C. A. Pitman, I. Mesones, and S. W. Kembel. 2010. "A Floristic Study of the White-Sand Forests of Peru." *Annals of the Missouri Botanical Garden* 97: 283–305.

Gaia, A. M., L. F. Yamaguchi, C. S. Jeffrey, and M. J. Kato. 2014. "Age-Dependent Changes from Allylphenol to Prenylated Benzoic Acid Production in *Piper gaudichaudianum* Kunth." *Phytochemistry* 106: 86–93.

Gaia, A. M., L. F. Yamaguchi, C. Guerrero-Perilla, and M. J. Kato. 2021. "Ontogenetic Changes in the Chemical Profiles of *Piper* Species." *Plants* 10: 1085.

Gentry, A. H. 1988. "Tree Species Richness of Upper Amazonian Forests." *Proceedings of the National Academy of Sciences* 85: 156–9.

Glassmire, A. E., C. Philbin, L. A. Richards, C. S. Jeffrey, J. S. Snook, and L. A. Dyer. 2019. "Proximity to Canopy Mediates Changes in the Defensive Chemistry and Herbivore Loads of an Understory Tropical Shrub, Piper Kelleyi." *Ecology Letters* 22: 332–41.

Hattas, D., J. Hjältén, R. Julkunen-Tiitto, P. F. Scogings, and T. Rooke. 2011. "Differential Phenolic Profiles in Six African Savanna Woody Species in Relation to Antiherbivore Defense." *Phytochemistry* 72: 1796–803.

Hyatt, L. A., M. S. Rosenberg, T. G. Howard, G. Bole, W. Fang, J. Anastasia, K. Brown, et al. 2003. "The Distance Dependence Prediction of the Janzen-Connell Hypothesis: A Meta-Analysis." *Oikos* 103: 590–602.

Janzen, D. 1970. "Herbivores and the Number of Tree Species in Tropical Forests." *American Naturalist* 104: 501–28.

Janzen, D. H. 1978. "Complications in Interpreting the Chemical Defenses of Trees against Tropical Arboreal Plant Eating Vertebrates." In *The Ecology of Arboreal Folivores*, edited by C. G. Montgomery, 73–84. Washington, D.C: Smithsonian Institute.

Jost, L. 2006. "Entropy and Diversity." *Oikos* 113: 363–75.

Karolewski, P., M. J. Giertych, M. Żmuda, A. M. Jagodziński, and J. Oleksyn. 2013. "Season and Light Affect Constitutive Defenses of Understory Shrub Species against Folivorous Insects." *Acta Oecologica* 53: 19–32.

Kato, M. J., and M. Furlan. 2007. "Chemistry and Evolution of the Piperaceae." *Pure and Applied Chemistry* 79: 529–38.

Kessler, A., and A. Kalske. 2018. "Plant Secondary Metabolite Diversity and Species Interactions." *Annual Review of Ecology, Evolution, and Systematics* 49: 115–38.

Koricheva, J., S. Larsson, E. Haukioja, and M. Keinänen. 1998. "Regulation of Woody Plant Secondary Metabolism by Resource Availability: Hypothesis Testing by Means of Meta-Analysis." *Oikos* 83: 212–26.

Kursar, T. A., K. G. Dexter, J. Lokvam, R. T. Pennington, J. E. Richardson, M. G. Weber, E. T. Murakami, C. Drake, R. McGregor, and P. D. Coley. 2009. "The Evolution of Antiherbivore Defenses and their Contribution to Species Coexistence in the Tropical Tree Genus Inga." *Proceedings of the National Academy of Sciences* 106: 18073–8.

Langeheim, J. H., and W. H. Stubblebine. 1983. "Variation in Leaf Resin Composition between Parent Tree and Progeny in *Hymenaea*: Implications for Herbivory in the Humid Tropics." *Biochemical Systematics and Ecology* 11: 97–106.

Langfelder, P., and S. Horvath. 2008. "WGCNA: An R Package for Weighted Correlation Network Analysis." *BMC Bioinformatics* 9: 1–13.

Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. Mack, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. "Negative Plant–Soil Feedback Predicts Tree-Species Relative Abundance in a Tropical Forest." *Nature* 466: 752–5.

Massad, T. J., and L. A. Dyer. 2010. "A Meta-Analysis of the Effects of Global Environmental Change on Plant-Herbivore Interactions." *Arthropod-Plant Interactions* 4: 181–8.

Massad, T. J., L. A. Dyer, and G. Vega C. 2012. "Costs of Defense and a Test of the Carbon-Nutrient Balance and Growth-Differentiation Balance Hypotheses for Two Co-Occurring Classes of Plant Defense." *PLoS One* 7: e47554.

Massad, T. J., J. K. Balch, E. A. Davidson, P. M. Brando, C. L. Mews, P. Porto, R. M. Quintino, S. A. Vieira, B. H. M. Junior, and S. E. Trumbore. 2013. "Interactions between Repeated Fire, Nutrients, and Insect Herbivores Affect the Recovery of Diversity in the Southern Amazon." *Oecologia* 172: 219–29.

Massad, T. J., J. K. Balch, C. L. Mews, P. Porto, B. H. Marimon Junior, R. M. Quintino, P. M. Brando, S. A. Vieira, and S. E. Trumbore. 2015. "Early Recruitment Responses to Interactions between Frequent Fires, Nutrients, and Herbivory in the Southern Amazon." *Oecologia* 178: 807–17.

Massad, T. J., M. M. de Moraes, C. Philbin, C. Oliveira, G. C. Torrejon, L. F. Yamaguchi, C. S. Jeffrey, L. A. Dyer, L. A. Richards, and M. J. Kato. 2017. "Similarity in Volatile Communities Leads to Increased Herbivory and Greater Tropical Forest Diversity." *Ecology* 98: 1750–6.

Massad, T. J., L. A. Richards, C. Philbin, L. F. Yamaguchi, M. J. Kato, C. S. Jeffrey, C. Oliveira, et al. 2022. "Data on Specialist and Generalist Herbivory, Environmental Variation, and Phytochemical Similarity from the Atlantic Rainforest of Brazil: 2013-2014 ver 1." Environmental Data Initiative. https://doi.org/10.6073/pasta/12ef61e0ca951d92aed76db57aa8bb7b.

Merkle, E. C., and Y. Rosseel. 2016. "Blavaan: Bayesian Structural Equation Models Via Parameter Expansion." arXiv 1511.05604. https://arxiv.org/abs/1511.05604.

Mittelbach, G. G., D. W. Schemske, H. V. Cornell, A. P. Allen, J. M. Brown, M. B. Bush, S. P. Harrison, et al. 2007. "Evolution and the Latitudinal Diversity Gradient: Speciation, Extinction and Biogeography." *Ecology Letters* 10: 315–31.

Moore, B. D., R. L. Andrew, C. Külheim, and W. J. Foley. 2014. "Explaining Intraspecific Diversity in Plant Secondary Metabolites in an Ecological Context." *New Phytologist* 201: 733–50.

Nichols-Orians, C. M. 1991. "The Effects of Light on Foliar Chemistry, Growth and Susceptibility of Seedlings of a Canopy Tree to an Attine Ant." *Oecologia* 86: 552–60.

Norghauer, J. M., J. R. Malcolm, and B. L. Zimmerman. 2008. "Canopy Cover Mediates Interactions between a Specialist Caterpillar and Seedlings of a Neotropical Tree." *Journal of Ecology* 96: 103–13.

Peel, M. C., B. L. Finlayson, and T. A. McMahon. 2007. "Updated World Map of the Köppen-Geiger Climate Classification." *Hydrology and Earth System Sciences* 11: 1633–44.

Pennington, R. T., M. Lavin, D. E. Prado, C. A. Pendry, S. K. Pell, and C. A. Butterworth. 2004. “Historical Climate Change and Speciation: Neotropical Seasonally Dry Forest Plants Show Patterns of both Tertiary and Quaternary Diversification.” *Philosophical Transactions. Biological Sciences* 359: 515–38.

Peters, K., A. Worrich, A. Weinhold, O. Alka, G. Balcke, C. Birkemeyer, H. Bruelheide, et al. 2018. “Current Challenges in Plant Eco-Metabolomics.” *International Journal of Molecular Sciences* 19: 1385–5.

Piper, F. I., S. H. Altmann, and C. H. Lusk. 2018. “Global Patterns of Insect Herbivory in Gap and Understorey Environments, and their Implications for Woody Plant Carbon Storage.” *Oikos* 127: 483–96.

R Core Team. 2021. “R: A Language and Environment for Statistical Computing.” In *R Foundation for Statistical Computing*. Vienna: Austria https://www.R-project.org/.

Ramos, C. S., S. A. Vanin, and M. J. Kato. 2009. “Sequestration of Prenylated Benzoic Acid and Chromenes by Naupactus Bipes (Coleoptera: Curculionidae) Feeding on *Piper gaudichaudianum* (Piperaceae).” *Chemoecology* 19: 73–80.

Richards, L. A., L. A. Dyer, M. L. Forister, A. M. Smilanich, C. D. Dodson, M. D. Leonard, and C. S. Jeffrey. 2015. “Phytochemical Diversity Drives Plant-Insect Community Diversity.” *Proceedings of the National Academy of Sciences of the United States of America* 112: 10973–8.

Richards, L. A., C. Oliveira, L. A. Dyer, A. Rumbaugh, F. Urbano-Muñoz, I. S. Wallace, C. D. Dodson, and C. S. Jeffrey. 2018. “Shedding Light on Chemically Mediated Tri-Trophic Interactions: A 1H-NMR Network Approach to Identify Compound Structural Features and Associated Biological Activity.” *Frontiers in Plant Science* 9: 1155.

Root, R. B. 1973. “Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (*Brassica Oleracea*).” *Ecological Monographs* 43: 95–124.

Rosseel, Y. 2012. “Lavaan: An R Package for Structural Equation Modeling.” *Journal of Statistical Software* 48: 1–36 http://www.jstatsoft.org/v48/i02/.

Salazar, D., and R. J. Marquis. 2012. “Herbivore Pressure Increases toward the Equator.” *Proceedings of the National Academy of Sciences - PNAS* 109: 12616–20.

Salazar, D., D. H. Kelm, and R. J. Marquis. 2013. “Directed Seed Dispersal of Piper by Carollia Perspicillata and its Effect on Understory Plant Diversity and Folivory.” *Ecology* 94: 2444–53.

Salazar, D., A. Jaramillo, and R. J. Marquis. 2016a. “The Impact of Plant Chemical Diversity on Plant–Herbivore Interactions at the Community Level.” *Oecologia* 181: 1199–208.

Salazar, D., M. A. Jaramillo, and R. J. Marquis. 2016b. “Chemical similarity and local community assembly in the species rich tropical genus *Piper*.” *Ecology* 97: 3176–83.

Salazar, D., J. Lokvam, I. Mesones, M. Vásquez Pilco, J. M. Ayarza Zuñiga, P. de Valpine, and P. V. A. Fine. 2018. “Origin and Maintenance of Chemical Diversity in a Species-Rich Tropical Tree Lineage.” *Nature Ecology & Evolution* 2: 983–90.

Sanchez-Hidalgo, M. E., M. Martinez-Ramos, and F. J. Espinosa-Garcia. 1999. “Chemical Differentiation between Leaves of Seedlings and Spatially Close Adult Trees from the Tropical Rain-Forest Species *Nectandra ambigens* (Lauraceae): An Alternative Test of the Janzen-Connell Model.” *Functional Ecology* 13: 725–32.

Sardi, A., A. M. Torres, and G. Corredor. 2018. “Diversidad florística en un paisaje rural del piedemonte de los Farallones de Cali, Colombia.” *Colombia Forestal* 21: 142–60.

Sedio, B. E., and A. M. Ostling. 2013. “How Specialised Must Natural Enemies Be to Facilitate Coexistence among Plants?” *Ecology Letters* 16: 995–1003.

Singer, M. S., Y. Carriere, C. Theuring, and T. Hartmann. 2004. “Disentangling Food Quality from Resistance against Parasitoids: Diet Choice by a Generalist Caterpillar.” *American Naturalist* 164: 423–9.

Song, X., J. Y. Lim, J. Yang, and M. S. Luskin. 2021. “When Do Janzen-Connell Effects Matter? A Phylogenetic Meta-analysis of Conspecific Negative Distance and Density Dependence Experiments.” *Ecology Letters* 24: 608–20.

Sullivan, J. J. 2003. “Density-Dependent Shoot-Borer Herbivory Increases the Age of First Reproduction and Mortality of Neotropical Tree Saplings.” *Oecologia* 136: 96–106.

Swamy, V., and J. W. Terborgh. 2010. “Distance-Responsive Natural Enemies Strongly Influence Seedling Establishment Patterns of Multiple Species in an Amazonian Rain Forest.” *The Journal of Ecology* 98: 1096–107.

Terreaux, C., M. P. Gupta, and K. Hostettmann. 1998. “Antifungal Benzoic Acid Derivatives from Piper Dilatatum in Honour of Professor G. H. Neil Towers 75th Birthday.” *Phytochemistry (Oxford)* 49: 461–4.

Torchiano, M. 2019. “Efficient effect size computation.” http://github.com/mtorchiano/effsize.

Wetzel, W. C., and S. R. Whitehead. 2020. “The Many Dimensions of Phytochemical Diversity: Linking Theory to Practice.” *Ecology Letters* 23: 16–32.

Wigley, B. J., J. A. Slingsby, S. Díaz, W. J. Bond, H. Fritz, and C. Coetsee. 2016. “Leaf Traits of African Woody Savanna Species across Climate and Soil Fertility Gradients: Evidence for Conservative Versus Acquisitive Resource-Use Strategies.” *Journal of Ecology* 104: 1357–69.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

**How to cite this article:** Massad, Tara Joy, Lora A. Richards, Casey Philbin, Lydia Fumiko Yamaguchi, Massuo J. Kato, Christopher S. Jeffrey, Celso Oliveira Jr, et al. 2022. “The Chemical Ecology of Tropical Forest Diversity: Environmental Variation, Chemical Similarity, Herbivory, and Richness.” *Ecology* 103(9): e3762. https://doi.org/10.1002/ecy.3762