Received: 11 April 2023 | Revised: 24 July 2023 | Accepted: 25 August 2023

DOI: 10.1111/ele.14308

ECOLOGY LETTERS WILEY

LETTER

Phytochemical diversity impacts herbivory in a tropical rainforest tree community

Xuezhao Wang[1,2,3] | **Yunyun He**[1,2] | **Brian E. Sedio**[4,5] | **Lu Jin**[6,7] | **Xuejun Ge**[7] | **Suphanee Glomglieng**[1,2] | **Min Cao**[1] | **Jianhong Yang**[8] | **Nathan G. Swenson**[9] | **Jie Yang**[1]

[1]Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, China
[2]University of Chinese Academy of Sciences, Beijing, China
[3]School of Ecology and Environment, Southwest Forestry University, Kunming, China
[4]Department of Integrative Biology, University of Texas at Austin, Texas, Austin, USA
[5]Smithsonian Tropical Research Institute, Ancón, Republic of Panama
[6]College of Life Sciences, South China Agricultural University, Guangzhou, China
[7]Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China
[8]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China
[9]Department of Biological Sciences, University of Notre Dame, Indiana, Notre Dame, USA

Correspondence
Jie Yang, Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming 650223, China.
Email: yangjie@xtbg.org.cn

Funding information
National Key R&D Program of China, Grant/Award Number: 2022YFF1302401; National Natural Science Foundation of China, Grant/Award Number: 31870410; NSF DEB award, Grant/Award Number: 2240430; NSF US-China Dimensions of Biodiversity grant, Grant/Award Number: DEB: 2124466; the Chinese Academy of Sciences Youth Innovation Promotion Association, Grant/Award Number: Y202080; the Distinguished Youth Scholar of Yunnan, Grant/Award Number: 202001AV070016; the NSFC China-US Dimensions of Biodiversity Grant, Grant/Award Number: DEB: 32061123003; the Ten Thousand Talent Plans for Young Top-notch Talents of Yunnan Province, Grant/Award Number: YNWR-QNBJ-2018-309

Editor: Peter H. Thrall

Abstract

Metabolomics provides an unprecedented window into diverse plant secondary metabolites that represent a potentially critical niche dimension in tropical forests underlying species coexistence. Here, we used untargeted metabolomics to evaluate chemical composition of 358 tree species and its relationship with phylogeny and variation in light environment, soil nutrients, and insect herbivore leaf damage in a tropical rainforest plot. We report no phylogenetic signal in most compound classes, indicating rapid diversification in tree metabolomes. We found that locally co-occurring species were more chemically dissimilar than random and that local chemical dispersion and metabolite diversity were associated with lower herbivory, especially that of specialist insect herbivores. Our results highlight the role of secondary metabolites in mediating plant–herbivore interactions and their potential to facilitate niche differentiation in a manner that contributes to species coexistence. Furthermore, our findings suggest that specialist herbivore pressure is an important mechanism promoting phytochemical diversity in tropical forests.

KEYWORDS
generalist herbivory, herbivore pressure, plant secondary metabolites, specialist herbivory, species diversity, tropical forest

INTRODUCTION

The complex interactions between plants and natural enemies are thought to play a central role in the maintenance of high local plant diversity in tropical forests (Connell, 1971; Janzen, 1980). Shared natural enemies mediate competitive interactions among plants (Holt, 1977). Yet species differences in

---

Xuezhao Wang and Yunyun He contributed equally to this work.

secondary metabolites and other traits can allow plants to avoid sharing herbivores (Endara et al., 2017; Salazar et al., 2018) and, therefore, promote defence-related niche differences that may play a large role in determining species abundances, composition, and diversity in tropical tree communities (Sedio & Ostling, 2013). Despite our understanding of phytochemical defences and their consequences for plant performance and herbivores (Hunter, 2016; Kessler & Kalske, 2018; Raguso et al., 2015), most previous studies have focused on one or a few clades, such as *Piper*, *Protium*, *Inga*, or *Ficus* (Endara et al., 2017; Salazar et al., 2016a, 2018; Sedio et al., 2017; Vleminckx et al., 2018; Volf et al., 2018), or on specific phytochemical classes (Aharoni et al., 2005; Hernandez et al., 2009). Thus, to date, we still know remarkably little about how metabolite diversity interacts with herbivore pressure and the consequences of these interactions for plant community structure and diversity (Dyer et al., 2018).

Recent studies have revealed that metabolite composition is a strong determinant of herbivore host use (Endara et al., 2017; Richards et al., 2015; Salazar et al., 2018), that high chemical diversity reduces specialist herbivory at the species level (Richards et al., 2015), that plant species tend to co-occur with chemically dissimilar species at fine spatial scales (Kursar et al., 2009; Salazar et al., 2016a; Vleminckx et al., 2018), that localised chemical dissimilarity reduces herbivory in some genera (Coley et al., 2018; Salazar et al., 2016a), that phylogenetic signal of metabolites varies at the community scale (Forrister et al., 2023; Sedio et al., 2018), and that community chemical diversity is associated with climatic variation over elevational gradients (Defossez et al., 2021; Volf et al., 2022, 2023) and continental geographic scales (Sedio et al., 2018, 2021). These pioneering works have enriched our understanding of the ecology of plant chemical defences. However, most of these studies have focused on single plant clades due to methodological limitations. The generality of these findings and how they are related to variation in herbivory and tree species co-occurrence at the scale of entire tree communities remain unclear. Fortunately, recent advances in metabolomics enable the rapid screening of tens of thousands of metabolites across hundreds to thousands of plant species, thus facilitating the comparison of metabolite diversity and composition on scale previously unattainable in the field of chemical community ecology (Sedio, 2017; van Dam & van der Meijden, 2011; Walker et al., 2022). This expansion of capabilities enhances our understanding of the ecological impacts stemming from plant chemical diversity.

Phylogenetic signal reflects the trait similarity of species due to common ancestry and can provide a window on the evolutionary lability of, or strength of selection acting on, a trait. On broad phylogenetic scales, secondary metabolites may be expected to exhibit phylogenetic signal (i.e. trait similarity that is higher than that expected from a Brownian model of trait evolution on a given phylogeny) for key metabolic pathways that have evolved only in one to a handful of clades (Liu et al., 2017; Vogt & Jones, 2000). For example, legumes (Fabaceae) are characterised by quinolizidine alkaloids and non-protein amino acids, whereas tropane and steroidal alkaloids prevail in the Solanaceae (Wink, 2003). That said, an extensive literature of studies on individual clades routinely reports generally rapid chemical evolution (e.g. Sedio et al., 2018; Volf et al., 2018), perhaps driven by a plant–herbivore coevolutionary arms race (Ehrlich & Raven, 1964). Additional work has shown that phylogenetic signal may vary depending on the chemical class studied (Forrister et al., 2023). For example, as alkaloids are not a biosynthetically related group, a strong phylogenetic signal may not be expected for this broad class of compounds, whereas we might expect to find phylogenetic signal in shikimates and phenylpropanoids as they are widely distributed in the plant kingdom and play vital roles in plant development by acting as essential components of the cell wall (Deng & Lu, 2017). Despite this work, we still have very little evidence demonstrating the presence or absence of phylogenetic signal in phytochemical groups across the broad phylogenetic sample that constitutes tropical tree communities.

While metabolomics has expanded our ability to conduct detailed community-level studies of phytochemical diversity, basic questions about the consequences of metabolomic variation for biotic interactions remain unanswered. How do herbivores respond to variation in phytochemical diversity and composition at the community scale? Several recent studies provide the foundation for predictions regarding the relationship between phytochemical diversity, herbivory, and herbivore specialisation. These studies demonstrate that high levels of phytochemical diversity reduce specialist herbivory (Glassmire et al., 2016; Richards et al., 2015; Whitehead et al., 2022), but not generalist herbivory (Glassmire et al., 2020; Richards et al., 2015; Salazar et al., 2016b), while local chemical dissimilarity reduces specialist herbivory (Coley et al., 2018; Massad et al., 2017; Salazar et al., 2016b) and reduces plant mortality in experimental plots with greater phytochemical diversity (Salazar & Marquis, 2022). Finally, observational studies have found that plant performance, growth, or survival increases with phytochemical dissimilarity of heterospecific neighbours in *Piper* (Salazar et al., 2016b) and *Inga* (Forrister et al., 2019). Such a relationship between phytochemical dissimilarity and herbivore-driven mortality is likely the result of specialist herbivores, as specialists are more likely to be sensitive to variation in the local density of suitable host plants with similar metabolites (Sedio & Ostling, 2013), whereas generalists may benefit from consuming a chemically diverse diet (Chambers et al., 1996). Hence, from these expectations we can derive testable predictions regarding the effect of local phytochemical diversity on herbivory by specialist and generalist herbivores. If plant metabolites mediate insect herbivore host ranges (Endara et al., 2017), and insect herbivores are sensitive to spatial variation in host-plant density (Janzen, 1970), we expect tree assemblages with diverse metabolites to experience reduced herbivory (Figure 1a). Furthermore, we expect metabolite diversity to reduce herbivore damage perpetrated by specialist insect herbivores (Figure 1b), but to increase herbivore damage due to generalist herbivores (Figure 1c).

In addition to serving as chemical defences against herbivores and pathogens, plant secondary metabolites also mediate plant responses to abiotic stress, such as drought, ultraviolet radiation (Tegelberg & Julkunen-Tiitto, 2001), and capture of abiotic resources (Vaughan et al., 2018). Plastic variation in the biosynthesis and accumulation of plant secondary metabolites occur in response to changing abiotic conditions, following the carbon–nutrient balance hypothesis (Bryant et al., 1983). Furthermore, the resource availability hypothesis describes evolutionary trade-offs between the intrinsic growth rate of plants, which is shaped by resource availability, and investment in defence (Coley et al., 1985). For example, species adapted to resource-poor environments grow intrinsically slower, invest more in constitutive defences, and experience less herbivory than species from more productive habitats (Endara & Coley, 2011). Therefore, we expect that resource availability is negatively associated with plant secondary metabolite diversity in the community (Figure 1d).

A growing literature on the abiotic and biotic drivers of phytochemical variation provides a sufficient foundation to form testable predictions, yet few studies have examined how phytochemical diversity affects herbivore damage at the community scale in tropical tree communities. Here, we used untargeted metabolomics to evaluate secondary metabolites and measured herbivory for 358 plant species in a 20-hectare (ha) forest dynamics plot in tropical rainforest at Xishuangbanna in southwest China. We used species-level metabolome and herbivory observations to calculate phytochemical diversity and dissimilarity and herbivory in local tree communities represented by 20 × 20 m quadrats within the plot and examined their relationships with light environment and soil nutrients at this spatial scale. We tested the following predictions: (i) Plant metabolites lack phylogenetic signal in diverse tropical tree communities, (ii) local tree assemblages will be composed of species that are less chemically similar than random at the quadrat scale, (iii) plant metabolite diversity will decrease with resource availability, as represented by light environment and soil nutrient content, and (iv) total and specialist herbivory will decrease, but generalist herbivory will increase, with plant metabolite diversity at the local scale.

MATERIALS AND METHODS

Study site

This study was conducted in a 20-ha seasonal tropical rainforest dynamics plot in Xishuangbanna, Yunnan Province, southwest China (21°36′42″-58″ N, 101°34′26″-47″ E; Figure S1). The plot is 400 × 500 m in which every tree ≥1 cm diameter at 1.3 m height is identified, measured, and mapped. The plot is dominated by large individuals of *Parashorea chinensis* (Dipterocarpaceae). Elevation in the plot ranges from 708.2 m to 869.1 m a.s.l,

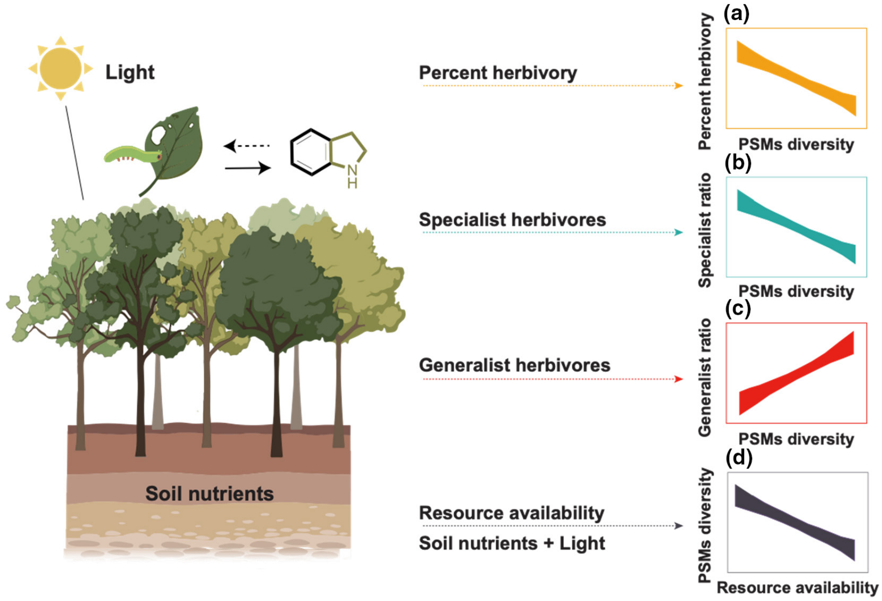

FIGURE 1 An overview of the expected relationships between plant secondary metabolite diversity (PSMs Diversity) with different factors. a: per cent herbivory; b: specialist herbivory; c: generalist herbivory; d: resource availability.

and three perennial streams intersecting and converging in the south-eastern corner contribute to habitat heterogeneity. A detailed description of the plot can be found in Cao et al. (2008). A total of 392 tree species, belonging to 196 genera and 69 families with DBH >1 cm in the plot based on the 2012 census, used in this study. The plot is demarcated into 500 20 × 20 m quadrats; we refer to trees recorded in the same quadrat as the "local-scale" community throughout.

Plant secondary metabolite sampling and analytical chemistry

Due to drought-associated mortality and species rarity, samples were collected from a total of 358 of the 392 tree species in the plot for the analysis of plant secondary metabolites (Table S1). Previous studies have found that five individuals typically represent >75% of the compounds observed in a species (Endara et al., 2022; Friberg et al., 2019; Karban et al., 2016). Therefore, for species in our plot with more than five adult individuals, five adult individuals were randomly sampled from the population inside the plot. If <5 individuals were observed in the plot, all were sampled. To capture maximum chemical diversity within each species and to avoid overlooking unique compounds, three to ten expanding, unlignified leaves were collected from different branch directions for each individual and immediately flash-frozen in liquid nitrogen.

Plant secondary metabolites were extracted and analysed from leaves using ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS), following Sedio et al. (2021) with slight modifications. The metabolomic data for all 358 tree species are publicly available as a MassIVE data set on the Global Natural Products Social (GNPS) Molecular Networking server (Wang et al., 2016) at: ftp://massive.ucsd.edu/MSV000091550/. Detailed methods on plant secondary metabolite analytical chemistry and informatics are provided in Appendix S1.

Insect herbivores

To quantify the relationship between herbivory and metabolite diversity at the local scale, we measured per cent herbivory and herbivore type for all 358 tree species (Coley & Barone, 1996). Leaf samples were collected from the same trees used for metabolite analysis. Three branches were taken from different directions, and 10 leaves per branch were sequentially selected from top to bottom on each branch to avoid overestimating the per cent herbivory and ignoring certain types of herbivores (Woodman & Fernandes, 1991). All collected leaves were scanned, and the leaf area was calculated using ImageJ software (Abramoff et al., 2004). The per cent herbivory for each leaf was calculated as the ratio of damaged area to estimated undamaged area, with higher percentages indicating greater herbivore damage (Kurokawa & Nakashizuka, 2008). We classified herbivore damage into broad categories (e.g. hole feeding and margin feeding) according to Labandeira et al. (2007) and further divided them into three diet-breadth categories: generalised, intermediate, or specialised (Labandeira et al., 2007; Wang et al., 2022). Mean per cent herbivory and diet-breadth values were calculated for each tree species. To incorporate differences in species abundance within each 20 × 20 m plot, we calculated a community-weighted mean of leaf damage (Miller et al., 2019). Finally, we calculated weighted means of per cent herbivory and diet breadth for each quadrat in the plot (Lavorel et al., 2008).

Abiotic environment

We used published data from our laboratory for 9 soil nutrient variables in the plot (Hu et al., 2012; Yang et al., 2014), including pH, total N, total P, total K, available N, extractable P, extractable K, total C, and bulk density. Light availability was measured using hemispherical photographs taken with a digital camera and analysed using the Gap Light Analyzer version 2.0 software to calculate the canopy gap fraction, representing the non-vegetated portion of the image (Frazer et al., 2000). To quantify variation in per cent herbivory and chemical diversity in different habitats, the plot was divided into slope, ridge, and valley habitats from Yang et al. (2014; Figure S1). Principal component analysis (PCA) was performed on the soil nutrient variables, with the first three principal components explaining over 85% of the variation (Table S2). The first component (PC1) was primarily influenced by total N, total P, available N, and extractable P, the second component (PC2) was associated with bulk density, and the third component (PC3) was mainly influenced by total K. Henceforth, we refer to these soil axes as "N-P" (PC1), "bulk density" (PC2), and "TK" (PC3) soil axes.

Metabolomics data and spatial pattern analysis

Compound annotation was performed using the biosynthetic "pathway"-level classifications of NPClassifier (Kim et al., 2021), which consisted of seven categories: alkaloids, amino acids and peptides, carbohydrates, fatty acids, polyketides, shikimates and phenylpropanoids, and terpenoids. These categories were further grouped into secondary metabolites (alkaloids, polyketides, shikimates and phenylpropanoids, amino acids and peptides, and terpenoids), and primary metabolites (carbohydrates and fatty acids). We followed a series of rules for describing compounds represented as products of multiple pathways. For example, if one category was a secondary metabolite category and the other was a primary metabolite category, we classified it as a secondary metabolite category. If both categories were secondary metabolite categories, we labelled it as "multiple." In total, we classified all compounds into eight pathway classifications, including "multiple." Coefficients of variation at the family and genus levels were reported for each category and for all metabolites (Table S3, 4).

Metabolite diversity for each tree species was quantified using the Shannon–Wiener diversity index: $H' = -\sum_{i=1}^{S} \left(p_i \times log_e p_i\right)$, where $P_i = a_i / \sum a_i$, a_i is the abundance of the ith metabolite, and S is the number of metabolites (Shannon & Weaver, 1949). Community-level metabolite diversity was calculated weighted mean Shannon–Wiener diversity for all metabolites and each class of metabolites, accounting for difference in species abundance (Lavorel et al., 2008; Miller et al., 2019).

To assess chemical similarity, we calculated the chemical structural and compositional similarity (CSCS) score for each pair of species, taking into account the structural similarity and ion intensity of the compound pairs, following Sedio et al. (2017). CSCS scores were calculated for all metabolites and for each of the major chemical classes. For the assessment of spatial dispersion patterns, we calculated the mean 1-CSCS value for species pairs co-occurring in each quadrat. To determine whether co-occurring species exhibited clustered or overdispersed in their chemical profiles, we calculated the net chemical similarity (NCS) as a standardised effect size of observed mean 1-CSCS compared with a null distribution obtained from 999 permutations of species assignments in the 1-CSCS matrix:

$$\text{NCS (S. E. S. CSCS)} = -\frac{\left(1-\text{CSCS}_{\text{obs}}\right)-\text{mean}\left(1-\text{CSCS}_{\text{null}}\right)}{\text{st. dev}\left(1-\text{CSCS}_{\text{null}}\right)} \quad (1)$$

where 1-CSCS_{obs} is the observed mean (1-CSCS), mean (1-$\text{CSCS}_{\text{null}}$) is the mean (1-CSCS) in the null distribution, and st.dev(1-CSCSnull) is the standard deviation of the null distribution. A positive S.E.S. value indicates that co-occurring species are more similar than expected, and a negative value indicates that co-occurring species are less similar than expected.

Phylogenetic signal tests

To quantify phylogenetic signal in metabolomic data, we first inferred the phylogenetic relationships of the tree species in our study using plastome sequence data generated by Jin et al. (2022) for this forest plot (Figure S2). Detailed methods for DNA extraction and phylogenetic tree construction are provided in Appendix S2 and Jin et al. (2022).

We assessed the phylogenetic signal of all metabolites and each class of metabolites using the plastome tree (Jin et al., 2022). We calculated Adams' (2014) K_{mult} metric, an approach based on an explicit model of multivariate trait evolution (i.e. Brownian) which accounts for the topology and branch lengths in the phylogeny. This approach differs from the traditional K metric (Blomberg et al., 2003), which is applied to a single trait, and from the Mantel tests of phylogenetic and trait distance matrices that have no explicit model of trait evolution underlying the expected relationship between phylogenetic and trait distances given a Brownian motion model (Blomberg et al., 2003; Swenson, 2014). When $K_{\text{mult}}<1$, this indicates that taxa are less chemically similar than expected by the Brownian motion evolution on the observed phylogeny, whereas $K_{\text{mult}}>1$ indicates that species are more chemically similar than expected under the Brownian motion.

Modelling soil nutrients, metabolites, and herbivory

To test our prediction that spatial variation in soil nutrients and plant metabolite diversity underpins variation in herbivory, we used a generalised linear mixed-effects model (GLMM). We fit separate models for per cent herbivory, generalist rate, and specialist rate as response variables, respectively, with terms representing metabolite diversity, soil nutrient content, and light environment as fixed independent variables. Habitat was used as a random independent variable. To identify highly correlated predictors, we calculated the variance inflation factor (VIF; Quinn & Keough, 2002). Factors exhibiting high collinearity ($\text{VIF}>10$) were subsequently removed. All variables were rescaled around their mean in the model, and a Gaussian distribution was chosen for the final models. The full model had the following form:

$$\begin{aligned}&\text{Percent herbivory OR generalised rate OR specialised rate}\\&=\beta_0+\beta_1\ \text{PSM diversity}+\beta_2\ \text{soil. pc1}+\beta_3\ \text{soil. pc2}\\&+\beta_{4.}\ \text{soil. pc3}+\beta_{5.}\ \text{light}+\gamma.\end{aligned} \quad (2)$$

where PSM diversity represents the Shannon–Wiener diversity of metabolites and β_0 is the intercept for all species. Soil pc1, Soil pc2, and Soil pc3 represent the first three axes of the PCA result of the soil nutrient content, and light represents the light environment. We utilised normally distributed random effects for habitat as γ.

All of the above analyses were performed using the R 4.2.0 (R Core Team, 2022) packages "*FD*" (Laliberté et al., 2014), *"lme4"* (Bates et al., 2014), *"lmerTest"* (Kuznetsova et al., 2017), *"MuMIn"* (Barton, 2010), "*spdep*" (Bivand, 2010), "*vegan*" (Oksanen et al., 2015), and "*picante*" (Kembel et al., 2010).

RESULTS

Chemical compound composition of tree species

We detected 25,810 compounds from 358 tree species in the tropical rainforest community. We generated a predicted molecular structure and a chemotaxonomic classification for 19,179 compounds (74.3%, Figure 2a); 6631 compounds (25.7%) were not classified. Compounds were classified into 8 categories (Figure 2b): "alkaloids," "shikimates and phenylpropanoids," "terpenoids," "fatty acids," "polyketides," "amino acids and peptides," "carbohydrates,' and "multiple." Among these 8 categories, alkaloids were the most numerous compounds at the community scale, accounting for 19.34% of metabolites, followed by shikimates and phenylpropanoids, which accounted for 19.03% of metabolites. Terpenoids were the third-most numerous class, representing approximately 15.27% of metabolites.

Phylogenetic signal of plant secondary metabolites

We quantified phylogenetic signal based on the composition and structural similarity of all metabolites and 8 classes of metabolites, which are, themselves, based on major biosynthetic pathways in plants. The metabolome considered as a whole did not exhibit significant phylogenetic signal (Table 1). Among metabolite classes, only shikimates and phenylpropanoids exhibited a K_{mult} value near the Brownian motion expectation of 1 (Table 1). The K_{mult} for every other pathway examined was much <1, indicating little-to-no phylogenetic signal in metabolomic similarity among species.

Spatial variation in chemical dissimilarity, chemical diversity, and herbivory

The dissimilarity of trees co-occurring within 20 × 20 m quadrats exhibited significantly higher chemical dissimilarity compared to random for all classified metabolites, indicating strong chemical overdispersion (classified;

TABLE 1 Phylogenetic signal of secondary metabolites using the Brownian motion evolutionary model test.

Secondary metabolites	K_{mult}
All plant secondary metabolites	0.991
Alkaloids	0.401
Amino acids and peptides	0.414
Carbohydrates	0.483
Fatty acids	0.816
Polyketides	0.410
Shikimates and phenylpropanoids	0.922
Terpenoids	0.721

Note: K_{mult}>1 indicates that species are more chemically similar than expected given a Brownian motion model of evolution on the phylogeny; $K_{\mathrm{mult}}=1$ indicates chemical similarity equal to that expected given Brownian motion; K_{mult}<1 indicates that taxa are less chemically similar than expected given Brownian motion.

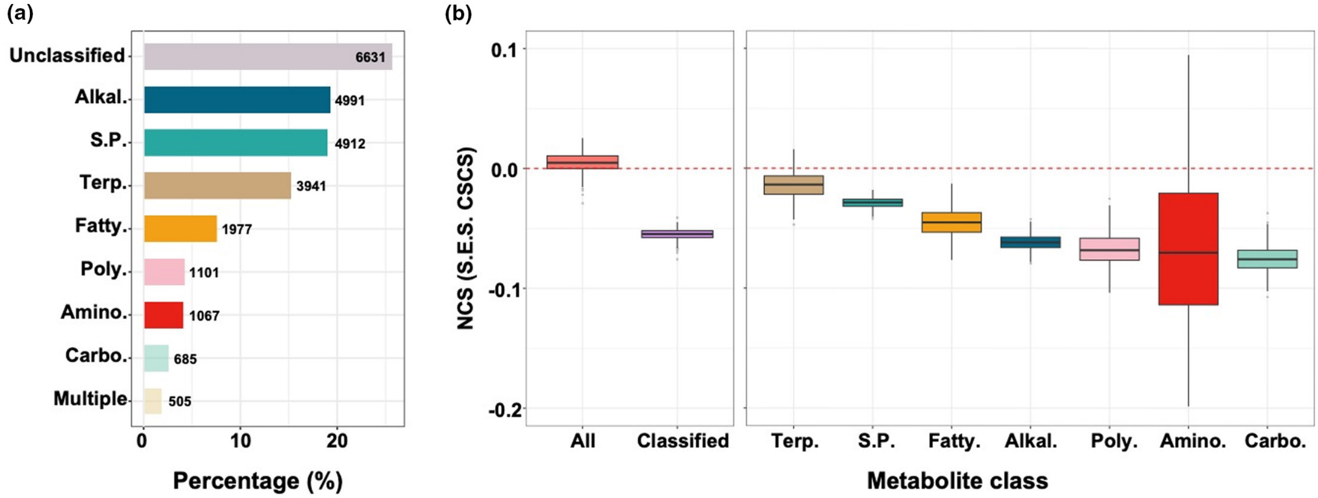

FIGURE 2 The spatial pattern of chemical compounds in the tree community at Xishuangbanna tropical seasonal forest. (a) The number of compounds found in each biosynthetic pathway; (b) Chemical similarity of trees in 20 × 20 m quadrats with respect to all metabolites and 7 major classes. *Note*: Box plots illustrate the standardised effect size (SES) of chemical dispersion calculated as difference between the observed mean pairwise chemical similarity of species in a quadrat and the expected mean pairwise chemical similarity given the plot-level species pool. Values <0 indicate that co-occurring species are less similar than random (are chemically overdispersed); values >0 indicate that co-occurring species are more similar than random (are chemically clustered). Classified represents the following: types of compounds that have been annotated, including Terp, S.P, Fatty, Alkal, Poly, Amino, and Carbo. All represent the following: classified and unclassified compounds.

Figure 2b). Moreover, this pattern of dissimilarity extended across various biosynthetic pathways, including "terpenoids," "shikimates and phenylpropanoids," "fatty acids," "alkaloids," "polyketides," "amino acids and peptides," and "carbohydrates" (Figure 2b).

The spatial variation we observed in per cent herbivory was negatively correlated with that of secondary metabolite diversity (Figure 3). The per cent herbivory in the valley was significantly higher than that in the slope and top ridge (Figure 3a,c), whereas the diversity of plant secondary metabolites in the valley habitat was significantly lower than that in the other two habitats (Figure 3b,d). Finally, we found that hole and margin herbivory ratio, and intermediate and specialist herbivory (but not generalist herbivory) were significantly higher in valleys than in the slopes (Figure S3). In brief, leaf per cent herbivory and secondary metabolite diversity exhibited incongruent variation associated with topographic habitats in the plot.

Abiotic and biotic factors driving spatial variation in herbivory

Model results indicate that both metabolite diversity and soil nutrient content had significant effects on per cent herbivory (Figure 4; Figure S4 and Table S5). Total herbivory increased with light and soil bulk density and decreased with metabolite diversity and soil N-P and TK (Figure 4). However, specialist herbivory and generalist herbivory both exhibited a strong response to metabolite diversity, but specialist herbivory decreased with metabolite diversity and generalist herbivory increased with metabolite diversity (Figure 4; Table S5). We also tested the relationship between metabolite diversity and abiotic factors. Plant metabolite diversity increased with soil N-P and TK, but decreased with soil bulk density; there was no significant relationship between metabolite diversity and light.

Total and specialist herbivory decreased with metabolite diversity measured with respect to "alkaloids," "shikimates and phenylpropanoids," and "terpenoids" (Figure S4). In contrast, total herbivory increased and generalist herbivory decreased with diversity of "polyketides." Polyketide diversity had no effect on specialist herbivory (Figure S4). In short, metabolite diversity was negatively associated with herbivory, especially specialist herbivory, and soil nutrient content had contrasting effects on metabolite diversity and herbivory.

DISCUSSION

Tropical forests are complex ecosystems, posing a challenge for ecologists to explain the remarkable diversity within them (Wiegand et al., 2007; Wills et al., 2006). Recent studies have revealed significant impacts of plant chemistry and shared herbivores on the coexistence of tree species in tropical forests (Forrister et al., 2019). In this study, we integrated untargeted metabolomics,

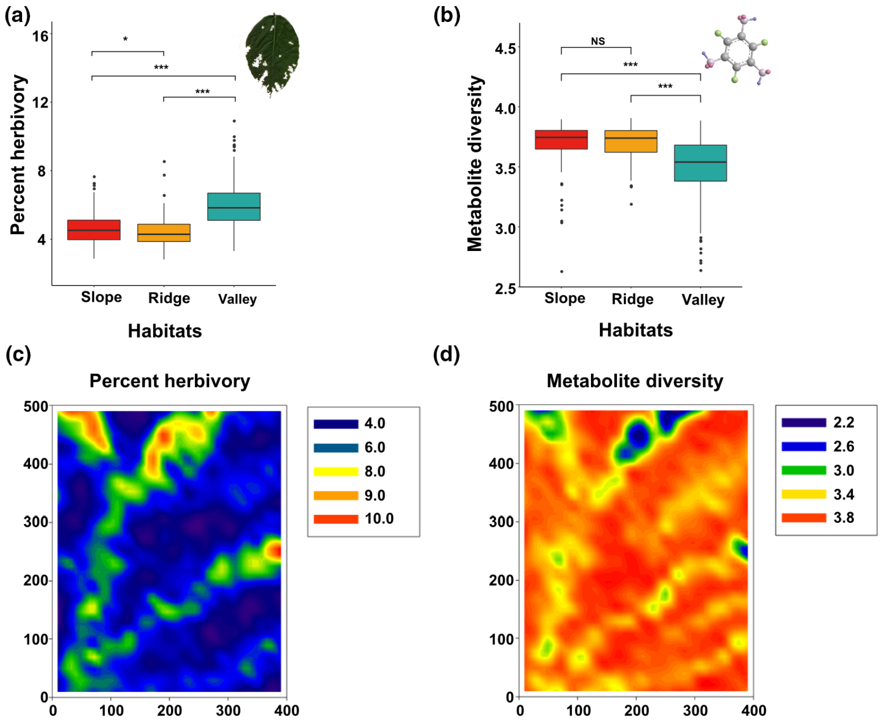

FIGURE 3 Differences in per cent herbivory and metabolite diversity in different habitats. (a) Differences in per cent herbivory in three habitat types; (b) Differences in metabolite diversity in three habitat types; (c) Spatial variation in per cent herbivory within the forest plot; (d) Spatial variation in metabolite diversity.

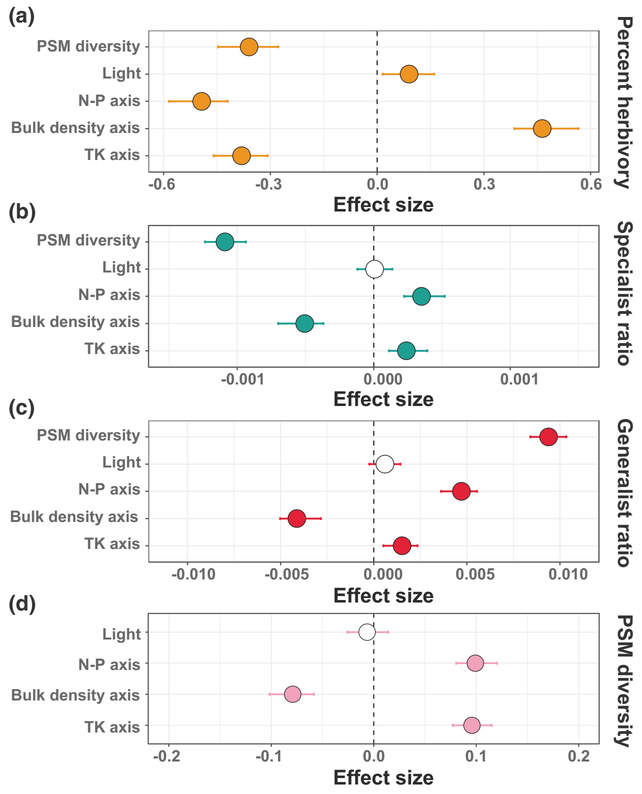

FIGURE 4 Effects of variation in the abiotic environment on metabolite diversity and effects of variation in the abiotic environment and plant metabolite diversity on herbivory. Plots illustrate the effect sizes of terms representing quadrat-level variation in metabolite diversity, light environment, and three principal components of variation in soil nutrients.

a plastome-based phylogeny, and a long-term tropical forest monitoring plot of 358 species to investigate the relationship between community phytochemical diversity and herbivore-induced leaf damage. Our results support the hypothesis that secondary metabolites represent a key component of niche. Local niche differentiation, here quantified as differentiation in metabolites, affords trees reduced herbivory, which may benefit individual performance and fitness in diverse assemblages and thereby function as a mechanism of diversity maintenance (Chesson & Kuang, 2008).

Overdispersion of metabolite diversity across phylogeny and space

Niche differentiation on local scales is believed to exert a profound influence on community assembly. Tree species distributions and co-occurrences in natural communities are the result of interactions among organisms within and between trophic levels (Seibold et al., 2018). Competition among plants for limiting resources such as light and soil nutrients can be intense, as the competitive interactions mediated by shared herbivores and pathogens (Chesson & Kuang, 2008; Holt, 1977). Hence, plants experience natural selection to diverge in their abiotic niche, defined by plant physiology and resource requirements, and their biotic niche, defined by numerous metabolites that shape the host ranges of herbivores and pathogens (Jia et al., 2018; Salazar et al., 2018). First, our results demonstrate a clear lack of phylogenetic signal in plant chemistry for the co-occurring species sampled (Table 1). Specifically, closely related species had a reduced tendency to share similar chemistry than expected by their time since a most recent common ancestor and a random walk, suggesting significant diversification in chemical profiles among co-occurring and closely related species. From a coevolutionary perspective, the divergence of secondary metabolites may potentially result from a plant–herbivore arms race (Endara et al., 2017).

Second, our findings demonstrate the locally co-occurring species are exceptionally dissimilar in their metabolomes. Specifically, we observed a strong pattern of overdispersion in both the secondary metabolites and all biosynthetic pathways (Figure 2b). These results align well with theoretical expectations derived from models of enemy-mediated competition in tropical forests (Sedio & Ostling, 2013). Such models propose that biotic interactions, including those mediated by insect herbivory, can act as pivotal niche dimensions that differentiate co-occurring species and serve as a mechanism for stabilising coexistence (Kondoh & Williams, 2001). Furthermore, our community-level findings are consistent with previous studies that have reported chemical overdispersion within specific genera in tropical communities. For instance, *Bursera* in Mexico (Becerra, 2007), *Inga* in Panama (Forrister et al., 2019; Kursar et al., 2009) and Peru (Endara et al., 2015), *Piper* in Costa Rica (Salazar et al., 2016b), and *Protium* in Peru (Vleminckx et al., 2018) have all exhibited exceptional chemical divergence, suggesting that the partitioning of niche space among natural enemies through the divergence of chemical defences may play a profoundly important role for explaining the generation and maintenance of tree diversity in tropical forests.

Metabolite diversity and herbivory

Insect herbivores and microbial pathogens with host ranges limited by plant defensive chemistry likely mediate "apparent" competition between species with shared metabolites (Holt, 1977) and select for chemical divergence among species (Becerra, 1997; Kursar et al., 2009). Our findings reveal a decline in overall herbivory as metabolite diversity increases within the community (Figure 3). This result is consistent with predictions that chemically diverse communities provide a reduced density of edible host plants to herbivores with intermediated to narrow host ranges (Sedio & Ostling, 2013) that are density-dependent (Janzen, 1970). This result may also be consistent with an effect of variation in chemical diversity at the species level, wherein species harbouring a rich array of secondary metabolites may be more likely to possess potent compounds, effectively deterring generalist herbivores (Volf et al., 2015) or synergistic combinations of compounds that make them better defended (Richards et al., 2012, 2016).

We observed a positive relationship between metabolite diversity and generalist herbivory for all metabolites and three major classes of secondary metabolites associated with defence: alkaloids, shikimates and phenylpropanoids, and terpenoids (Figure 4c; Figure S4). This result is consistent with previous studies, which found that generalist herbivores benefit from a chemically diverse diet that prevents over-exposure to any single toxin (Chambers et al., 1996; Sedio et al., 2020). In contrast, we observed a decline in specialist herbivory with greater metabolite diversity for all metabolites, alkaloids, shikimates and phenylpropanoids, and terpenoids (Figure 4; Figure S4). Although other research has indicated greater herbivore specialisation in communities with high plant diversity (Forister et al., 2015), chemically diverse plant assemblages are likely to include fewer host plants for specialist insects with narrow, chemically constricted host ranges (Ali & Agrawal, 2012; Becerra, 2007). Our study observed that more than 90% of herbivores measured in this study are generalists, while specialists comprise less than 10% (Figure S3). By attacking host plants in chemically similar assemblages (Figure 4a,b), specialist herbivores likely promote chemical diversity on the landscape (Sedio & Ostling, 2013) and link local metabolite diversity and dissimilarity to neighbouring trees to individual fitness (Forrister et al., 2019). Hence, by promoting metabolite diversity at the community scale, specialist herbivores may link diversity maintenance over ecological time (Janzen, 1970) to selection for novel metabolites and even lineage diversification over evolutionary time (Ehrlich & Raven, 1964).

Resource availability and the diversity of secondary metabolites

In addition to defence against biotic stresses imposed by natural enemies, plant secondary metabolites mediate responses to abiotic stresses, such as deficiencies in soil nitrogen and phosphate (Dixon & Paiva, 1995). In this study, we found that plant secondary metabolite diversity was positively affected by soil N-P and TK, while bulk density exhibited a negative impact on PSMs diversity at local scale (Figure 4d). Furthermore, the "pathway"-level chemical classes, such as alkaloids, shikimates and phenylpropanoids, and terpenoids, we examined also showed greater diversity in areas with higher soil N-P and TK levels, but decreased diversity with increasing bulk density (Figure S4; Table S5). Although soil carbon fertilisation did not show significant effect in the model, our findings partially support the carbon–nutrient balance hypothesis and the growth–differentiation balance hypothesis, which propose a positive relationship between the availability of carbon and nitrogen and secondary metabolite production (Lerdau et al., 1994; Lerdau & Coley, 2002). The positive correlation between soil N-P and TK levels and PSMs suggests a proportional allocation of soil nutrients to carbon-based secondary compounds, such as terpenoids, phenolic compounds, and flavonoids, in areas where there is an abundant supply of nitrogen and phosphate. For example, Ormeño and Fernandez (2012) found a positive correlation between soil N-P content and monoterpene and sesquiterpene levels in *Pinus halepensis*. Nitrogen fertilisation increased alkaloid content in *Catharanthus roseus*, while flavonoid synthesis, a component of shikimates and phenylpropanoids, was influenced by soil K availability (Chong et al., 2013; Sreevalli et al., 2004). These results challenge the resource availability hypothesis, suggesting that as trees mature, additional nutrients are allocated to secondary metabolism, potentially leading to higher carbon fixation, protein synthesis, enzyme activity, and secondary metabolite production (Litvak et al., 1996; Massad et al., 2014). These results suggest that greater nutrient availability, greater community metabolomic diversity, and the greater niche differences must be to stabilise coexistence among competitors. In addition, we found light had no significant effect on all chemical diversity (Figure 4), but significantly increased the diversity of polyketides (Figure S4). This may be due to the fact that some compounds in polyketides need to react via oxidative and photocyclisation pathways (Torres et al., 2020; Zuidema & Jones, 2005). It is thought that these reactions protect the species from damage associated with photosynthesis and that they may thus be necessary for a photosynthetic lifestyle (Zuidema & Jones, 2006).

CONCLUSION

Recent innovations in metabolomics have opened up new possibilities to understand the ecological and evolutionary importance of the diverse metabolomes of plants, even in tropical tree communities. We leveraged these innovations in untargeted metabolomics to study the foliar metabolomes of 358 tree species to evaluate the interaction between the abiotic environment, metabolite diversity, and herbivore damage due to specialist and generalist insect herbivores. Our results suggest that plant neighbourhoods with diverse secondary metabolites reduce the rate of damage by herbivores, especially specialist herbivores with limited host ranges and the potential for density-dependent impacts on plants. The role of specialist herbivores in promoting local neighbourhoods of chemically dissimilar and diverse trees suggests that chemically mediated plant–herbivore interactions represent a key component of the niche with the potential to contribute to the maintenance of tree species diversity in tropical forests.

AUTHOR CONTRIBUTIONS

J.Y and X.Z.W designed the study; X.Z.W, Y.Y.H, and B.E.S. performed the analysis; X.Z.W, S.G., M.C, L.J, X.J.G, and J.H.Y collected and measured the data; X.Z.W., J.Y, Y.Y.H, N.G.S, and B.E.S. wrote the manuscript; and all authors provided comments.

ACKNOWLEDGEMENTS

This research was supported by the National Key R&D Program of China (2022YFF1302401), the NSFC China-US Dimensions of Biodiversity Grant (DEB: 32061123003) and NSF US-China Dimensions of Biodiversity Grant (DEB: 124466), the National Natural Science Foundation of China (31870410), the Chinese Academy of Sciences Youth Innovation Promotion Association (Y202080), the Distinguished Youth Scholar of Yunnan (202001AV070016), the Ten Thousand Talent Plans for Young Top-notch Talents of Yunnan Province (YNWR-QNBJ-2018-309), the XTBG 145 program and NSF DEB award 2240430 to B.E.S. We are grateful for support from Xishuangbanna Station for Tropical Rain Forest Ecosystem Studies. Plant secondary metabolites were analysed at State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Science.

CONFLICT OF INTEREST STATEMENT

The author(s) declare that they have no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ele.14308.

DATA AVAILABILITY STATEMENT

All data supporting the main findings of this study are publicly available on Dryad: doi:10.5061/dryad.n2z34tn32.

ORCID

Brian E. Sedio https://orcid.org/0000-0002-1723-9822
Nathan G. Swenson https://orcid.org/0000-0003-3819-9767
Jie Yang https://orcid.org/0000-0002-4444-8240

REFERENCES

Abramoff, M., Magalhães, P. & Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International*, 11, 36–42.

Adams, D.C. (2014) Quantifying and comparing phylogenetic evolutionary rates for shape and other high-dimensional phenotypic data. *Systematic Biology*, 63, 166–177.

Aharoni, A., Jongsma, M.A. & Bouwmeester, H.J. (2005) Volatile science? Metabolic engineering of terpenoids in plants. *Trends in Plant Science*, 10, 594–602.

Ali, J.G. & Agrawal, A.A. (2012) Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, 17, 293–302.

Barton, K. (2010) MuMIn: multi-model inference. R package version 0.13. 17. http://CRAN.R-project.org/package=MuMIn

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2014) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Becerra, J.X. (1997) Insects on plants: macroevolutionary chemical trends in host use. *Science*, 276, 253–256.

Becerra, J.X. (2007) The impact of herbivore-plant coevolution on plant community structure. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 7483–7488.

Bivand, R. (2013) *spdep: Spatial dependence: weighting schemes, statistics and models*. R package version 0.5-58. http://CRAN.R-project.org/package=spdep

Blomberg, S.P., Garland, T., Jr. & Ives, A.R. (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57, 717–745.

Bryant, J.P., Chapin, F.S., III & Klein, D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, 40, 357–368.

Cao, M., Zhu, H., Wang, H., Lan, G., Hu, Y., Zhou, S. et al. (2008) *Xishuangbanna tropical seasonal rainforest dynamics plot: tree distribution maps, diameter tables and species documentation*. Kunming: Yunnan Science and Technology Press, pp. 1–266.

Chambers, P., Sword, G., Angel, J., Behmer, S. & Bernays, E. (1996) Foraging by generalist grasshoppers: two different strategies. *Animal Behaviour*, 52, 155–165.

Chesson, P. & Kuang, J.J. (2008) The interaction between predation and competition. *Nature*, 456, 235–238.

Chong, P., Jaafar, H. & Ibrahim, M. (2013) Relationship of chlorophyll and flavonoids content in Labisia pumila Benth. Under nitrogen and potassium fertilization. *Plant Physiology in Addressing Green Economy: Proceedings of the 23rd Malaysian Society of Plant Physiology Conference 2012 (MSPPC 2012)*, 21, 88–91.

Coley, P.D. & Barone, J. (1996) Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics*, 27, 305–335.

Coley, P.D., Bryant, J.P. & Chapin, F.S., III. (1985) Resource availability and plant antiherbivore defense. *Science*, 230, 895–899.

Coley, P.D., Endara, M.-J. & Kursar, T.A. (2018) Consequences of interspecific variation in defenses and herbivore host choice for the ecology and evolution of *Inga*, a speciose rainforest tree. *Oecologia*, 187, 361–376.

Connell, J.H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. *Dynamics of Populations*, 298, 312.

Defossez, E., Pitteloud, C., Descombes, P., Glauser, G., Allard, P.M., Walker, T.W. et al. (2021) Spatial and evolutionary predictability of phytochemical diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 118, e2013344118.

Deng, Y. & Lu, S. (2017) Biosynthesis and regulation of phenylpropanoids in plants. *Critical Reviews in Plant Sciences*, 36, 257–290.

Dixon, R.A. & Paiva, N.L. (1995) Stress-induced phenylpropanoid metabolism. *The Plant Cell*, 7, 1085.

Dyer, L.A., Philbin, C.S., Ochsenrider, K.M., Richards, L.A., Massad, T.J., Smilanich, A.M. et al. (2018) Modern approaches to study plant–insect interactions in chemical ecology. *Nature Reviews Chemistry*, 2, 50–64.

Ehrlich, P.R. & Raven, P.H. (1964) Butterflies and plants: a study in coevolution. *Evolution*, 18, 586–608.

Endara, M.J. & Coley, P.D. (2011) The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology*, 25, 389–398.

Endara, M.J., Coley, P.D., Ghabash, G., Nicholls, J.A., Dexter, K.G., Donoso, D.A. et al. (2017) Coevolutionary arms race versus host defense chase in a tropical herbivore-plant system. *Proceedings of* *the National Academy of Sciences of the United States of America*, 114, E7499–E7505.

Endara, M.J., Soule, A.J., Forrister, D.L., Dexter, K.G., Pennington, R.T., Nicholls, J.A. et al. (2022) The role of plant secondary metabolites in shaping regional and local plant community assembly. *Journal of Ecology*, 110, 34–45.

Endara, M.-J., Weinhold, A., Cox, J.E., Wiggins, N.L., Coley, P.D. & Kursar, T.A. (2015) Divergent evolution in antiherbivore defences within species complexes at a single Amazonian site. *Journal of Ecology*, 103, 1107–1118.

Forister, M.L., Novotny, V., Panorska, A.K., Baje, L., Basset, Y., Butterill, P.T. et al. (2015) The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 442–447.

Forrister, D.L., Endara, M.J., Soule, A.J., Younkin, G.C., Mills, A.G., Lokvam, J. et al. (2023) Diversity and divergence: evolution of secondary metabolism in the tropical tree genus *Inga*. *New Phytologist*, 237, 631–642.

Forrister, D.L., Endara, M.-J., Younkin, G.C., Coley, P.D. & Kursar, T.A. (2019) Herbivores as drivers of negative density dependence in tropical forest saplings. *Science*, 363, 1213–1216.

Frazer, G.W., Canham, C. & Lertzman, K. (2000) Gap light analyzer (GLA), version 2.0. *Bulletin of the Ecological Society of America*, 81, 191–197.

Friberg, M., Schwind, C., Guimarães, P.R., Jr., Raguso, R.A. & Thompson, J.N. (2019) Extreme diversification of floral volatiles within and among species of *Lithophragma* (Saxifragaceae). *Proceedings of the National Academy of Sciences of the United States of America*, 116, 4406–4415.

Glassmire, A.E., Jeffrey, C.S., Forister, M.L., Parchman, T.L., Nice, C.C., Jahner, J.P. et al. (2016) Intraspecific phytochemical variation shapes community and population structure for specialist caterpillars. *New Phytologist*, 212, 208–219.

Glassmire, A.E., Zehr, L.N. & Wetzel, W.C. (2020) Disentangling dimensions of phytochemical diversity: alpha and beta have contrasting effects on an insect herbivore. *Ecology*, 101, e03158.

Hernandez, I., Alegre, L., Van Breusegem, F. & Munne-Bosch, S. (2009) How relevant are flavonoids as antioxidants in plants? *Trends in Plant Science*, 14, 125–132.

Holt, R.D. (1977) Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology*, 12, 197–229.

Hu, Y.H., Sha, L.Q., Blanchet, F.G., Zhang, J.L., Tang, Y., Lan, G.Y. et al. (2012) Dominant species and dispersal limitation regulate tree species distributions in a 20-ha plot in Xishuangbanna, Southwest China. *Oikos*, 121, 952–960.

Hunter, M.D. (2016) The phytochemical landscape. In: *The phytochemical landscape*. New Jersey: Princeton University Press.

Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *The American Naturalist*, 104, 501–528.

Janzen, D.H. (1980) When is it coevolution. *Evolution*, 34, 611–612.

Jia, S., Wang, X., Yuan, Z., Lin, F., Ye, J., Hao, Z. et al. (2018) Global signal of top-down control of terrestrial plant communities by herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, 115, 6237–6242.

Jin, L., Liu, J.-J., Xiao, T.-W., Li, Q.-M., Lin, L.-X., Shao, X.-N. et al. (2022) Plastome-based phylogeny improves community phylogenetics of subtropical forests in China. *Molecular Ecology Resources*, 22, 319–333.

Karban, R., Wetzel, W.C., Shiojiri, K., Pezzola, E. & Blande, J.D. (2016) Geographic dialects in volatile communication between sagebrush individuals. *Ecology*, 97, 2917–2924.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D. et al. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, 26, 1463–1464.

Kessler, A. & Kalske, A. (2018) Plant secondary metabolite diversity and species interactions. *Annual Review of Ecology, Evolution, and Systematics*, 49, 115–138.

Kim, H.W., Wang, M., Leber, C.A., Nothias, L.-F., Reher, R., Kang, K.B. et al. (2021) NPClassifier: a deep neural network-based structural classification tool for natural products. *Journal of Natural Products*, 84, 2795–2807.

Kondoh, M. & Williams, I.S. (2001) Compensation behaviour by insect herbivores and natural enemies: its influence on community structure. *Oikos*, 93, 161–167.

Kurokawa, H. & Nakashizuka, T. (2008) Leaf herbivory and decomposability in a Malaysian tropical rain forest. *Ecology*, 89, 2645–2656.

Kursar, T.A., Dexter, K.G., Lokvam, J., Pennington, R.T., Richardson, J.E., Weber, M.G. et al. (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 of the United States of America*, 106, 18073–18078.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H. (2017) lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software*, 82, 1–26.

Labandeira, C.C., Wilf, P., Johnson, K.R. & Marsh, F. (2007) *Guide to insect (and other) damage types on compressed plant fossils*. Washington, DC: Smithsonian Institution, National Museum of Natural History, Department of Paleobiology.

Laliberté, E., Legendre, P., Shipley, B. & Laliberté, M.E. (2014) FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R package v. 1.0–11, http://CRAN.R-project.org/package=FD

Lavorel, S., Grigulis, K., McIntyre, S., Williams, N.S.G., Garden, D., Dorrough, J. et al. (2008) Assessing functional diversity in the field-methodology matters! *Functional Ecology*, 22, 134–147.

Lerdau, M. & Coley, P.D. (2002) Benefits of the carbon-nutrient balance hypothesis. *Oikos*, 98, 534–536.

Lerdau, M., Litvak, M. & Monson, R. (1994) Plant chemical defense: monoterpenes and the growth-differentiation balance hypothesis. *Trends in Ecology & Evolution*, 9, 58–61.

Litvak, M.E., Loreto, F., Harley, P.C., Sharkey, T.D. & Monson, R.K. (1996) The response of isoprene emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white oak trees. *Plant Cell and Environment*, 19, 549–559.

Liu, M., Wang, W.-G., Sun, H.-D. & Pu, J.-X. (2017) Diterpenoids from *Isodon* species: an update. *Natural Product Reports*, 34, 1090–1140.

Massad, T.J., de Morass, M.M., Philbin, C., Oliveira, C., Torrejon, G.C., Yamaguchi, L.F. et al. (2017) Similarity in volatile communities leads to increased herbivory and greater tropical forest diversity. *Ecology*, 98, 1750–1756.

Massad, T.J., Trumbore, S.E., Ganbat, G., Reichelt, M., Unsicker, S., Boeckler, A. et al. (2014) An optimal defense strategy for phenolic glycoside production in *Populus trichocarpa*—isotope labeling demonstrates secondary metabolite production in growing leaves. *New Phytologist*, 203, 607–619.

Miller, J.E., Damschen, E.I. & Ives, A.R. (2019) Functional traits and community composition: a comparison among community-weighted means, weighted correlations, and multilevel models. *Methods in Ecology and Evolution*, 10, 415–425.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, B. et al. (2015) Vegan: community ecology package. R Package Version *2.2-1*, 2, 1–2.

Ormeño, E. & Fernandez, C. (2012) Effect of soil nutrient on production and diversity of volatile terpenoids from plants. *Current Bioactive Compounds*, 8, 71–79.

Quinn, G.P. & Keough, M.J. (2002) *Experimental design and data analysis for biologists*. Cambridge: Cambridge university press.

R Core Team. (2022) R: a language and environment for statistical computing. https://www.R-project.org/

Raguso, R.A., Agrawal, A.A., Douglas, A.E., Jander, G., Kessler, A., Poveda, K. et al. (2015) The raison d'être of chemical ecology. *Ecology*, 96, 617–630.

Richards, L.A., Dyer, L.A., Forister, M.L., Smilanich, A.M., Dodson, C.D., Leonard, M.D. et al. (2015) Phytochemical diversity drives plant–insect community diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 10973–10978.

Richards, L.A., Glassmire, A.E., Ochsenrider, K.M., Smilanich, A.M., Dodson, C.D., Jeffrey, C.S. et al. (2016) Phytochemical diversity and synergistic effects on herbivores. *Phytochemistry Reviews*, 15, 1153–1166.

Richards, L.A., Lampert, E.C., Bowers, M.D., Dodson, C.D., Smilanich, A.M. & Dyer, L.A. (2012) Synergistic effects of iridoid glycosides on the survival, development and immune response of a specialist caterpillar, *Junonia coenia* (Nymphalidae). *Journal of Chemical Ecology*, 38, 1276–1284.

Salazar, D., Jaramillo, A. & Marquis, R.J. (2016b) The impact of plant chemical diversity on plant–herbivore interactions at the community level. *Oecologia*, 181, 1199–1208.

Salazar, D., Jaramillo, M.A. & Marquis, R.J. (2016a) Chemical similarity and local community assembly in the species rich tropical genus *piper*. *Ecology*, 97, 3176–3183.

Salazar, D., Lokvam, J., Mesones, I., Pilco, M.V., Zuniga, J.M.A., de Valpine, P. et al. (2018) Origin and maintenance of chemical diversity in a species-rich tropical tree lineage. *Nature Ecology & Evolution*, 2, 983–990.

Salazar, D. & Marquis, R.J. (2022) Testing the role of local plant chemical diversity on plant-herbivore interactions and plant species coexistence. *Ecology*, 103(9), e3765.

Sedio, B.E. (2017) Recent breakthroughs in metabolomics promise to reveal the cryptic chemical traits that mediate plant community composition, character evolution and lineage diversification. *New Phytologist*, 214, 952–958.

Sedio, B.E., Devaney, J.L., Pullen, J., Parker, G.G., Wright, S.J. & Parker, J.D. (2020) Chemical novelty facilitates herbivore resistance and biological invasions in some introduced plant species. *Ecology and Evolution*, 10, 8770–8792.

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

Sedio, B.E. & Ostling, A.M. (2013) How specialised must natural enemies be to facilitate coexistence among plants? *Ecology Letters*, 16, 995–1003.

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

Sedio, B.E., Spasojevic, M.J., Myers, J.A., Wright, S.J., Person, M.D., Chandrasekaran, H. et al. (2021) Chemical similarity of co-occurring trees decreases with precipitation and temperature in North American forests. *Frontiers in Ecology and Evolution*, 9, 679638.

Seibold, S., Cadotte, M.W., MacIvor, J.S., Thorn, S. & Müller, J. (2018) The necessity of multitrophic approaches in community ecology. *Trends in Ecology & Evolution*, 33, 754–764.

Shannon, C.E. & Weaver, W. (1949) *The mathematical theory of communication*. Urbana: University of Illinois Press, p. 96.

Sreevalli, Y., Kulkarni, R., Baskaran, K. & Chandrashekara, R. (2004) Increasing the content of leaf and root alkaloids of high alkaloid content mutants of periwinkle through nitrogen fertilization. *Industrial Crops and Products*, 19, 191–195.

Swenson, N.G. (2014) *Functional and phylogenetic ecology in R*. Berlin, Germany: Springer.

Tegelberg, R. & Julkunen-Tiitto, R. (2001) Quantitative changes in secondary metabolites of dark-leaved willow (*Salix myrsinifolia*) exposed to enhanced ultraviolet-B radiation. *Physiologia Plantarum*, 113, 541–547.

Torres, J.P., Lin, Z., Winter, J.M., Krug, P.J. & Schmidt, E.W. (2020) Animal biosynthesis of complex polyketides in a photosynthetic partnership. *Nature Communications*, 11, 2882.

van Dam, N.M. & van der Meijden, E. (2011) A role for metabolomics in plant ecology. *Annual Plant Reviews: Biology of Plant Metabolomics*, 43, 87–107.

Vaughan, M.M., Block, A., Christensen, S.A., Allen, L.H. & Schmelz, E.A. (2018) The effects of climate change associated abiotic stresses on maize phytochemical defenses. *Phytochemistry Reviews*, 17, 37–49.

Vleminckx, J., Salazar, D., Fortunel, C., Mesones, I., Davila, N., Lokvam, J. et al. (2018) Divergent secondary metabolites and habitat filtering both contribute to tree species coexistence in the Peruvian Amazon. *Frontiers in Plant Science*, 9. https://doi.org/10.3389/fpls.2018.00836

Vogt, T. & Jones, P. (2000) Glycosyltransferases in plant natural product synthesis: characterization of a supergene family. *Trends in Plant Science*, 5, 380–386.

Volf, M., Hrcek, J., Julkunen-Tiitto, R. & Novotny, V. (2015) To each its own: differential response of specialist and generalist herbivores to plant defence in willows. *Journal of Animal Ecology*, 84, 1123–1132.

Volf, M., Leong, J.V., de Lima Ferreira, P., Volfová, T., Kozel, P., Matos-Maraví, P. et al. (2023) Contrasting levels of β-diversity and underlying phylogenetic trends indicate different paths to chemical diversity in highland and lowland willow species. *Ecology Letters*, 26, 1559–1571.

Volf, M., Segar, S.T., Miller, S.E., Isua, B., Sisol, M., Aubona, G. et al. (2018) Community structure of insect herbivores is driven by conservatism, escalation and divergence of defensive traits in *Ficus*. *Ecology Letters*, 21, 83–92.

Volf, M., Volfová, T., Hörandl, E., Wagner, N.D., Luntamo, N., Salminen, J.P. et al. (2022) Abiotic stress rather than biotic interactions drives contrasting trends in chemical richness and variation in alpine willows. *Functional Ecology*, 36, 2701–2712.

Walker, T.W.N., Alexander, J.M., Allard, P.M., Baines, O., Baldy, V., Bardgett, R.D. et al. (2022) Functional traits 2.0: the power of the metabolome for ecology. *Journal of Ecology*, 110, 4–20.

Wang, M., Carver, J.J., Phelan, V.V., Sanchez, L.M., Garg, N., Peng, Y. et al. (2016) Sharing and community curation of mass spectrometry data with global natural products social molecular networking. *Nature Biotechnology*, 34, 828–837.

Wang, X.Z., Sun, S.W., Sedio, B.E., Glomglieng, S., Cao, M., Cao, K.F. et al. (2022) Niche differentiation along multiple functional-trait dimensions contributes to high local diversity of Euphorbiaceae in a tropical tree assemblage. *Journal of Ecology*, 110, 2731–2744.

Whitehead, S.R., Schneider, G.F., Dybzinski, R., Nelson, A.S., Gelambi, M., Jos, E. et al. (2022) Fruits, frugivores, and the evolution of phytochemical diversity. *Oikos*, e08332.

Wiegand, T., Gunatilleke, C.S., Gunatilleke, I.N. & Huth, A. (2007) How individual species structure diversity in tropical forests. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 19029–19033.

Wills, C., Harms, K.E., Condit, R., King, D., Thompson, J., He, F. et al. (2006) Nonrandom processes maintain diversity in tropical forests. *Science*, 311, 527–531.

Wink, M. (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry*, 64, 3–19.

Woodman, R.L. & Fernandes, G.W. (1991) Differential mechanical defense: herbivory, evapotranspiration, and leaf-hairs. *Oikos*, 60, 11–19.

Yang, J., Zhang, G.C., Ci, X.Q., Swenson, N.G., Cao, M., Sha, L.Q. et al. (2014) Functional and phylogenetic assembly in a Chinese tropical tree community across size classes, spatial scales and habitats. *Functional Ecology*, 28, 520–529.

Zuidema, D.R. & Jones, P.B. (2005) Photochemical relationships in Sacoglossan polypropionates. *Journal of Natural Products*, 68, 481–486.

Zuidema, D.R. & Jones, P.B. (2006) Triplet photosensitization in cyercene a and related pyrones. *Journal of Photochemistry and Photobiology B: Biology*, 83, 137–145.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Wang, X., He, Y., Sedio, B.E., Jin, Lu, Ge, X., Glomglieng, S. et al. (2023) Phytochemical diversity impacts herbivory in a tropical rainforest tree community. *Ecology Letters*, 26, 1898–1910. Available from: https://doi.org/10.1111/ele.14308