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ARTICLE

Testing the role of biotic interactions in shaping elevational diversity gradients: An ecological metabolomics approach

David Henderson[1] | **J. Sebastián Tello**[2] | **Leslie Cayola**[2,3] | **Alfredo F. Fuentes**[2,3] | **Belen Alvestegui**[4] | **Nathan Muchhala**[4] | **Brian E. Sedio**[5,6] | **Jonathan A. Myers**[1]

[1]Department of Biology, Washington University in St. Louis, St. Louis, Missouri, USA

[2]Center for Conservation and Sustainable Development, Missouri Botanical Garden, St. Louis, Missouri, USA

[3]Herbario Nacional de Bolivia, Universidad Mayor de San Andrés, La Paz, Bolivia

[4]Department of Biology, University of Missouri St. Louis, St. Louis, Missouri, USA

[5]Department of Integrative Biology, University of Texas at Austin, Austin, Texas, USA

[6]Smithsonian Tropical Research Institute, Panama, Panama

Correspondence
Jonathan A. Myers
Email: jamyers@wustl.edu

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Abstract

Seminal hypotheses in ecology and evolution postulate that stronger and more specialized biotic interactions contribute to higher species diversity at lower elevations and latitudes. Plant-chemical defenses mediate biotic interactions between plants and their natural enemies and provide a highly dimensional trait space in which chemically mediated niches may facilitate plant species coexistence. However, the role of chemically mediated biotic interactions in shaping plant communities remains largely untested across large-scale ecological gradients. Here, we used ecological metabolomics to quantify the chemical dissimilarity of foliar metabolomes among 473 tree species in 16 tropical tree communities along an elevational gradient in the Bolivian Andes. We predicted that tree species diversity would be higher in communities and climates where co-occurring tree species are more chemically dissimilar and exhibit faster evolution of secondary metabolites (lower chemical phylogenetic signal). Further, we predicted that these relationships should be especially pronounced for secondary metabolites known to include antiherbivore and antimicrobial defenses relative to primary metabolites. Using structural equation models, we quantified the direct effects of rarefied median chemical dissimilarity and chemical phylogenetic signal on tree species diversity, as well as the indirect effects of climate. We found that chemical dissimilarity among tree species with respect to all metabolites and secondary metabolites had positive direct effects on tree species diversity, and that climate (higher temperature and precipitation, and lower temperature seasonality) had positive indirect effects on species diversity by increasing chemical dissimilarity. In contrast,

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chemical dissimilarity of primary metabolites was unrelated to species diversity and climate. Chemical phylogenetic signal of all metabolite classes had negative direct effects on tree species diversity, indicating faster evolution of metabolites in more diverse communities. Climate had a direct effect on species diversity but did not indirectly affect diversity through chemical phylogenetic signal. Our results support the hypothesis that chemically mediated biotic interactions shape elevational diversity gradients by imposing stronger selection for chemical divergence in more diverse communities and maintaining higher chemical dissimilarity among species in warmer, wetter, and more stable climates. Our study also illustrates the promise of ecological metabolomics in the study of biogeography, community ecology, and complex species interactions in high-diversity ecosystems.

KEYWORDS
Andes mountains, biotic interactions, chemical community ecology, climate, elevational diversity gradient, environmental gradient, functional traits, metabolomics, natural enemies, plant chemical defenses, species diversity, tropical forest

INTRODUCTION

Foundational hypotheses in ecology and evolution posit that stronger and more specialized biotic interactions contribute to large-scale gradients in species diversity (Schemske et al., 2009). Wallace (1878) and Dobzhansky (1950) proposed that biotic interactions comprise a stronger selective force than the abiotic environment in the tropics. However, the mechanisms by which tropical forests may facilitate the coexistence of hundreds to thousands of tree species remain unclear (Wright, 2002). Unlike animals, which can exploit distinct resources, nearly all plants require light, water, and nutrients, so opportunities for resource-based niche differentiation are few (Hubbell, 2001). In contrast to resource-based niche axes, the nearly infinite variety of insect herbivores and microbial pathogens provides a highly multidimensional space within which plant species can carve out a distinct niche defined by the enemies they support and the enemies they avoid (Chesson & Kuang, 2008; Holt, 1977). Specialized natural enemies can maintain species-rich plant communities by attacking host plants where they are abundant, impeding their fitness relative to competitors that avoid the enemy (Bever et al., 2015; Connell, 1971; Janzen, 1970). Hence, gradients in biodiversity may be attributed to greater pressure from specialized herbivores and pathogens at lower elevations and latitudes with warmer, wetter, and more stable climates (Levi et al., 2019; Schemske et al., 2009; Terborgh, 2012).

Recent advances in ecological metabolomics offer a promising approach to understanding complex biotic interactions and the chemical ecology of plant communities across biodiversity gradients (Sedio, 2017; Sedio et al., 2021; Volf et al., 2023). Plant-chemical defenses (secondary metabolites) mediate biotic interactions and host-use relationships between plants and their natural enemies (Becerra, 1997; Kursar et al., 2009; Salazar et al., 2016). Secondary metabolites are organic molecules that mediate plant responses to abiotic or biotic stress and can function as defenses against herbivores and pathogens. In contrast, primary metabolites are involved in resource-acquisitive metabolism and essential cellular function and include compounds that are classified as carbohydrates, fatty acids, and nucleotides. Herbivores and pathogens can evolve counters to plant chemical defenses, but often at the cost of generality (Ehrlich & Raven, 1964; Schemske et al., 2009). Plant-enemy coevolution can result in host-use patterns that track plant secondary metabolites and thus will promote chemical diversity and species richness in plant communities (Sedio & Ostling, 2013) and mediate selection for chemical divergence among closely related plants (Becerra, 1997; Endara et al., 2017; Kursar et al., 2009; Salazar et al., 2016). Plant secondary metabolites have been shown to be more evolutionarily labile than other traits, as even closely related species can have very different metabolomes (Becerra, 1997; Kursar et al., 2009). At evolutionary timescales, this evolutionary lability is expected to result in less phylogenetic conservatism of secondary metabolites among species found in warmer, wetter, and more stable climates, where plant-enemy interactions are hypothesized to be stronger and more specialized (Connell, 1971; Dobzhansky, 1950; Janzen, 1970; Wallace, 1878).

Along elevational gradients, the abundance of herbivores and pathogens tends to decrease with elevation (Sam et al., 2020), while abiotic stress tends to increase with elevation (Lomolino, 2001). This can result in a trade-off (Coley et al., 1985) in which high-elevation plants may be expected to invest more in chemical defenses because compensatory regrowth of biomass lost to natural enemies is relatively more costly under unfavorable abiotic conditions and low nutrients (Defossez et al., 2018; Salgado et al., 2016). Furthermore, abiotic stress itself may select for investment in specialized secondary metabolites that mediate plant stress response or protect against damage, such as from ultraviolet light (Volf et al., 2020). Yet, unlike plant-enemy interactions that may undergo reciprocal coevolution, abiotic stress should select for convergence on shared, optimal traits (Bakhtiari et al., 2021). On the other hand, high-elevation conditions may select for unique metabolites not found in lowland plants (Defossez et al., 2021). Perhaps because of such discordant selection, some studies have found nonlinear, hump-shaped relationships between herbivory, plant secondary metabolite dissimilarity, and elevational gradients (Sam et al., 2020; Volf et al., 2020).

Despite the importance of plant chemistry in mediating community dynamics, key gaps remain in our understanding of the chemical ecology of plant communities along elevational gradients. Few studies have simultaneously examined the roles of climate, species diversity, and phylogeny in shaping plant chemical variation along elevational gradients. Prior studies have examined chemical variation at the community scale in herbaceous grassland communities (Defossez et al., 2018, 2021), but most have focused on single genera of woody plants (Sam et al., 2020; Volf et al., 2020, 2023). In addition, insights into the role of plant secondary metabolites in generating biodiversity patterns have, until recently, been limited by their overwhelming diversity and the lack of untargeted approaches to study them at macroecological scales. Here, we overcome this obstacle using recent innovations in untargeted metabolomics based on mass spectrometry (Dührkop et al., 2019; Wang et al., 2016) that enable the study of chemical ecology at the scale of species-rich ecological communities such as tropical forests (Sedio, 2017; Sedio et al., 2018).

In this study, we explored the hypothesis that stronger selection by natural enemies at lower elevations and in warmer, wetter, and more stable climates shapes gradients in species diversity and the evolution of plant secondary metabolites in tropical forests. We utilized data from a network of forest plots along an elevational gradient in the Bolivian Andes (Figure 1a,b). Using large-scale untargeted metabolomics techniques (Sedio, 2017; Sedio et al., 2021), we compared patterns of primary and secondary foliar metabolites in 473 tree species to tree species diversity (Figure 1c), climate (Figure 1d), and phylogeny along the gradient to test four predictions: (1) warmer, wetter, and less variable climates are associated with greater species dissimilarity in plant secondary metabolites; (2) species dissimilarity in plant secondary metabolites is associated with greater species diversity in communities; (3) warmer, wetter, and less variable climates are associated with more rapid evolution of plant secondary metabolites (lower phylogenetic signal); and (4) rapid evolution of plant secondary metabolites (lower phylogenetic signal) is associated with greater species diversity in communities. Evidence in favor of these predictions would support the hypothesis that variation in the strength of selection for interspecific divergence in secondary metabolites associated with climatic gradients contributes to the widespread elevational diversity gradient in trees (Figure 1e).

METHODS

Forest plot data were collected as part of the Madidi Project, a large-scale survey of the flora of the Madidi region in the central Andes Mountains of Bolivia (Figure 1a; Tello et al., 2015). We selected a subset of 16 1-ha permanent plots in which leaves were sampled in 2010 for chemical analyses and dried in silica gel. The 16 plots span an elevational gradient from 662 to 3324 m (elevational range: 2662 m) and include three seasonally dry, low-elevation forest plots (elevational range: 218 m) and 13 moist, montane forest plots (elevational range: 2270 m) (Figure 1b; Appendix S1: Table S1). Tree species diversity exhibits the typical negative relationship with elevation among the 13 moist forest plots, whereas the three seasonally dry forest plots exhibit a unique pattern of low species richness at low elevations (Figure 1c). In contrast, tree species diversity increases toward warmer, wetter, and less variable climates along the elevational gradient (Figure 1d). By including plots that encompassed a range of elevations and climates, our study design allowed us to disentangle the effects of climate from elevation per se in driving the chemical composition and species diversity of communities (Predictions 1 & 3; Figure 1e). Within each plot, all woody plants (hereafter trees) with a diameter at breast height of at least 10 cm were mapped, measured, and identified to a valid species or morphospecies.

Analytical chemistry, untargeted metabolomics, and chemical similarity among species

Across the 16 forest plots, we collected 3506 total leaf samples from 473 tree species, including 906 unique

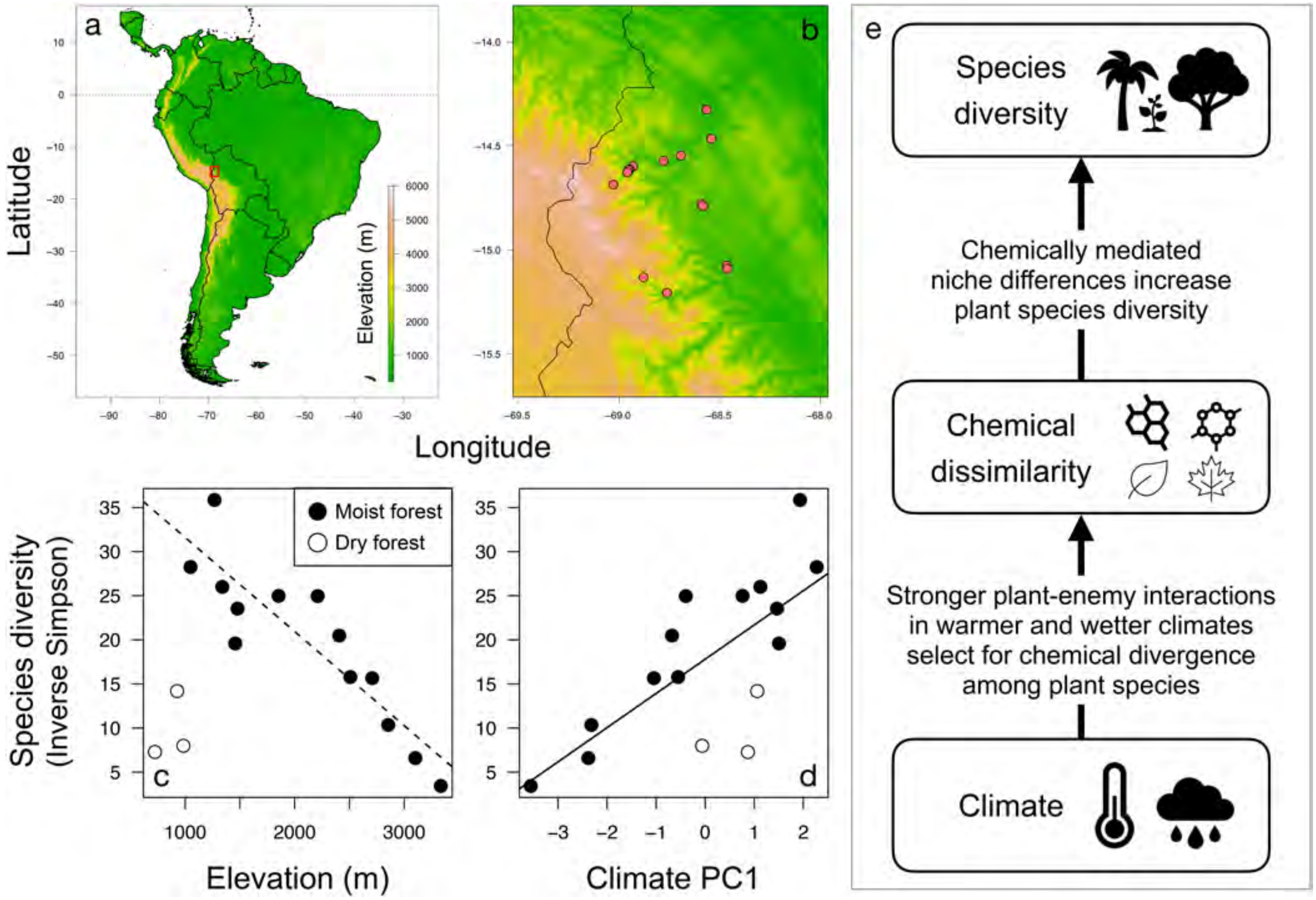

FIGURE 1 Overview of 1-ha forest plots used to test effects of climate and chemical dissimilarity on tree species diversity. (a) Location of study region in northwest Bolivia. (b) Distribution of plots along the eastern slopes of the Andes Mountains (662–3324 m) in and around the Madidi region. (c) Relationship between tree species diversity (inverse Simpson's index) and elevation. (d) Relationship between tree species diversity (inverse Simpson's index) and climate (PC1: precipitation and temperature). The dashed line in (c) shows a linear regression excluding the three seasonally dry forest plots (white circles) ($df = 11$, $p < 0.0001$, adjusted $R^2 = 0.77$). The solid line in (d) shows a linear regression including all 16 forest plots ($df = 14$, $p = 0.0019$, adjusted $R^2 = 0.47$). Elevation data (color scale bar) from WorldClim (www.worldclim.org). (e) Overview of the hypothesis linking climate and chemical dissimilarity to tree species diversity, illustrated with the meta model for the piecewise structural equation model (SEM) used to test Prediction 1 (bottom arrow: indirect effect of climate on species diversity through chemical dissimilarity) and Prediction 2 (top arrow: direct effect of chemical dissimilarity on species diversity). (e) created by J. Myers. PC, principal component.

species-by-plot samples for metabolomics analyses. We collected leaf samples from 62% to 90% of the tree species in each plot (Appendix S1: Table S1) and up to five individual trees per species in each plot. A total of 217 tree species occurred in more than one forest plot, and 29 tree species occurred in five or more forest plots. The total number of leaf samples ranged from 1 to 55 per tree species (mean ± 1 SD: 7.3 ± 6.5 samples per species).

Our goal was to quantify interspecific variation in foliar metabolites in each forest plot using individuals sampled in the same forest plot. Hence, for each forest plot, we pooled up to five individuals per species per plot to create 906 extract pools representing each unique species-by-plot for subsequent analysis. This approach accounts for population-level chemical variation within species that occur in multiple plots. While our study focused on interspecific variation in foliar metabolites, we include figures in Appendix S1: Figures S1 and S2 to illustrate intraspecific variation among individuals and species for two species-rich, high-elevation genera (*Ilex* [Aquifoliaceae] and *Weinmannia* [Cunoniaceae]) and two species-rich, low-elevation genera (*Inga* [Fabaceae] and *Nectandra* [Lauraceae]).

Leaf samples were extracted for chemical analyses following the methods detailed in Sedio et al. (2021). We analyzed filtered extract pools using ultra-high-performance liquid chromatography–tandem mass spectrometry. Raw LC–MS data were centroided using MZmine2 (Pluskal et al., 2010). Aligned chromatograms were used to create a "feature-based molecular network"

(FBMN; Nothias et al., 2020) using GNPS (Wang et al., 2016). The resulting network was used to create a dendrogram in which the structural similarities of all metabolites were reflected in one phylogeny-like dendrogram using Qemistree (Tripathi et al., 2021; Appendix S1: Figure S3). Metabolites were annotated by predicting molecular formulae using Sirius (Dührkop et al., 2019), predicting molecular structures using CSI:FingerID (Dührkop et al., 2015), and classifying compounds chemically using NPClassifier (Kim et al., 2021). We used the "pathway"-level classifications of NPClassifier to group metabolites into primary and secondary metabolite categories (Appendix S1: Table S2). Our classification scheme was based on the broad likelihood of a metabolite being associated with antiherbivore or antimicrobial defense.

Sedio et al. (2017) developed a metric that quantifies chemical structural-compositional similarity (CSCS) over all compounds among species pairs. Conventional distance or similarity indices such as Bray–Curtis incorporate shared compounds but ignore the structural similarity of unique compounds, and hence underestimate the similarity of species with distinct but very structurally similar, and perhaps functionally redundant, metabolites (Sedio et al., 2017). For each pair of the 906 species-by-plot samples, we calculated CSCS for three chemical classes: (1) the whole metabolome (all metabolites); (2) secondary metabolites; and (3) primary metabolites. We transformed CSCS matrices into dissimilarity matrices by calculating 1-CSCS. Lastly, we calculated the abundance-weighted median 1-CSCS for each of the 16 forest plots.

To disentangle chemical dissimilarity from the effect of species richness per se, we carried out rarefaction based on 12 species, the number of tree species sampled for chemical analyses in the most species-poor plot (Appendix S1: Table S1). For each forest plot, we calculated rarefied 1-CSCS by taking a random sample of 12 species and calculating their median chemical dissimilarity. We repeated this procedure 1000 times for each plot and used the mean of the distribution to represent the rarefied median chemical dissimilarity of species in each forest plot. Observed and rarefied median CSCS values were highly correlated among forest plots (Pearson $r = 0.96$ and 0.98 for secondary and primary metabolites, respectively) and we obtained qualitatively similar results using both metrics. For simplicity, we present results for rarefied 1-CSCS (hereafter rarefied chemical dissimilarity).

Climate data

We selected four variables to represent variation in climate along the elevational gradient. Temperature variables included annual mean temperature and temperature annual range obtained from WorldClim Version 2.1 (Fick & Hijmans, 2017). Precipitation variables included annual precipitation and precipitation seasonality, which is calculated as the ratio between the SD and the mean precipitation of each month. Precipitation data were obtained from the Tropical Rainfall Measuring Mission (TRMM; Huffman et al., 2023), a regional database that provides greater accuracy compared with WorldClim data in the Madidi region. To reduce the dimensionality of the climate data, we performed a principal components analysis (PCA) on standardized (centered and scaled) values of the four climate variables and used the first principal component of climatic variation (Climate PC1) in the following analyses. Climate PC1 explained 71.2% of the variation among the 16 forest plots and primarily represents annual temperature, precipitation, and temperature range (Appendix S1: Figure S4).

Chemical phylogenetic signal

To quantify the phylogenetic signal of metabolites, we constructed a phylogenetic tree using the R V.Phylomaker package (Jin & Qian, 2019). The tree was generated from all 50 of the Madidi permanent plots and had 1123 species as tips. The tree was then rooted and transformed into a distance matrix using the cophenetic function in R (R Core Team, 2024), to be directly comparable to the chemical distance matrices. The tree was pruned to include only the species recorded in the 16 plots for all analyses, which included 892 species-by-plot combinations.

For each plot, we calculated Adams' (2014) K_{mult} metric of phylogenetic signal for multivariate trait data. This technique compares a Brownian motion model of evolution in multivariate trait space to the observed trait data, accounting for the topology and branch lengths of the phylogeny. When $K_{\mathrm{mult}} < 1$, taxa are less chemically similar to one another than expected by Brownian motion evolution on the observed phylogeny, whereas $K_{\mathrm{mult}} > 1$ indicates that species are more chemically similar to each other than expected. The K_{mult} test is an improvement over the Mantel test, which does not consider an explicit model of trait evolution underlying the expected relationship between phylogenetic and trait distance (Swenson, 2014).

Hypothesis testing

We tested our predictions using piecewise structural equation models (SEMs). First, we calculated tree species diversity in each forest plot as the inverse Simpson's index using the vegan package in R (Oksanen et al., 2024). This index provides a scale-independent measure of diversity that is insensitive to differences in numbers of individuals (Chase et al., 2018). Second, we used piecewise SEMs to quantify the indirect effect of climate on tree species diversity through its effect on rarefied chemical dissimilarity (Prediction 1) and the direct effect of rarefied chemical dissimilarity on tree species diversity (Prediction 2) (Figure 1e). Third, we used separate piecewise SEMs to quantify the indirect effect of climate on tree species diversity through its effect on chemical phylogenetic signal (Prediction 3) and the direct effect of chemical phylogenetic signal on tree species diversity (Prediction 4). We fitted separate piecewise SEMs for each group of metabolites (all metabolites, secondary metabolites, primary metabolites) using the R piecewiseSEM package version 2.3.0 (Lefcheck, 2016), where each forest plot is a replicate ($n = 16$). Therefore, we fitted six SEMs: one for each combination of chemistry variable (rarefied chemical dissimilarity & chemical phylogenetic signal) and group of metabolites. We inspected whether bivariate relationships in each SEM were approximately linear by plotting regressions for each path (e.g., Appendix S1: Figures S5 and S6) and assessed normality of residuals using Shapiro–Wilk tests. Fourth, we evaluated the goodness-of-fit of each SEM by comparing our initial models (two paths) to a saturated model (three paths, including the direct effect of climate on tree species diversity) using Fisher's C (Lefcheck, 2016). Four of the six initial models were inconsistent with the data (i.e., poor model fit, Fisher's $C = 11.9–14.8$, $p \leq 0.002$, $\Delta\text{AIC} = 9.6–13.0$; Appendix S1: Table S3). For these models, we included the third path testing the direct effect of climate on tree species diversity. Last, we extracted the standardized path coefficients from each SEM that represent the relative importance of the direct effects of rarefied chemical dissimilarity and chemical phylogenetic signal on species diversity, the direct effects of climate on species diversity, and the indirect effects of climate through its effect on chemistry.

RESULTS

Across the 906 unique species-by-plot samples, we identified a total of 20,571 unique metabolites. Of the metabolites classified to a biosynthetic pathway, 94% were classified as secondary metabolites, and 6% were classified as primary metabolites (Appendix S1: Table S2). Of the secondary metabolites, 36% were terpenoids, 32% were shikimates and phenylpropanoids, 23% were alkaloids, 3% were polyketides, 2% were amino acids and peptides, and <1% were derived from more than one secondary metabolite pathway. The overall composition of the metabolome varied among species, within the same species in different forest plots, and among species within species-rich genera at high (*Ilex* and *Weinmannia*) and low (*Inga* and *Nectandra*) elevations (Appendix S1: Figures S1 and S2).

Predictions 1 and 2: Chemical dissimilarity among co-occurring tree species

For all metabolites and secondary metabolites, rarefied chemical dissimilarity had positive direct effects on tree species diversity (Figure 2a,b). Climate PC1 also had positive indirect effects on tree species diversity through its effects on rarefied chemical dissimilarity. The strength of these relationships was similar for all metabolites (Figure 2a; standardized [std.] coefficients $= 0.68$ and 0.70, $\text{df} = 14$, $p = 0.003$ and 0.002 for direct effect of chemical dissimilarity and indirect effect of climate, respectively) and secondary metabolites (Figure 2b; std. coefficients $= 0.71$ and 0.63, $\text{df} = 14$, $p = 0.001$ and 0.008, respectively) (Appendix S1: Table S4).

In contrast to all metabolites and secondary metabolites, rarefied chemical dissimilarity with respect to primary metabolites was unrelated to tree species diversity and Climate PC1 (Figure 2c). In the SEM for primary metabolites, Climate PC1 had a positive direct effect on tree species diversity (std. coefficient $= 0.71$, $\text{df} = 13$, $p = 0.002$) but no significant indirect effect on species diversity through its effect on rarefied chemical dissimilarity (std. coefficient $= 0.01$, $\text{df} = 14$, $p = 0.969$) (Appendix S1: Table S4).

Predictions 3 and 4: Chemical phylogenetic signal

Chemical phylogenetic signal was low for all secondary and primary metabolites, as none of the plots approached the Brownian motion expectation for any of the three metabolite classes (Appendix S1: Table S5). Chemical phylogenetic signal appeared greatest for low-elevation, low-diversity seasonally dry forests and the highest elevation, low-diversity moist montane forests along the gradient (Appendix S1: Table S5).

Chemical phylogenetic signal of all three metabolite classes had direct effects on tree species diversity (Figure 3; Appendix S1: Table S6), indicating faster evolution of metabolites in more diverse communities. In the SEM for all three metabolite classes, Climate PC1 had positive direct effects on tree species diversity but did not

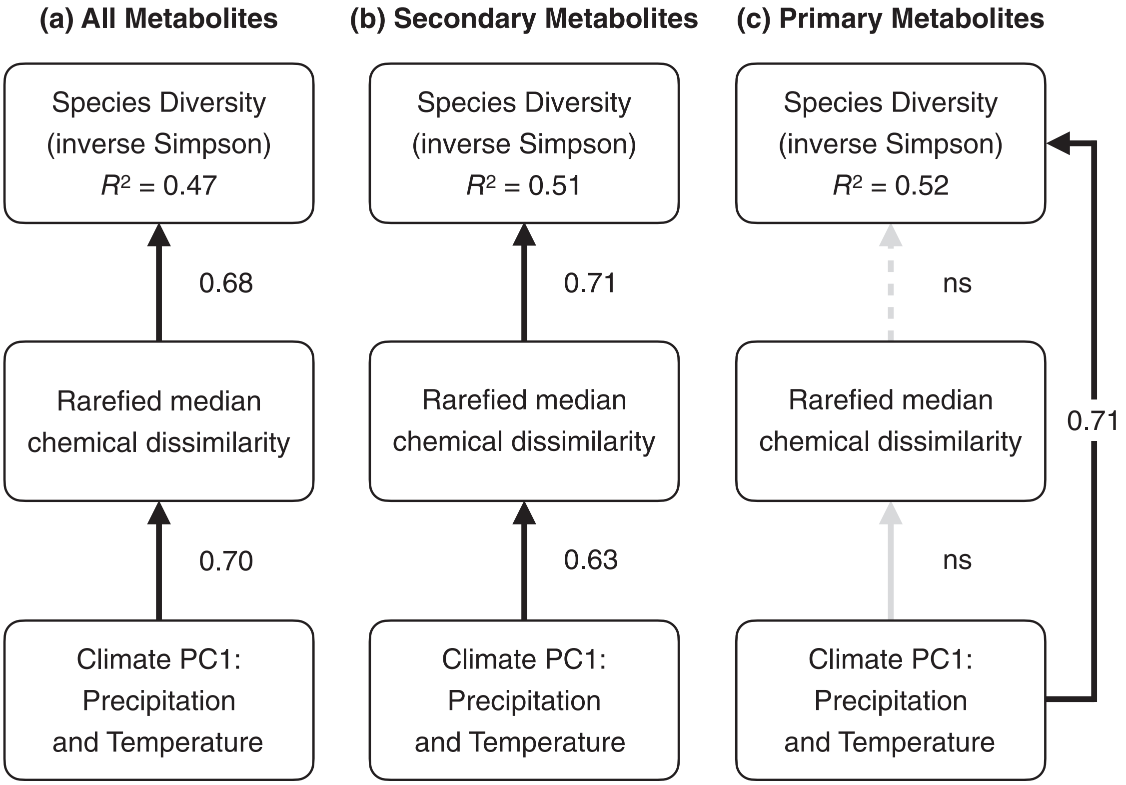

FIGURE 2 Piecewise structural equation models (SEMs) showing the effects of rarefied chemical dissimilarity (1 − chemical structural-compositional similarity [CSCS] index) and climate (PC1: precipitation and temperature) on tree species diversity (inverse Simpson's index) in 16 forest plots along an elevational gradient in the Bolivian Andes. A separate SEM was fit for each of three groups of metabolites: (a) all metabolites (whole metabolome including primary and secondary metabolites); (b) secondary metabolites; and (c) primary metabolites. Secondary metabolites are defined as those derived from the alkaloids, amino acid and peptides, polyketides, shikimates and phenylpropanoids, and terpenoids biosynthetic pathways. Primary metabolites are defined as those derived from the carbohydrates and fatty acids pathways. Each SEM tested the direct effect of chemical dissimilarity on tree species diversity (top arrow) and the indirect effect of climate on tree species diversity through chemical dissimilarity (bottom arrow). Black arrows represent positive effects, and gray arrows represent nonsignificant (NS) effects ($p > 0.5$). Effects are relative (standardized) path coefficients. R^2 is the total amount of variation in tree species diversity explained by chemical dissimilarity and climate. Goodness-of-fit tests, unstandardized effects, SEs, and significance for each path are provided in Appendix S1: Tables S3 and S4. PC, principal component.

indirectly affect tree species diversity through chemical phylogenetic signal. In addition, the direct effect of climate was stronger than the direct effect of chemical phylogenetic signal, and the strength of both of these relationships was similar for all metabolites (Figure 3a; std. coefficients = −0.45 and 0.63, df = 13, $p = 0.010$ and 0.001 for direct effects of phylogenetic signal and climate, respectively), secondary metabolites (Figure 3b; std. coefficients = −0.51 and 0.62, df = 13, $p = 0.002$ and 0.0006, respectively), and primary metabolites (Figure 3c; std. coefficients = −0.47 and 0.63, df = 13, $p = 0.006$ and 0.0008, respectively) (Appendix S1: Table S6).

DISCUSSION

Elevational diversity gradients are a striking feature of our planet and have inspired the development of ideas in ecology for centuries (Lomolino, 2001; Rahbek, 2005; von Humboldt & Bonpland, 2010). Classical hypotheses posit that large-scale diversity gradients are shaped by geographic variation in the relative strength and nature of selection imposed by the abiotic and biotic environment (Dobzhansky, 1950; Lim et al., 2015; Schemske et al., 2008; Wallace, 1878). In turn, these processes are predicted to create systematic differences in the interspecific dissimilarity and phylogenetic signal of secondary metabolite profiles among co-occurring plant species along ecological gradients. In this study, we tested these predictions using 16 tropical forest plots that span a wide range of variation in elevation, species diversity, and climate within a regional biodiversity hotspot in the central Andes Mountains. Our results broadly support three of four specific predictions concerning relationships between chemical dissimilarity, chemical phylogenetic signal, and gradients in species diversity and climate.

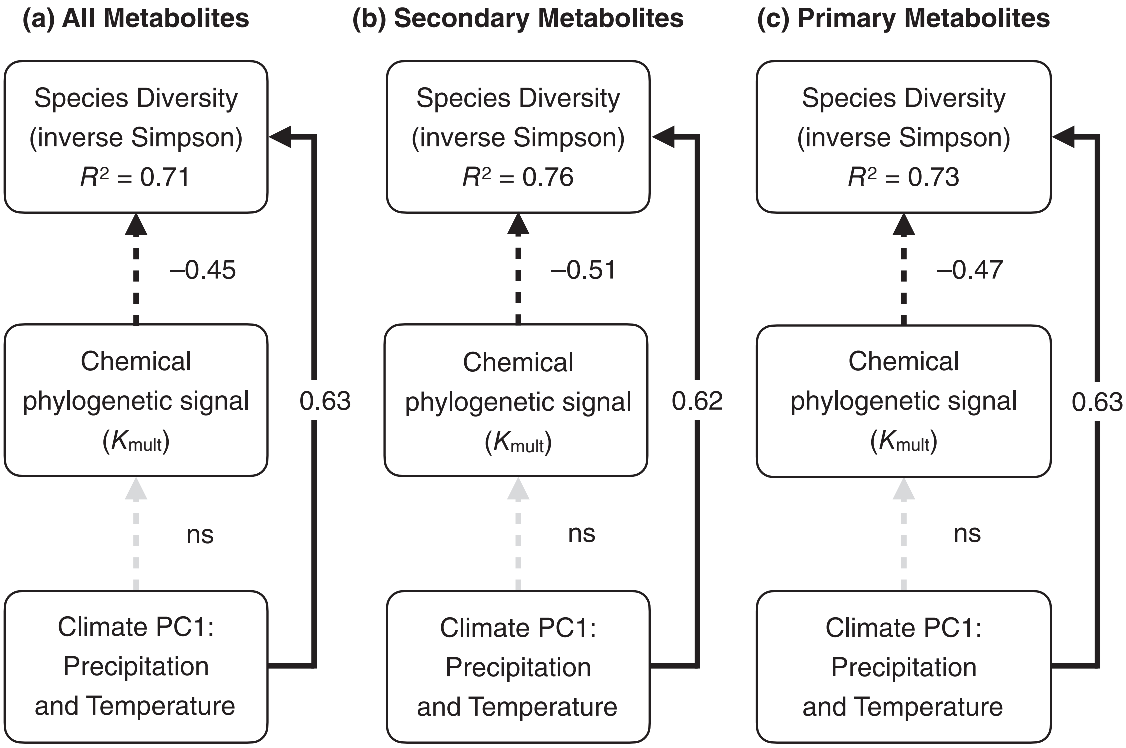

FIGURE 3 Piecewise structural equation models (SEMs) showing the effects of tree chemical phylogenetic signal (K_{mult}) and climate (PC1: precipitation and temperature) on tree species diversity (inverse Simpson's index) for (a) all metabolites (whole metabolome including primary and secondary metabolites), (b) secondary metabolites, and (c) primary metabolites. Secondary and primary metabolites are defined in Figure 2. Each SEM tested the direct effect of chemical phylogenetic signal on tree species diversity (top arrow), the indirect effect of climate on tree species diversity through chemical phylogenetic signal (bottom arrow), and the direct effect of climate on tree species diversity (right arrow). Black solid arrows represent positive effects, black dashed arrows represent negative effects, and gray arrows represent nonsignificant (NS) effects. Effects are relative (standardized) path coefficients. R^2 is the total amount of variation in tree species diversity explained by chemical phylogenetic signal and climate. Goodness-of-fit tests, unstandardized effects, SEs, and significance for each path are provided in Appendix S1: Tables S3 and S6. PC, principal component.

Communities in more benign climates are composed of more chemically dissimilar species

We predicted that warmer, wetter, and less variable climates would exhibit greater chemical dissimilarity of co-occurring species (Prediction 1), based on the hypothesis that chemically mediated plant-enemy coevolution that selects for chemical divergence among plants plays a greater role in these abiotically benign climates (Dobzhansky, 1950; Wallace, 1878). This prediction was supported for the whole metabolome (Figure 2a) and secondary metabolites (Figure 2b). Recent studies of metabolomic variation along elevational gradients have considered single genera of woody plants, such as *Ficus* in Papua New Guinea (Volf et al., 2020) and *Salix* in Europe (Volf et al., 2023), and herbaceous plant communities in Europe (Defossez et al., 2018, 2021). However, none of these studies have examined the chemical dissimilarity of co-occurring species at the level of entire tropical tree communities. Volf et al. (2023) found that low-elevation *Salix* were more dissimilar with respect to salicinoids, an important class of phenolic chemical defenses, a result consistent with our finding for secondary metabolites (Figure 2b). In contrast, we found that the chemical dissimilarity of primary metabolites was unrelated to climate (Figure 2c), suggesting that tree communities are more constrained by temperature and precipitation with respect to primary metabolites.

High-diversity communities are composed of more chemically dissimilar species

We predicted a positive direct effect of chemical dissimilarity on tree species diversity (Prediction 2), based on the hypothesis that species diversity is increased by antagonistic biotic interactions that select for chemical divergence among species (Dobzhansky, 1950; Ehrlich & Raven, 1964), reduce natural-enemy overlap among species (Becerra, 1997; Endara et al., 2017), and promote competitive coexistence (Sedio & Ostling, 2013). This prediction was supported for the whole metabolome (Figure 2a) and secondary metabolites (Figure 2b). Furthermore, the best fit SEM models indicated that warmer, wetter, and less variable climates enhance tree species diversity indirectly by increasing dissimilarity of plant secondary metabolites, rather than through a direct effect on species diversity (Figure 2a,b). These results are consistent with hypotheses that attribute variation in species diversity to variation in the strength of mechanisms that promote chemical divergence among species, such as pressure from relatively host-specific but oligophagous herbivores and pathogens (Lim et al., 2015; Schemske et al., 2009). Furthermore, species diversity was unrelated to dissimilarity of primary metabolites (Figure 2c). This contrast suggests that species differences in secondary metabolites—including alkaloids, phenolics, polyketides, terpenoids, and nonprotein amino acids that can function as antiherbivore and/or antimicrobial defenses—contribute to the diversity gradient.

Previous studies have shown that co-occurring species are less chemically similar than expected by chance. This result has been reported for numerous species-rich tree and shrub genera in the lowland Neotropics (Becerra, 2007; Kursar et al., 2009; Salazar et al., 2016). Similar patterns have been found in *Ficus* in Papua New Guinea (Volf et al., 2018) and Euphorbiaceae (principally *Macaranga*) in China (Wang et al., 2022). In addition to studies focused on single lineages, community-wide studies have found that plants that co-occur within meters are more chemically dissimilar than expected from a community-wide sample (Wang et al., 2023) and that the chemical dissimilarity of co-occurring species tends to increase from temperate to tropical latitudes (Sedio et al., 2018) and with temperature and precipitation within the temperate zone (Sedio et al., 2021). Our study contrasts with these previous studies focused on single lineages and comparative studies focused on variation among whole plant communities at continental scales (Sedio et al., 2018; Sedio et al., 2021) in that we focused on variation along a local elevational gradient within the same biogeographic region. Nevertheless, our results are consistent with the widely observed chemical dissimilarity of tree species in low-elevation tropical forests. However, it is worth noting that Sedio et al. (2018, 2021) found differences in both the diversity of metabolites and in the chemical dissimilarity of species among geographically distant plant communities with very different biogeographic histories and little possibility for dispersal over ecological timescales. Our findings suggest that variation in temperature, precipitation, and seasonality over distances of kilometers may generate variation in community chemical dissimilarity comparable to that of climatic gradients on a continental scale, and hence that the underlying mechanisms that link climate to chemical evolution and competitive coexistence are likely general and operate over a wide range of spatial scales.

Chemical divergence among closely related species is greater in high-diversity communities

Chemical phylogenetic signal was much lower than expected based on a model of Brownian motion without selection, even in high-elevation plots with comparatively higher phylogenetic signal than wetter, low-elevation plots (Appendix S1: Table S5). Plants do exhibit phylogenetic signal with respect to broad chemical classes that tend to occur in certain plant families or genera (e.g., quinolizidine alkaloids in some lineages of legumes; Wink, 2003). However, our result is consistent with other recent studies that have concluded that plant metabolite composition can be highly evolutionarily labile, especially in tropical climates when phylogenetic signal is measured among confamilial species (Becerra, 1997; Kursar et al., 2009; Salazar et al., 2018; Volf et al., 2018; Wang et al., 2022), which can degrade phylogenetic signal when measured in the context of a species-rich forest community characterized by many co-occurring congeneric and confamilial species (Sedio et al., 2018, 2021; Wang et al., 2023).

We predicted a negative effect of warmer, wetter, and less variable climates on chemical phylogenetic signal (Prediction 3) and a negative effect of chemical phylogenetic signal on tree species diversity (Prediction 4) based on the hypothesis that selection by natural enemies for chemical divergence among closely related species is relatively stronger in such environments (Sedio et al., 2018). Our results supported Prediction 4 for all three metabolite classes but did not support Prediction 3 for any metabolite class (Figure 3). Instead, Climate PC1 had positive direct effects on tree species diversity. These patterns could be explained by several processes. First, the low chemical phylogenetic signal observed in most plots (Appendix S1: Table S5) could indicate a strong but similar role for biotic interactions as an agent of natural selection and chemical divergence in most climates along this tropical elevational gradient. Second, the agents or strength of selection could differ between wet and seasonally dry forests in a way that is difficult to distinguish with the small number of seasonally dry forests represented in our metabolomics dataset. Third, the climatic data used in our PCA may not completely represent the climates that individual trees experience in these forests, especially the seasonally dry forests. Climate can vary significantly over finer spatial scales in the topographically heterogeneous Andes and their foothills compared with the variation captured by remote sensing, and other climatic variables may better capture major sources of abiotic stress in dry forests, such as short periods of intense drought or strong interannual variation in drought. More broadly, our results highlight the importance of considering both direct effects of climate and indirect effects of climate on chemically mediated biotic interactions in shaping patterns of species diversity.

In conclusion, our results support the hypothesis that chemically mediated biotic interactions shape elevational diversity gradients by imposing stronger selection for interspecific divergence in plant chemical defenses in more diverse communities and maintaining higher chemical dissimilarity among plant species in warmer, wetter, and less variable climates. Abiotic stress associated with high elevations may select for convergent secondary metabolite evolution distinct from that imposed by biotic stressors (Defossez et al., 2021; Volf et al., 2020, 2022, 2023), but antagonistic interactions among plants and their shared herbivores and pathogens are expected to select for chemical divergence. Our results suggest that the strength of this mechanism varies in a manner that affects character evolution and plant species diversity. Our study also illustrates the promise of ecological metabolomics in the study of biogeography, community ecology, and complex species interactions in high-diversity ecosystems.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data for each forest plot in Appendix S1: Table S1 can be accessed via the Madidi Project Plot Search page in Tropicos, the botanical database of the Missouri Botanical Garden, at http://tropicos.org/PlotSearch.aspx?projectid=20. The metabolomics data (Henderson et al., 2022) are available in the Mass Spectrometry Interactive Virtual Environment (MassIVE) database in MassIVE MSV000090549 at https://doi.org/10.25345/C52R3P21H.

ORCID

David Henderson https://orcid.org/0000-0003-3523-0175
J. Sebastián Tello https://orcid.org/0000-0003-2539-6796
Leslie Cayola https://orcid.org/0009-0006-8543-1994
Alfredo F. Fuentes https://orcid.org/0000-0003-4848-4182
Belen Alvestegui https://orcid.org/0000-0001-7514-1682
Nathan Muchhala https://orcid.org/0000-0002-4423-5130
Brian E. Sedio https://orcid.org/0000-0002-1723-9822
Jonathan A. Myers https://orcid.org/0000-0002-2058-8468

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SUPPORTING INFORMATION

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