



## Research



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# Chemical properties of foliar metabolomes represent a key axis of functional trait variation in forests of the tropical Andes

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Plants interact with their environment through diverse specialized metabolites that protect them from abiotic stressors, like drought or radiation, and biotic stressors, like herbivores or pathogens. However, few studies have considered the chemical properties of metabolites as a potential axis of functional trait variation along environmental gradients. Here, we examined how the chemical properties of foliar metabolomes, such as mean aromaticity, hydrophobicity and polarity, as well as commonly used morphological traits, vary with climate and elevation among 16 forest plots in the tropical Andes of Bolivia. We found that chemical properties were weakly related to morphological traits among tree species, yet both varied significantly with climate and elevation. In particular, abundance-weighted mean hydrophobicity decreased, and polar surface area increased with elevation and in colder and drier climates. Additionally, co-occurring species showed increasing chemical similarity with elevation for the most-aromatic and most-polar metabolites. These results suggest that abiotic stress associated with colder, drier climates and solar radiation acts as a filter for metabolome chemical properties. This contrasts with chemical dissimilarity observed at lower elevations, which is likely driven by pressure from host-specialized enemies in warmer, wetter climates. Our results introduce the possibility that chemical defences may be constrained by abiotic stressors.

## 1. Introduction

Plants interact with the world around them through diverse specialized metabolites that make up the plant metabolome. Plant metabolites mediate abiotic stress response and adaptations to drought, heat, freezing temperatures and solar radiation [1–5]. They also mediate biotic interactions, acting as

attractants and defences that affect enemies such as herbivores and pathogens [6,7] and mutualists such as pollinators and endophytes [8,9]. The biosynthesis of metabolites requires energetic investment on the part of the plant; hence, metabolites contribute to fundamental trade-offs in resource allocation that define alternative defence syndromes [10,11] and life-history strategies [12]. Specialized metabolites are associated with species distributions over environmental gradients [4,5] and the effect of neighbouring plants on individual performance [13,14], indicating a role as functional traits. A major goal of ecology is to predict plant performance as a function of traits [13], yet the deep reservoir of plant trait variation represented by the plant metabolome remains a little-explored frontier in trait-based ecology [15] and functional biogeography [16].

Since von Humboldt documented the dramatic turnover in plant communities along elevational gradients in the tropical Andes [17], biologists have recognized the unique value of elevational gradients in understanding the mechanisms responsible for variation in plant species distributions, traits and community diversity [18]. Recent findings from temperate ecosystems suggest that countervailing gradients in the relative importance of stress due to abiotic conditions, on the one hand, and due to specialized herbivores and pathogens, on the other, may select for different features of plant metabolome function and diversity [5,19]. Abiotic stress increases with elevation owing to a decrease in temperature and moisture and an increase in solar radiation, promoting investment in metabolites that serve as protection against ultraviolet radiation, anti-oxidants or metabolites that otherwise function in abiotic stress mediation [5,19]. Abiotically stressful and resource-poor environments are also frequently associated with greater investment in defence against herbivores to protect hard-earned biomass [12,20,21]. Abiotic pressures can act as an environmental filter that selects for trait similarity, resulting in similar physiological, morphological and chemical traits in plants growing under similar conditions [22–25]. Furthermore, the environmental filter effect of abiotic stress should select for evolutionary convergence on the traits that are most effective at ameliorating the stress.

In contrast to the increase in abiotic stressors with elevation, biotic stressors such as specialized herbivores and pathogens generally decrease with elevation [19,26–28]. The stronger selective pressure exerted by such enemies at lower elevations may favour the evolution of dissimilarity in chemical composition, reducing the proportion of enemies shared with other species [29–31]. Indeed, specialized herbivores can overcome high concentrations or numerous unique but structurally related defensive metabolites and even preferentially feed on hosts sharing them [25,31–33]. Hence, owing to the benefits of expressing unique defences, biotic stress due to natural enemies should not select for convergence on a shared, effective defence, but rather divergence in metabolome composition [7,30,34]. The resulting chemical dissimilarity among plant species should allow ‘chemical niche partitioning’ with respect to natural enemies and hence facilitate species coexistence and the maintenance of species diversity in communities [13,35,36]. Variation in this mechanism associated with climate may contribute to widespread elevation- and climate-diversity gradients [37–39].

Countervailing gradients in abiotic and biotic stress may select for different features of the metabolome over elevational gradients, but the nature of this variation remains unexplored. New innovations in metabolomics [40–43] make it possible to examine numerous facets of the metabolome, including chemical richness, structural or functional diversity [44], structural similarity [45] and composition with respect to chemical class or metabolic pathway of origin [5,14,34]. In addition, molecular structures predicted using these metabolomics methods can be used to calculate chemical and physical properties of individual metabolites [46], including those related to biological functions such as light absorbance, hydrophobicity, reactivity, cell-membrane transport and pharmaceutical bioactivity [47]. Recently, Walker *et al.* [47] explored the relationships between these properties and commonly measured morphological functional traits of plants, concluding that chemical properties of the metabolome and morphological traits represent several orthogonal axes of plant trait variation at the global scale. Yet, it remains unknown whether the chemical properties of plant metabolomes represent functional traits that determine variation in community composition over environmental gradients. Furthermore, it is unclear whether such variation may constrain the chemical space in which plant metabolomes may differentiate among co-occurring species with similar environmental tolerances.

Here, we used untargeted metabolomics to calculate five foliar metabolome chemical properties with clear functional hypotheses (box 1) that we combined with five leaf and stem morphological traits for 473 tree species in 16 forest plots that represent variation in tree communities over an elevational and climatic gradient spanning 2680 m in elevation in Madidi National Park in the tropical Andes of Bolivia [39]. We tested the following hypotheses and their predictions: (i) metabolome chemical properties represent functional trait variation distinct from that encompassed by morphological traits. This hypothesis predicts that metabolome chemical properties will vary independently of morphological variation among species after controlling for phylogeny. (ii) The increase in abiotic stress and decrease in biotic stress with elevation create opposing filters for metabolome chemical properties. This hypothesis predicts that community-weighted mean chemical properties should vary with elevation and climate; and (iii) the increase in abiotic stress and decrease in biotic stress with elevation create opposing selection pressures on metabolome chemical properties. This hypothesis predicts two distinct patterns of chemical similarity. First, we predict that community-weighted mean chemical similarity with respect to metabolites that exhibit properties related to abiotic stress tolerance will increase with elevation. This is due to selection for convergence in high-elevation, cold and dry climates. Second, we expect similarity in metabolites related to herbivore and pathogen defence to also increase with elevation. This pattern arises because selection favours chemical divergence at lower elevations in warmer, wetter climates.

**Box 1.** Foliar functional traits: metabolome chemical properties and morphological traits.

Chemoinformatics make it possible to predict the structure of unknown metabolites based on evidence from isotopic variation [41] and molecular fragmentation profiles detected using tandem mass spectrometry [40]. A predicted or known molecular structure can then be used to calculate a set of chemical properties [46] that shed light on metabolite function. We selected five properties, each of which represents a distinct axis of variation among plant species at the global scale [47]. In addition, we selected five morphological traits that reflect a species position on a trade-off axis from conservative traits associated with defence and survival to acquisitive traits associated with fast growth [48].

**Metabolome chemical properties**

**nAtomP**—the number of atoms in the largest aromatic, conjugated  $\pi$  system, which is high in pigments [49] and light-absorbing molecules [50] that function to protect plants from ultraviolet radiation [51,52].

**AlogP**—hydrophobicity measured as the log-ratio of solubility in octanol versus water [53], which may be related to a plant-defence spectrum defined by unsaturated, aromatic and non-polar metabolites versus saturated and polar metabolites [47] and negatively correlated with polarity and hence desiccation resistance [2,3].

**TopoPSA**—topological polar surface area, the sum of the surface area of polar atoms in ångströms (Å) [54], which may contribute to desiccation resistance [2,3], but is negatively correlated with passive transport through cell membranes [55].

**Fsp3**—the fraction of carbon atoms with  $sp^3$  electron orbits (i.e. only single bonds) to the total number of carbon atoms in the molecule, which is positively correlated with melting point, solubility and the likelihood of a compound to exhibit bioactivity in pharmaceutical assays [56].

**MW**—molecular weight in daltons (Da); greatest in peptides and lignans [47]; may be related to leaf longevity because peptides and lignans function as long-lived physical and storage structures [57].

**Morphological traits**

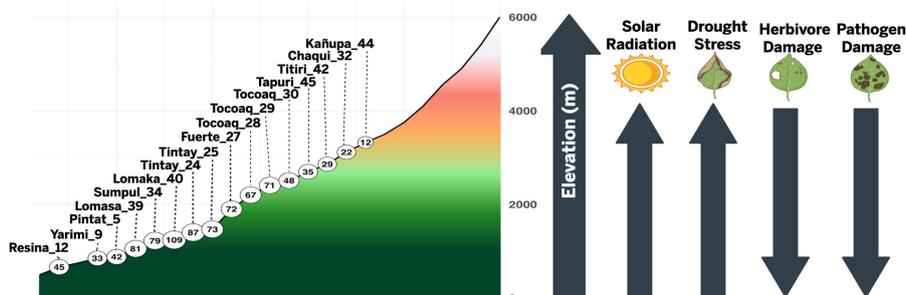
**Leaf area**—associated with a resource-acquisitive life-history strategy.

**Specific leaf area (SLA)**—a leaf economics–resource capture trait associated with high photosynthetic rates, high relative growth rates, low carbon investment in lignin or tannins, and resource-rich environments.

**Leaf thickness**—associated with tolerance to disturbance and nutrient stress and a resource-conservative life-history strategy.

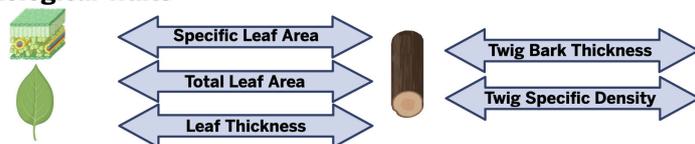
**Twig bark thickness**—associated with protection from attack by pathogens and herbivores.

**Twig specific density**—associated with low relative growth rates, high survival, and resistance to pathogens, herbivores or physical damage.

**A) Distribution of Madidi plots along elevation gradient**

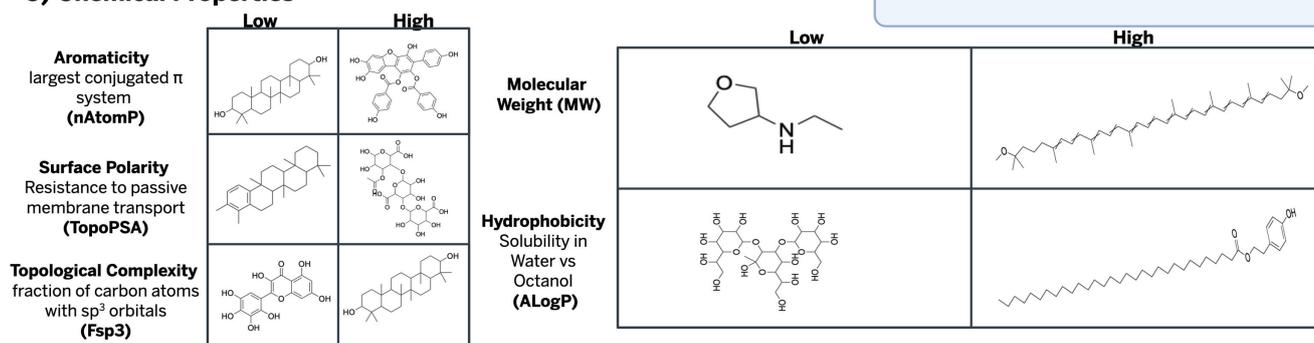
**H1:** Metabolomic traits vary independently of morphological traits and are phylogenetically uncorrelated

**H2:** Community-weighted mean chemical traits shift with elevation and climate owing to opposing abiotic and biotic stress filters.

**B) Morphological Traits**

**H3 a:** Chemical similarity in abiotic stress-related metabolites increases with elevation (convergent selection in cold, dry climates).

**H3 b:** Chemical similarity in biotic defence-related metabolites increases with elevation (divergent selection in warm, wet climates).

**C) Chemical Properties**

**FigureCSI.** (A) Distribution of forest plots with respect to elevation and direction of abiotic and biotic gradients thought to influence functional traits and metabolome chemical properties of plants. Ovals represent species richness of each plot. (B) Morphological traits measured in this study. (C) Description of the five metabolome chemical properties used in this study, along with examples of compounds that represent the lowest and highest values for each property.

## 2. Material and methods

### (a) Forest plot data: the Madidi Project

Floristic data were collected as part of the Madidi Project (<https://www.missouribotanicalgarden.org/plant-science/plant-science/south-america/the-madidi-project>), a collaboration of more than two decades between the Herbario Nacional de Bolivia and the Missouri Botanical Garden to document the flora of the Madidi region in the Andes of Bolivia [58]. The region features wide variation in plant communities over an extreme elevational gradient, from lowland rainforests located at 200 m a.s.l. to alpine environments above the tree line at 6000 m a.s.l. [59]. The Madidi Project includes 50 1 ha permanent forest plots ranging in elevation from 212 to 3334 m a.s.l. We selected 16 1 ha permanent plots in which leaves were sampled for chemical analysis and that represent broad variation in elevation (662–3324 m a.s.l.), climate and tree species richness (17–137 species per 1 ha plot). The 16 plots include three seasonally dry, low-elevation forest plots and 13 moist, montane forest plots [39]. Abundant genera include: *Miconia* (Melastomataceae), *Sloanea* (Elaeocarpaceae) and *Ocotea* (Lauraceae) in the low-elevation moist plots; *Weinmannia* (Cunoniaceae), *Hedyosmum* (Chloranthaceae) and *Clethra* (Clethraceae) in the high-elevation (>2500 m) plots; and *Weinmannia*, *Hedyosmum* and *Clethra* in the seasonally dry plots. Tree species richness declines with elevation among the 13 moist forest plots, whereas the three seasonally dry lowland plots display low species richness [39]. In each 1 ha plot, all free-standing woody plants with a diameter at breast height of  $\geq 10$  cm were mapped, measured and identified to a botanically valid species or morphospecies.

### (b) Morphological functional traits

Protocols for morphological functional trait data collection are described in detail in the Madidi Project manual (<https://www.missouribotanicalgarden.org/plant-science/plant-science/south-america/the-madidi-project>). We selected five morphological leaf and stem traits that reflect a species position on a trade-off axis from conservative traits associated with defence and survival to acquisitive traits associated with fast growth (box 1). Leaf area and specific leaf area (SLA; area per unit mass) are associated with a resource-acquisitive life-history strategy; leaf thickness, bark thickness and twig specific density are associated with a resource-conservative life-history strategy [48]. Morphological traits for each species-by-plot are reported in electronic supplementary material, dataset S1. Community-weighted mean trait values are reported in electronic supplementary material, dataset S2.

### (c) Chemical analysis

We collected leaf samples from 473 tree species representing 906 unique species-by-plot. Within each forest plot, we collected leaf samples from 62 to 90% of the species in the plot (mean = 80% of the species per plot; [39]). Leaves of up to five individual trees per species per plot were collected between 2010 and 2019 and dried with silica gel upon collection in the field. Leaf samples were extracted for untargeted metabolomics analysis following Sedio *et al.* [38]. Briefly, 50 mg of dried leaf tissue was ground to a fine powder and 10 mg weighed for extraction in 1800  $\mu$ l 90 : 10 methanol : water pH 5 overnight at 4°C. Extracts of up to five individuals per species per plot were pooled to create 906 pools representing unique species-by-plot.

All individual extracts and species pools were filtered and analysed using ultra-high performance liquid chromatography–heated electrospray ionization–tandem mass spectrometry (UHPLC–HESI–MS/MS) using a Thermo Fisher Scientific (Waltham, MA, USA) Vanquish UHPLC with a C18 column and a Thermo QExactive quadrupole-orbitrap MS. Separation of metabolites by UHPLC was followed by HESI ionization in positive mode using full scan MS1 and data-dependent acquisition of MS2. Detailed instrumental methods are described by Sedio *et al.* [38]. Spectra were deposited as a public MassIVE dataset on the Global Natural Products Social (GNPS) Molecular Networking server (doi: <https://doi.org/10.25345/C52R3P21H>).

Raw spectra were centroided and processed for peak detection, peak alignment and filtering using MZmine 2 [60]. Aligned chromatograms were used to create a feature-based molecular network (FBMN [42]) using GNPS [61]. The structural similarities of all metabolites as represented in the resulting network were used to create a dendrogram using the software Qemistree [62], which is reported in electronic supplementary material, dataset S3. Metabolites were annotated by predicting molecular formulae using Sirius [41], predicting molecular structures using CSI:FingerID [40] and classifying compounds using Canopus [43] according to the organic chemical taxonomy scheme of ClassyFire [63] and according to biosynthetic origins using NPClassifier [64]. For a comparison of intra- and inter-specific variation for selected species-rich high- and low-elevation genera, see [39].

To calculate chemical properties of metabolites, we used the highest-confidence molecular structure predicted by CSI:FingerID, represented as a SMILES text string, to query the Chemistry Development Kit (CDK) [46] using the R package ‘rckd’ [65]. The CDK library includes 51 variables that describe chemical and physical properties, but Walker *et al.* [47] found that many of these are highly correlated and hence represent five major axes of variation. A correlation matrix of 21 chemical properties for metabolites in our data closely matched that of Walker *et al.* [47]. Hence, like Walker *et al.* [47], we chose one of each of five major dimensions of variation (box 1). Molecular formulae, predicted structures, classifications, chemical properties and sample-level abundances for 20 571 unique metabolites are provided in electronic supplementary material, dataset S4. Foliar metabolome chemical properties for each species-by-plot are reported in electronic supplementary material, dataset S1. Community-weighted mean values are reported in electronic supplementary material, dataset S2.

We calculated the chemical structural–compositional similarity (CSCS) of species, which accounts for the structural similarity of unique metabolites [45]. We calculated CSCS with respect to metabolites in the upper and/or lower quartile of nAtomP, ALogP, TopoPSA and Fsp3, respectively, for the species co-occurring in each of the 16 forest plots.

#### (d) Climate data

We selected four climatic variables to represent variation among the 16 forest plots in temperature, precipitation and seasonality. Annual mean temperature and annual range in temperature were derived from WorldClim v. 2.1 [66]. Annual precipitation and precipitation seasonality, calculated as the ratio of the standard deviation to the mean precipitation of each month, were derived from the Tropical Rainfall Measuring Mission (TRMM), a regional database that for the Bolivian Andes provides greater accuracy in precipitation measurements than WorldClim [39]. We scaled and centred the four variables and carried out a principal components analysis, of which the first principal component (PC1) represented 71.2% of the variation and was clearly interpretable as a gradient from cold, dry environments (values <0) to warm, wet environments (values >0) [39]. Elevation and position on climate PC1 for each of the 16 forest plots are reported in electronic supplementary material, dataset S2.

#### (e) Hypothesis testing

To test the prediction that chemical properties of species' foliar metabolomes and morphological functional traits evolve independently, we extracted a community phylogeny representing the 473 tree species from the GBOTB.extended.TPL phylogeny using 'V.PhyloMaker2' [67] and calculated the phylogenetic variance–covariance matrix using the 'phyl.vcv' function in the R package 'phytools' [68]. In addition, we visualized the relationships among these variables as a correlation plot using the R package 'corrplot' [69].

To test the prediction that community-weighted mean chemical properties and morphological functional traits vary with underlying climatic and elevational gradients among the 16 forest plots, we employed redundancy analysis using the 'rda' function in the R package 'vegan' [70]. We calculated phylogenetic similarity using the 'dsimTree' function in the R package 'adiv' [71] and used the phylogenetic similarity matrix as a conditioning variable in the RDA. In addition, we used linear regression to model the relationships between community-weighted mean chemical properties and elevation and climate, respectively, in two-variable, response (chemical property) versus predictor (gradient) models.

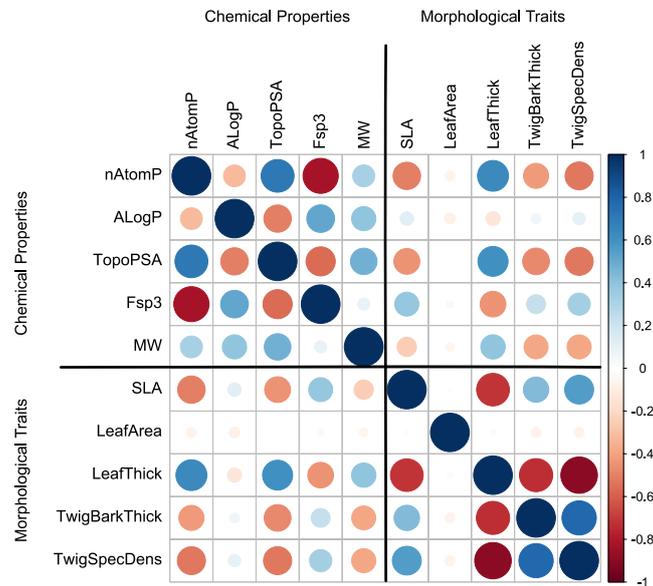
We tested three predictions concerning the effects of gradients in abiotic and biotic stress on the chemical similarity of co-occurring species: (i) to test the prediction that high-elevation trees are similar with respect to the most light-absorbing metabolites, we regressed community-weighted mean CSCS based on the upper quartile of nAtomP against elevation and climate PC1; (ii) to test the prediction that trees at high elevations and in colder and drier climates are similar with respect to the most hydrophilic or polar metabolites, we regressed community-weighted mean CSCS based on the lower quartile of ALogP and the upper quartile of TopoPSA against elevation and climate PC1; and (iii) to test the prediction that trees at low elevations and warmer and wetter environments are dissimilar with respect to metabolites with the greatest potential for passive cell-membrane transport and bioactivity or pharmaceutical potential, we regressed community-weighted mean CSCS based on the lower quartile of TopoPSA and the upper quartile of Fsp3 against elevation and climate PC1. Linear regressions modelled the relationship between community (abundance)-weighted mean CSCS and underlying gradients in two-variable, response (mean CSCS) versus predictor (elevational or climatic gradient) models.

Finally, we used the R package 'plotly' [72] to generate a three-dimensional (3D) regression plot to illustrate the relationships between chemical similarity of the most light-absorbing (upper quartile nAtomP) and most hydrophilic (lower quartile AlogP) metabolites and elevation. We recreated this figure in additional variations to highlight five species-rich families with broad distributions over the elevational gradient, five species-rich genera with broad distributions over the gradient, and five distantly related genera that are diverse in high-elevation forest plots.

### 3. Results

#### (a) Metabolome chemical properties and morphological traits represent orthogonal axes of variation among species

To test our first hypothesis, we examined interspecific correlations among five metabolome chemical properties and five morphological functional traits (box 1). We observed strong covariance among metabolome chemical properties (maximum absolute covariance  $-0.83$ , mean  $0.47$ ) and strong covariance among morphological traits (maximum absolute covariance  $0.89$ , mean  $0.43$ ), but weaker covariances between chemical properties and morphological traits (maximum absolute covariance  $-0.64$ , mean  $0.30$ ) (figure 1). The largest conjugated  $\pi$  system (nAtomP), which reflects the capacity of a molecule to absorb light, was strongly negatively correlated ( $\text{cor} = -0.83$ ) with Fsp3, which reflects pharmaceutical potential or bioactivity [56]. nAtomP was also positively correlated with TopoPSA ( $\text{cor} = 0.55$ ), indicating that species with aromatic, light-absorbing metabolomes also tend to display metabolomes with greater polar surface area. Furthermore, TopoPSA was negatively correlated with ALogP ( $\text{cor} = -0.49$ ), indicating that mean polar surface area and hydrophobicity negatively covary among species' metabolomes. Molecular weight displayed weak covariances overall but was positively correlated with TopoPSA ( $\text{cor} = 0.52$ ). The strongest covariances among morphological traits were among leaf thickness, twig bark thickness and twig-specific density (figure 1).



**Figure 1.** Interspecific variation in foliar metabolome chemical properties is weakly related to leaf and twig traits. Point size reflects the phylogenetic trait variance–covariance among species for five foliar metabolome chemical properties and five leaf and twig morphological traits. Phylogenetic variance–covariance was calculated among species means for 437 species.

## (b) Opposing gradients in abiotic and biotic stress create opposing filters for metabolome chemical properties

To test our second hypothesis, we analysed correlations and performed redundancy analysis (RDA) to assess community-level relationships among the underlying climatic and elevational gradients for each site and the five metabolome chemical properties and five morphological traits (box 1). To account for evolutionary history, we conditioned the RDA on the phylogenetic similarity among forest plots. We found strong associations among community-weighted mean chemical properties and morphological traits (figure 2A), and most traits, whether chemical or morphological, were clearly associated with climate and elevation (figure 2B).

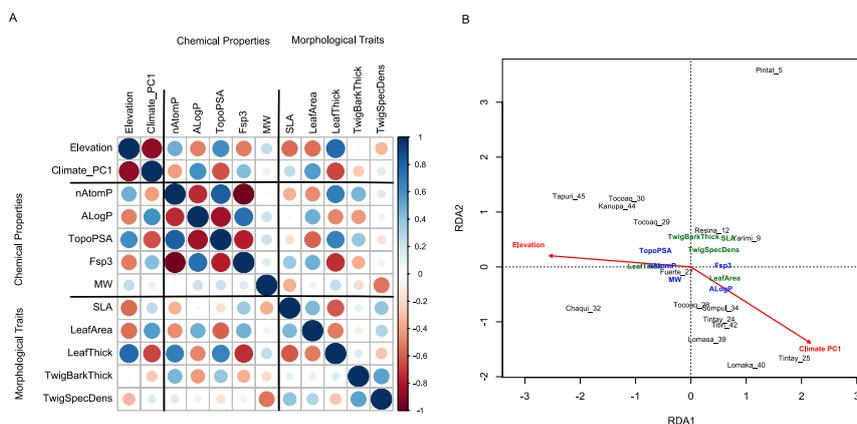
We predicted that community-weighted mean foliar metabolome chemical properties would vary with elevation and climate in a manner consistent with greater pressure by abiotic sources of stress (ultraviolet radiation, lower precipitation) at higher elevations and greater pressure by biotic sources of stress (insect herbivores, microbial pathogens) at lower elevations. Our results were broadly consistent with these predictions. We did not observe a significant relationship between community-weighted mean largest  $\pi$  system (nAtomP) of the metabolomes of tree communities and either elevation ( $R^2 = 0.18$ ,  $p = 0.056$ ; figure 3a) or climate PC1 (figure 3b), along which mean precipitation and temperature load positively [39]. In contrast, hydrophobicity (ALogP) decreased with elevation ( $R^2 = 0.20$ ,  $p = 0.05$ ; figure 3c) and increased with climate PC1 ( $R^2 = 0.32$ ,  $p = 0.01$ ; figure 3d). Similarly, topological polar surface area (TopoPSA), which was negatively correlated with ALogP (figures 1 and 2), increased with elevation ( $R^2 = 0.33$ ,  $p = 0.01$ ; figure 3e) and decreased with climate PC1 ( $R^2 = 0.37$ ,  $p < 0.01$ ; figure 3f). Potential bioactivity (Fsp3) decreased with elevation ( $R^2 = 0.24$ ,  $p = 0.01$ ; figure 3g) but was unrelated to climate PC1. Community-weighted mean molecular weight was unrelated to elevation or climate (figure 3i,j).

## (c) Opposing gradients in abiotic and biotic stress drive convergence in metabolome chemical properties at high elevations

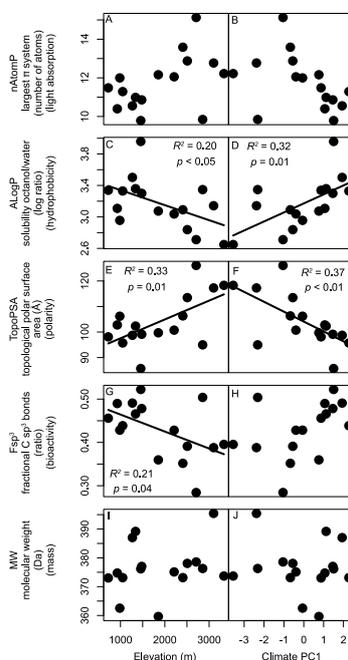
For our third hypothesis, we expected to find convergence in metabolites associated with radiation, drought and cold stress at higher elevations in cold, dry climates because these selective agents should not impose divergent selection among closely related or coexisting species. Conversely, we anticipated greater divergence in metabolites linked to defence against herbivores and pathogens at lower elevations in warm, wet climates. To quantify these patterns, we calculated the chemical similarity of co-occurring species within each forest plot representing the upper or lower quartile of variation with respect to particular chemical properties. We used the CSCS metric [45], which accounts for the structural similarity of unique metabolites.

Consistent with these predictions, we observed strong associations between community-weighted mean chemical similarity based on the most light-absorbing (nAtomP) and polar (ALogP and TopoPSA) metabolites and underlying elevational and climatic gradients (figure 4; electronic supplementary material, figure S1). Specifically, chemical similarity based on the upper quartile of nAtomP, representing the most aromatic, light-absorbing metabolites, increased strongly with elevation ( $R^2 = 0.62$ ,  $p < 0.001$ ; figure 4a), but did not vary with climate PC1 (figure 4b). Chemical similarity based on the lower quartile of AlogP and the upper quartile of TopoPSA, representing the most polar metabolites, increased strongly with elevation (ALogP:  $R^2 = 0.67$ ,  $p < 0.001$ ; figure 4c; TopoPSA:  $R^2 = 0.56$ ,  $p < 0.001$ ; figure 4e) and decreased with climate PC1 (ALogP:  $R^2 = 0.48$ ,  $p = 0.002$ ; figure 4d; TopoPSA:  $R^2 = 0.28$ ,  $p = 0.019$ ; figure 4f).

Contrary to our expectations, chemical similarity based on the lower quartile of TopoPSA, which represents the metabolites with the greatest potential for passive cell-membrane transport, did not vary with elevation or climate PC1 (figure 4g,h).



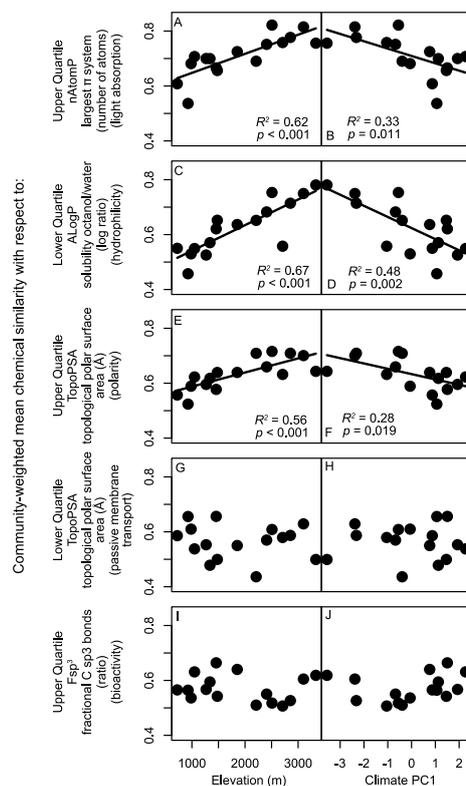
**Figure 2.** Community-weighted mean foliar metabolome chemical properties and morphological traits exhibit co-varying relationships with elevation and climate. (A) Correlation plot of coefficient of Pearson's correlation among community abundance-weighted mean foliar chemical properties and leaf and twig morphological traits. (B) Redundancy analysis (RDA) of the relationships among foliar metabolome chemical properties, leaf and twig morphological traits, climate and elevation, conditioned on phylogenetic similarity. Values used for correlations and the RDA were community abundance-weighted mean trait values for 16 forest plots. Climate is represented by the first principal component (PC1) of a principal components analysis of variation among the 16 forest plots in annual mean and range in temperature, annual precipitation and precipitation seasonality. Positive values on climate PC1 represent warmer, wetter and less seasonal climates.



**Figure 3.** Community-weighted mean foliar metabolome chemical properties vary with elevation (left column) and climate (right column). Solid lines illustrate significant linear regressions ( $p < 0.05$ ). Linear regression response variables represent the abundance-weighted mean of co-occurring species with respect to: (a,b) largest conjugated  $\pi$  system (aromaticity, light absorbance; nAtomP), (c,d) solubility in octanol versus water (hydrophobicity; ALogP), (e,f) topological polar surface area (surface polarity, resistance to passive membrane transport; TopoPSA), (g,h) fraction of carbon atoms with sp<sup>3</sup> electron orbits (topological complexity, bioactivity; Fsp3), and (i,j) molecular weight (MW). Positive values on climate PC1 represent warmer, wetter and less seasonal climates.

Likewise, chemical similarity based on the upper quartile of Fsp3, which represents the metabolites with the greatest pharmaceutical potential or bioactivity, also did not vary with elevation or climate PC1 (figure 4i,j).

We explored the relationships between species abundances (number of individuals in a forest plot), community-weighted mean chemical similarity based on the upper quartile of nAtomP and ALogP, and elevation through an interactive 3D scatterplot (electronic supplementary material, figure S1), which illustrates the species-level variation underpinning the regressions in figure 4a,c. Three additional figures highlight trends within species-rich families (electronic supplementary material, figure S2), species-rich genera (electronic supplementary material, figure S3), and five distantly related genera that are diverse and abundant in high-elevation communities (electronic supplementary material, figure S4). In these 3D plots, each data point represents a tree species in a forest plot, with point size scaled by abundance. Two of the axes represent the abundance-weighted similarity of co-occurring tree species with respect to the most-polar metabolites (lower quartile ALogP) and most-aromatic, light-absorbing metabolites (upper quartile nAtomP); the third, vertical axis represents elevation of the forest plots in which the species occur. Species in high-elevation forest plots are remarkably similar with respect to the most polar and most aromatic metabolites, indicating shared or very structurally similar metabolites with these properties (electronic supplementary material, figure S1). This relationship between elevation and metabolome similarity for the most



**Figure 4.** Similarity in metabolome composition of co-occurring species with respect to the most aromatic and polar metabolites increases with elevation (left column) and in cold and dry climates (right column). Linear regression response variables represent the CSCS for the upper quartile of metabolites with respect to: (a,b) largest conjugated  $\pi$  system (aromaticity, light absorbance; nAtomP) and (e,f) topological polar surface area (surface polarity, resistance to passive membrane transport; TopoPSA) and the lower quartile of metabolites with respect to (c,d) solubility in octanol versus water (hydrophobicity; ALogP) and (g,h) the fraction of carbon atoms with  $sp^3$  electron orbits (topological complexity, bioactivity; Fsp3). Positive values on climate PC1 represent warmer, wetter and less seasonal climates.

polar and aromatic metabolites is repeated within the species-rich families Fabaceae, Lauraceae, Melastomataceae, Myrtaceae and Rubiaceae (electronic supplementary material, figure S2) and the species-rich genera *Inga* (Fabaceae), *Miconia* (Melastomataceae), *Myrcia* (Myrtaceae), *Ocotea* and *Nectandra* (Lauraceae) and *Pouteria* (Sapotaceae) (electronic supplementary material, figure S3). Furthermore, species that represent distantly related plant lineages, including Aquifoliaceae (asterids: Aquifoliales), Clethraceae (asterids: Ericales), Cloranthaceae (Chloranthales), Clusaceae (rosids: Malpighiales), Cunoniaceae (rosids: Oxidales), Ericaceae (asterids: Ericales), Lauraceae (magnoliids: Laurales), Melastomataceae (rosids: Myrtales), Rosaceae (rosids: Rosales), Solanaceae (asterids: Solanales) and Symplocaceae (asterids: Ericales) (electronic supplementary material, figures S1 and S4), display strong convergence in nAtomP and ALogP at high elevations (electronic supplementary material, figures S1 and S4).

## 4. Discussion

We tested the hypothesis that abiotic stressors in colder, drier and higher-radiation environments impose strong selection on metabolome properties, while biotic pressures from specialized herbivores and pathogens drive chemical differentiation in warmer, wetter environments. Our results suggest that foliar metabolome chemical properties, such as hydrophobicity and aromaticity, represent unique facets of functional trait variation [47] that mediate plant responses to abiotic and biotic stress in ways not reflected by morphological traits. Our results suggest that opposing gradients in abiotic and biotic stress may constrain metabolome composition in abiotically stressful environments while promoting interspecific divergence in abiotically mild environments where biotic stress is high [39]. This has important implications for our understanding of diversity, as it suggests that plant metabolomes may play a central role in the mechanisms underlying elevational and climatic diversity gradients [39].

The chemical properties of species' foliar metabolomes appear to represent orthogonal axes of functional trait variation compared with morphological traits. This result is consistent with other studies that have compared interspecific variation in metabolomic and morphological traits in temperate forests [38,73] and globally [47]. However, despite the absence of strong correlations between chemical properties and morphological traits among species, community-weighted mean chemical properties and morphological traits per site both exhibited strong associations with elevation and climate (figures 2 and 3). Previous studies in the tropical Andes [74–76] and elsewhere [23,76] have interpreted turnover in morphological traits with elevation as evidence of trait-based filtering as a driver of community composition. Our results suggest that foliar metabolome chemical properties display similar effects of environmental filtering on community assembly along environmental gradients in the tropical Andes.

Our results suggest that high-elevation plants have converged in various aspects of their metabolomes. One especially strong pattern is that they tend to be rich in light-absorbing compounds, likely owing to the need to protect against ultraviolet

radiation [51,52]. This was evidenced by the increase with elevation in abundance-weighted chemical similarity based on the upper quartile of compounds ranked by nAtomP (figure 4a; electronic supplementary material, figures S1–S4). High-elevation communities also displayed less hydrophobic (ALogP; figure 3b) and more polar (TopoPSA; figure 3c) metabolomes and greater similarity with respect to the least-hydrophobic and most-polar metabolites (figure 4c,e; electronic supplementary material, figures S1–S4). These elevational clines suggest that colder, drier environments at high elevations select for metabolomes that are not only richer overall in polar metabolites but composed of many of the same or structurally related polar metabolites. Hydrophobic non-polar compounds are known to be adaptive in arid environments when expressed as waxy cuticles that form a hydrophobic barrier to water loss on the leaf surface. Yet, polar metabolites within the cortex of the leaf may function to stabilize proteins and other cellular structures during desiccation [2], which could explain the pattern we observed here.

We observed stronger interspecific correlations among metabolome chemical properties than between chemical properties and morphological traits (figure 1). We also found stronger associations between chemical properties and elevation and climate than between morphological traits and these factors (figure 2B). Because these patterns hold when controlling for phylogenetic similarity, they suggest that similarity has arisen through convergent evolution rather than simply from turnover of phylogenetic lineages along the gradient. A closer examination of electronic supplementary material, figures S1–S4 provides further insight. Species in high-elevation forest plots are remarkably similar with respect to the most polar and most aromatic metabolites, indicating shared or very structurally similar metabolites with these properties (electronic supplementary material, figure S1). This relationship between elevation and metabolome similarity for the most polar and aromatic metabolites is repeated within species-rich families (electronic supplementary material, figure S2) and species-rich genera (electronic supplementary material, figure S3). Furthermore, the species that display such remarkable similarity in the high-elevation plots represent distantly related plant lineages (electronic supplementary material, figures S1 and S4). Together, these results suggest that similarity in high-elevation communities has arisen through convergent evolution across distantly related lineages.

Damage due to herbivores and pathogens is greatest at low elevations and in warm and wet climates [26–28,77]. It follows that this pressure should select for more effective chemical defences at low elevations. This may be reflected by low TopoPSA, which correlates with greater capacity for passive cell-membrane transport [55], a characteristic of many toxins, or high Fsp3, which correlates with greater topological complexity and potential bioactivity in the context of enzyme inhibition [56]. The increase we observed in community-weighted mean TopoPSA and the decrease in Fsp3 with elevation are consistent with the prediction that plants at lower elevations and in warmer and wetter climates are better defended (figure 3e–g). This contrasts with several studies that instead observed that plants at higher elevations had increased defensive metabolites and concluded that this may reflect adaptation for defence against herbivores under limited resources [5,19,27]. A promising avenue for future studies would be to further explore the relationships between energetic investment, concentration and bioactivity or toxicity of chemical defences, and variation in these relationships along environmental gradients, including gradients extending the one considered here into lowland Amazonian forests.

Low elevations and warm, wet climates also show increased host specialization of herbivores and pathogens [27,78]. In such habitats, reciprocal coevolution between plants and their natural enemies is thought to select for chemical divergence among plants [7,30], resulting in chemical-defence niche partitioning that can facilitate species coexistence and diversity maintenance [36]. Hence, we might predict that chemical similarity of co-occurring species would increase with elevation as well as with cold and dry climates. However, chemical similarity showed no relationship with elevation or climate (figure 4g–j) for either the lower quartile of compounds based on TopoPSA (i.e. those with the highest potential for passive membrane transport) or the upper quartile based on Fsp3 (i.e. those with the highest potential bioactivity). This suggests that the gradient we observed in community-weighted mean TopoPSA is driven primarily by selection for polar metabolomes at higher elevations rather than by selection for less polar metabolites with greater capacity for passive membrane transport at lower elevations. Likewise, Fsp3 is strongly negatively correlated with nAtomP (figure 1); hence, selection for aromatic, light-absorbing metabolites at high elevations may be sufficient to explain the observed elevational gradient in mean Fsp3 without invoking selection for greater bioactivity in lower-elevation communities. Overall, we suggest that the observed trends in metabolome properties were primarily driven by abiotic selection pressures rather than by specialized herbivores or pathogens [19].

On the other hand, a previous study of the Madidi elevational gradient found that chemical similarity of co-occurring species with respect to secondary metabolites (those classified as alkaloids, amino acids and peptides, polyketides, shikimates and phenylpropanoids, and terpenoids) decreased with precipitation, temperature and tree species diversity [39]. This suggests that divergence in chemical composition from competing species may be more important than acute toxicity of individual chemical defences in shaping elevational and other climate–diversity gradients. This conclusion would be broadly consistent with studies of species-rich tropical tree genera, which display remarkable chemical diversity and divergence in composition among closely related species [6,31,34,37,79,80]. It remains unknown whether these genera exhibit more toxic or bioactive compounds than other trees. It may be that simple metrics such as TopoPSA and Fsp3 may not represent strong indicators of metabolite function in defence against herbivores and pathogens. Rather, divergence with respect to numerous metabolites from diverse biosynthetic origins may contribute to the avoidance of what would otherwise be shared natural enemies [7]. Further research, especially the use of natural product chemistry-style toxicity assays, may yet identify chemical properties associated with defensive function.

The divergence in plant metabolome composition that pathogens and herbivores can select for is thought to promote species diversity of both plants and their natural enemies [13,30,36]. However, our results point to convergence in metabolome composition in sites with high abiotic stress. This raises the possibility that these pressures will act in opposition, with high-stress habitats limiting the ability of plants to diverge in defences, and thus in turn limiting species diversity. On the one hand, perhaps the sheer number of metabolites found in leaves allows simultaneous response to both pressures. For instance, there may be ample opportunity for plants to evolve the most effective light-absorbing flavonoids, while diverging

with respect to effective defences, such as salicinoids in the case of alpine willows [5]. Indeed, previous studies have shown that individual plant defences can follow different evolutionary trajectories, which seem to be linked to differences in their effects on insect herbivores [31]. On the other hand, abiotic stress in cold, dry climates may actively reduce opportunities for chemical differentiation by selecting for convergence, limiting the potential for biotic niche segregation within communities in abiotically stressful environments, and thus in turn limiting species diversity in these communities.

Metabolomics approaches to chemical ecology have facilitated the use of classification as a proxy for function [47]. However, similar functions, such as anti-herbivore defence, can be derived from metabolites with very different biosynthetic origins [30]. This is not surprising given the fact that plant secondary metabolism is a complex web of intertwined and often convergently evolved biosynthetic intermediates, enzymes and pathways. Our results suggest that molecular descriptors, such as chemical properties calculated for individual metabolites, offer a distinct perspective compared with approaches that focus on classification with respect to biosynthetic origin. Considering the chemical properties of metabolites accounts for functional convergence across biosynthetic classes, as well as the multifunctionality of many compound groups (e.g. phenolics that function in both radiation protection and herbivore defence). Furthermore, despite broad differences in mean properties among major chemical classes, such as alkaloids, carbohydrates, fatty acids, shikimates and terpenoids [47], our results highlight that some chemical properties of metabolomes may be better measures of functional trait variation than any one chemical class and provide additional perspective not represented by metabolite classification in studies of chemical ecology.

In summary, we observed that foliar metabolome chemical properties and morphological traits varied independently, with community mean chemical properties shifting along elevation and climate gradients. Notably, we found strong convergence in the composition of the most hydrophilic, aromatic and light-absorbing metabolites at high elevations. Taken together, our results suggest that foliar metabolome chemical properties represent functional traits that mediate plant tolerance of abiotic and biotic sources of stress, and that function uniquely and independently of commonly measured morphological traits. Our findings raise new questions regarding the constraints imposed on metabolome composition by abiotic stressors and its role in shaping diversity gradients and suggest a greater role for metabolomics in trait-based ecology and functional biogeography.

**Ethics.** This work did not require ethical approval from a human subject or animal welfare committee.

**Data accessibility.** R code and supplemental datasets S1–S4 are available on Dryad [81]. The raw data from the Madidi Project are stored and managed in Tropicos (<https://tropicos.org/home>), the botanical database of the Missouri Botanical Garden. The data for each forest plot can be accessed via the Madidi Project Plot Search page (<http://tropicos.org/PlotSearch.aspx?projectid=20>). Links to the data page for each of the 16 forest plots used in this study can be found in dataset S2. Spectra were deposited as a public MassIVE dataset on the Global Natural Products Social (GNPS) Molecular Networking server ([https://massive.ucsd.edu/ProteoSAFe/dataset\\_files.jsp?task=a4a891a0f3b3467fb790a9c335783205#%7B%22table\\_sort\\_history%22%3A%22main.collection\\_asc%22%7D](https://massive.ucsd.edu/ProteoSAFe/dataset_files.jsp?task=a4a891a0f3b3467fb790a9c335783205#%7B%22table_sort_history%22%3A%22main.collection_asc%22%7D)) with doi (<https://doi.org/10.25345/C52R3P21H>).

Supplementary material is available online [82].

**Declaration of AI use.** We have not used AI-assisted technologies in creating this article.

**Authors' contributions.** S.C.: formal analysis, writing—original draft, writing—review and editing; D.H.: conceptualization, writing—review and editing; D.L.F.: conceptualization, formal analysis, writing—review and editing; L.C.: data curation, investigation; A.F.: data curation, investigation; B.A.: conceptualization; N.M.: conceptualization, funding acquisition, writing—review and editing; J.S.T.: conceptualization, data curation, funding acquisition, investigation, writing—review and editing; M.V.: conceptualization, formal analysis, writing—review and editing; J.A.M.: conceptualization, funding acquisition, writing—review and editing; B.E.S.: conceptualization, formal analysis, funding acquisition, methodology, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed herein.

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