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Paul V.A. Fine
University of California, Berkeley

Diego Salazar
Florida International University

Roberta E. Martin
Arizona State University

Margaret R. Metz
Lewis & Clark College, Portland

Tracy M. Misiewicz
University of California, Berkeley

See next page for additional authors

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Authors

Paul V.A. Fine, Diego Salazar, Roberta E. Martin, Margaret R. Metz, Tracy M. Misiewicz, and Gregory P. Asner

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Exploring the links between secondary metabolites and leaf spectral reflectance in a diverse genus of Amazonian trees

Paul V. A. Fine [1,†] Diego Salazar [2] Roberta E. Martin [3,5] Margaret R. Metz [4] Tracy M. Misiewicz [1] and Gregory P. Asner [3,5]

[1]*Department of Integrative Biology and Jepson and University Herbaria, University of California, Berkeley, 3040 Valley Life Sciences Building #3140, Berkeley, California 94720 USA*
[2]*Department of Biological Sciences, Institute of the Environment, and International Center for Tropical Botany, Florida International University, 11200 S.W. 8th Street, Miami, Florida 33199 USA*
[3]*School of Geographical Sciences and Urban Planning, Arizona State University, P.O. Box 875302, Tempe, Arizona 85287 USA*
[4]*Department of Biology, Lewis & Clark College, 615 S Palatine Hill Rd, Portland, Oregon 97219 USA*
[5]*Center for Global Discovery and Conservation Science, Arizona State University, Tempe, Arizona 85287 USA*

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Abstract. Plant defense chemistry is often hypothesized to drive ecological and evolutionary success in diverse tropical forests, yet detailed characterizations of plant secondary metabolites in tropical plants are logistically challenging. Here, we explore a new integrative approach that combines visible-to-shortwave infrared (VSWIR) spectral reflectance data with detailed plant metabolomics data from 19 *Protium* (Burseraceae) tree species. Building on the discovery that different *Protium* species have unique chemistries yet share many secondary metabolites, we devised a method to test for associations between metabolites and VSWIR spectral data. Given species-level variation in metabolite abundance, we correlated the concentration of particular chemicals with the reflectance of the spectral bands in a wavelength band per secondary metabolite matrix. We included 45 metabolites that were shared by at least 5 *Protium* species and correlated their per-species foliar abundances against each one of 210 wavelength bands of field-measured VSWIR spectra. Finally, we tested whether classes of similar metabolites showed similar relationships with spectral patterns. We found that many secondary metabolites yielded strong correlations with VSWIR spectra of *Protium*. Furthermore, important *Protium* metabolite classes such as procyanidins (condensed tannins) and phytosterols were grouped together in a hierarchical clustering analysis (Ward's algorithm), confirming similarity in their associations with plant spectral patterns. We also found a significant correlation in the phenolics content between juvenile and canopy trees of the same species, suggesting that species-level variation in defense chemistry is consistent across life stages and geographic distribution. We conclude that the integration of spectral and metabolic approaches could represent a powerful and economical method to characterize important aspects of tropical plant defense chemistry.

Key words: Burseraceae; chemical defenses; metabolomics; *Protium*; secondary metabolites; spectranomics; VSWIR.

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Introduction

Tropical rainforest trees comprise extraordinarily high species diversity and exhibit exceedingly large variation in functional traits (Lamanna et al. 2014). Recent studies have shown that the turnover in tree species composition across edaphic and elevational gradients is strongly correlated with functional traits (Fortunel et al. 2014, Asner et al. 2014*a*, 2016). At the same time, species-level variation in traits at the local scale is also extremely high (Kraft et al. 2008), especially traits that relate to protection against natural enemies (Becerra 2007, Asner and Martin 2011, Asner et al. 2014*a*, Endara et al. 2015, Vleminckx et al. 2018). However, our understanding of many important functional traits and their effect on modulating natural processes has been hampered by the lack of detailed studies of foliar chemistry, arguably the largest source of variation in tropical tree species both within and across habitats (Sedio et al. 2017). Additionally, our ability to understand the drivers of such large variation among tropical tree species is further limited by the logistical and economic challenges associated with the collection and analysis of plant functional traits at large geographical scales and with enough detail to be informative.

Advances in remote sensing have increased the potential of a more affordable and integrated measure of functional traits of tropical trees at large scales. Most notably, spectroscopic approaches that measure the spectrally detailed light reflectance and/or transmittance of plant foliage provide accurate predictions of several functional chemical traits, including those linked to plant growth and primary metabolism such as leaf carbon, nitrogen, phosphorus, and chlorophyll content, as well as traits linked to defense and secondary metabolism such as phenolics and lignin (Asner and Martin 2011, Asner et al. 2014*a*, 2015). The use of spectral data to assess species traits in order to understand their ecology, evolution, and distribution is called s*pectranomics* (Asner and Martin 2009). Although a relatively recent field of inquiry, spectranomics approaches have yielded important discoveries about how variation in foliar chemistry is associated with environmental gradients and is structured across tropical forests. For example, spectranomics approaches found that plant species with a similar phosphorus and calcium leaf content closely tracked edaphic and elevational gradients. By contrast, broad groups of plant metabolites related to defense (e.g., phenolics) exhibited high variation within sites (Asner et al. 2014*b*).

Plant secondary metabolites are known to represent a large energy investment for trees, up to 40% or more by dry weight (Lokvam and Kursar 2005, Salazar et al. 2018). Importantly, each tree species produces dozens of diverse secondary metabolites from different metabolic pathways that, individually and in combination, deter natural enemies such as fungal and microbial pathogens, viruses, and vertebrate and invertebrate herbivores (Richards et al. 2015, 2016). Assays to characterize these chemicals in the laboratory are expensive and time-consuming, and for this reason, only a few tropical tree genera and species have been studied in detail. Nevertheless, a more complete characterization of plant secondary metabolite diversity will allow us to look into this important black box and make advances in our understanding of the role of plant chemical defenses in community assembly. Toward this end, new untargeted metabolomics approaches have been developed to determine non-model plant species' secondary chemical traits in more efficient and informative ways (Richards et al. 2016, Salazar et al. 2016*a*, *b*, 2018, Sedio et al. 2017).

Here, we explore a new integrative approach that combines the scalability of spectranomics advances with a detailed plant metabolomics dataset. This proof of concept aims to determine the effectiveness of spectranomics approaches for capturing fine-grain chemical information from plant species. Specifically, in this study we use spectroscopic data and plant chemical foliar traits from multiple species from a common and diverse clade of tropical trees. The genus *Protium* (Burseraceae) comprising the formerly more narrowly circumscribed genus *Protium*, and the formerly recognized genera *Tetragastris* and *Crepidospermum* (Daly and Fine 2018), represents an ideal study system to investigate the link between chemical diversity and spectranomics. This group has a solid taxonomic foundation, with a well-supported molecular phylogeny that includes almost three quarters of published taxa

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(Fine et al. 2005, 2014). The Burseraceae (Frankincense and Myrrh family) are well known for the production of terpene resins, and *Protium* is no exception, producing a wide array of volatile and nonvolatile secondary compounds, including mono-, sesqui-, and triterpenes, flavones, oxidized terpenes, and quinic acid derivatives, among others (Lokvam and Fine 2012, Siani et al. 2012, Lokvam et al. 2015). Recently, Salazar et al. (2018) published a detailed characterization of more than 600 putative secondary metabolites from 32 species of *Protium*. Here, we combine metabolite data with phenolics data and visible-to-shortwave infrared (VSWIR) spectral data (collected in the Spectranomics Project; Asner et al. 2014*a*, *b*) from 19 of these same *Protium* species to investigate how foliar spectra correlate with specific secondary metabolites. We ask: Can a spectranomics approach inform our understanding of plant secondary metabolites within *Protium*? Are specific spectral bands associated with particular secondary metabolites or broader groups of chemical compounds?

Methods

We integrated three different datasets to explore these predictions: (1) total phenolics data collected from 19 species of *Protium* by the Spectranomics Project in Peru and Ecuador (hereafter "phenolics data"), (2) VSWIR (visible-to-shortwave infrared, 350–2500 nm) spectral data for all 19 species (also from the Spectranomics Project in Peru and Ecuador, hereafter "spectral data"), and (3) a metabolomics dataset from the same 19 species of *Protium* from the University of California, Berkeley Project in Peru (including GC-MS and HPLC-MS-DAD-ELSD; see below; hereafter "metabolomics data"). All of the samples from the Spectranomics Project had voucher specimens housed at the Carnegie Institution for Science (now at Arizona State University), and all of the samples from the Berkeley metabolomics project have voucher specimens housed at the University of California, Berkeley (UC). The first author reviewed all of the *Protium* vouchers from both projects and standardized (and updated) the taxonomic identifications to be able to compare species-level chemistry for the 19 *Protium* species across the two datasets (Daly and Fine 2011, 2018, Misiewicz and Fine 2014, Daly 2019). The Allpahuayo-Mishana Reserve near Iquitos Peru was the location where all of the *Protium* metabolomics data were collected. This same reserve was one of the field sites for the Spectronomics Project where phenolics and spectral data were collected. All of the other sites were in lowland Peru and Ecuador (see Appendix S1: Fig. S1, and Asner et al. 2014*a*, *b* for more details).

Protium *chemical characterization using metabolomics*

Chemical analysis was conducted at the University of California, Berkeley, using leaf samples of *Protium* trees collected from the Allpahuayo-Mishana Reserve near Iquitos, Peru. Young and mature leaf samples from 6 to 10 different individuals per species were collected and subjected to separate chemical analyses for high and low molecular weight metabolites. All details on the methodology of chemical characterization are available in a recent publication (Salazar et al. 2018).

Spectral data

Samples from the Spectranomics spectral dataset were processed as follows for foliar spectroscopic reflectance signatures. For each *Protium* species sampled, electromagnetic energy reflectance (hereafter "spectra") was measured on the adaxial surface of six randomly selected leaves per individual immediately after acquiring each canopy branch in the field. Multiple individuals per species were analyzed. The spectral measurements were taken at or close to the midpoint between the main vein and the leaf edge, and approximately halfway from the petiole to the leaf tip. Care was taken to avoid large primary or secondary veins, while allowing for smaller veins to be incorporated in the measurement. Each of the six reflectance spectra for an individual was averaged, and then, the spectra of all sampled individuals were also averaged to obtain an average species spectral signature. When possible, multiple individuals were sampled for each species (average 3 individuals per *Protium* species; see Appendix S1: Tables S1, S2).

The spectra were collected with a field spectrometer (FS-3 with custom detectors and exit slit configuration to maximize signal-to-noise performance; Analytical Spectra Devices, Boulder,

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Colorado, USA), an integrating sphere designed for high-resolution spectroscopic measurements, and an illumination collimator (Asner and Martin 2011). The spectrometer records in 2151 bands spanning the 350- to 2500-nm wavelength region, which includes the visible and near-infrared, and shortwave infrared portions of the electromagnetic spectrum. Measurements were collected with 136-ms integration time per spectrum. The spectra were calibrated for dark current and stray light, referenced to a calibration block (Spectralon; LabSphere, Durham, New Hampshire, USA) in the integrating sphere, resampled to 10-nm bandwidth, and trimmed to the 400–2500 nm range. The high-fidelity measurement capability of our system resulted in calibrated spectra that did not require smoothing or other filters. For this study, the 2150 bands were binned and then averaged into 210 larger bands in order to simplify the analysis and interpretation of the results.

Phenolics

Following spectral measurements, leaves were sealed in polyethylene bags to maintain moisture, stored on ice in coolers, and transported to a local site for processing within 3 h, and often <30 min. At the mobile laboratory, leaf disks (at least 15–30 per leaf) were immediately taken from 12 to 18 randomly selected leaves and transferred to −80°C cryogenic containers and then to climate-controlled −80°C freezers until chemical assays were performed in the laboratory. Leaf disks for the Spectranomics phenolics dataset were analyzed to calculate the total phenolics concentration of *Protium* individuals colorimetrically using the Folin-Ciocalteu method. All details on the methodology are available in a recent publication (Asner et al. 2014*a*).

Data analysis

All collection sites were primary rain forests. Sample sizes and locations of each individual are included in Appendix S1: Tables S1, S2. Although plant species were often sampled in multiple geographic locations (Appendix S1: Table S2), species exhibited very little intraspecific variation with respect to leaf chemistry (Asner et al. 2014*a*). However, given that the *Protium* individuals sampled for in the Spectranomics dataset were not the same sampled for the metabolomics dataset, we wanted to assess the equivalency between both sets of samples. Accordingly, we assessed the degree by which the species total phenolics data from the Spectranomics dataset predicted the species phenolics fraction data from the Berkeley dataset using a simple linear regression. Phenolics data were used here because it is the only comparable analysis between both datasets. It is important to note that these two broad measures of species phenolics composition were assessed via two very different assays and that the phenolics fraction assessed in the Berkeley dataset could contain other metabolites.

In order to determine how much information on plant foliar chemical composition can be obtained from foliar reflectance data, we used information on the interspecific variation in secondary chemical composition for particular chemicals across different *Protium* species to determine how the foliar content of specific secondary metabolites may be associated with the different spectroscopic foliar reflectance values across the visible–shortwave infrared electromagnetic spectrum (350–2500 nm; hereafter "spectral data"). Nevertheless, we emphasize that this particular approach limits the number of secondary compounds that can be used in the analysis, because the chemicals that are included in the analysis are based on high relative frequencies across the focal *Protium* species (i.e., found in multiple *Protium* species). For example, for compounds that are only present in one species of *Protium*, it would be impossible to determine whether the spectroscopic signal comes from the presence of a putative compound, or from any other associated traits or even from stochastic patterns. Similarly, if a particular secondary metabolite is only present in a very small fraction of the total number of *Protium* taxa, the likelihood of observing a false signal due to stochastic patterns among the compounds, the spectroscopic signal, and other unassociated traits would be relatively high. Thus, in order to have a robust and conservative analysis we decided to include in our analysis only those metabolites that are present in at least five of all the *Protium* species sampled. Furthermore, in order to reduce the noise resulting from the potential differences between the different individuals sampled by the Spectranomics and

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metabolomics datasets, the analysis was done at the species level, averaging spectral and metabolite data across all individuals of each species. The data were then cross-referenced to select the maximum number of *Protium* species with complete spectral and chemical data. This yielded a significantly smaller but very robust final dataset from the 19 *Protium* species of 45 secondary metabolites and 210 wavelength bands from the spectral data.

Using this curated dataset, we aimed to assess whether changes in the relative abundance of particular metabolites were mirrored by changes in the foliar light reflectance at specific wavelengths (Fig. 1). We expected that at least a subset of secondary metabolites present in the foliar tissues of our samples would change the amount of electromagnetic energy (light) reflected by the plant tissue on specific sections of the sampled spectra (hereafter "wavelength bands"). Furthermore, we expected that different species sharing the same metabolites would show similar foliar reflectance patterns at specific wavelength bands. Finally, we tested whether classes of similar secondary metabolites would also show similar patterns of reflectance across the sampled wavelength bands.

To evaluate these relationships, we correlated the changes in abundance of specific metabolites with the spectral data across all our study species similar to some molecular biology approaches where the intensity of the expression of specific metabolites can be linked to the presence of particular alleles (Saito et al. 2008). For each one of our 45 target metabolites, we correlated their per-species foliar abundances against each one of the 210 wavelength bands on our spectral data. This resulted in a total of 9450 individual correlations, each one with 19 data points corresponding to the 19 *Protium* species sampled (see Fig. 1). If there was an association between the spectral values and foliar metabolite abundance, we expected the correlation line to show a strong negative or positive slope. By contrast, if there was no relationship, we expected the correlation line to have a slope close to zero. It is important to emphasize that the slope of a correlation is not necessarily an indicator of statistical significance; nevertheless, the slope allowed us to determine how changes in the chemical composition of

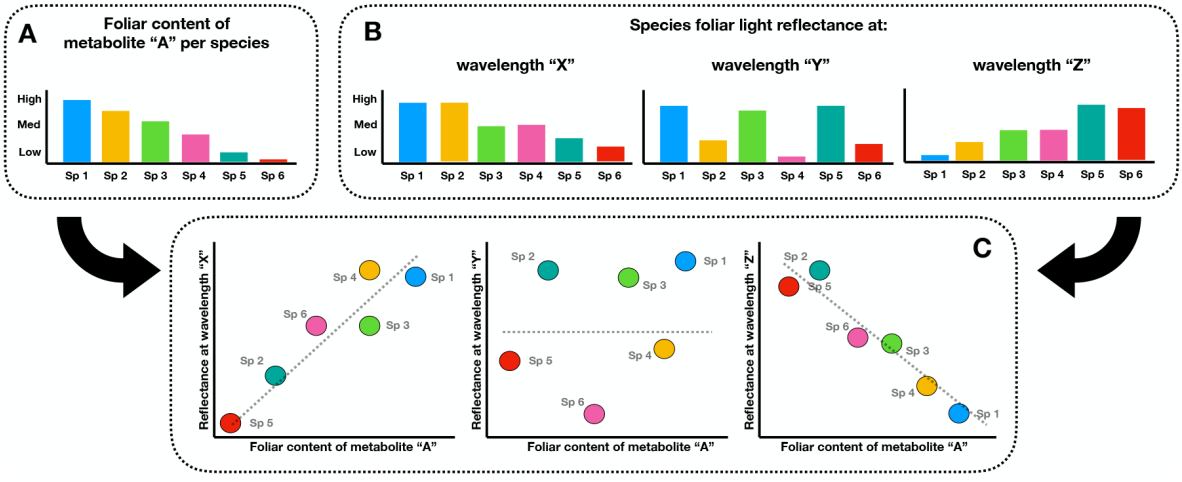

Fig. 1. Conceptual figure illustrating our analytical approach used to identify the association between foliar spectral data and leaf secondary metabolite composition from the metabolomics dataset. First, leaf samples from multiple species are analyzed to determine the concentration of a particular secondary metabolite (panel A). Second, leaf spectral data across a broad range of wavelengths are recorded for leaf samples for all species (panel B). Finally, the variation in metabolite composition across the sampled species is correlated with the variation in spectral data across all sampled wavelengths (panel C). By plotting the slope of the correlation line, one can identify associations between specific spectral bands and the leaf secondary chemical composition.

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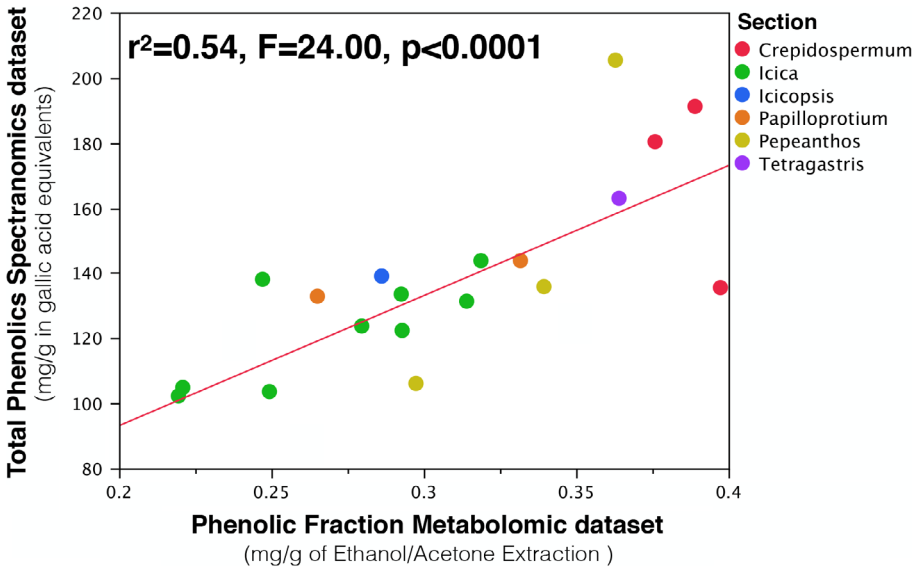

Fig. 2. Correlation between Spectranomics dataset measurement of total phenols (Folin-Ciocalteu test, milligram of gallic acid equivalents per gram of leaf) and metabolomics dataset of total mass of phenolics fraction (milligram of per gram of leaf). Colors of the dots correspond to the published taxonomic sections of *Protium* (Daly and Fine 2018).

Protium leaves were associated with the changes in their spectral profile (Fig. 1).

To assess whether similar secondary metabolites showed comparable patterns of foliar reflectance across the measured wavelength bands, we used the resulting data to construct a wavelength band per secondary metabolite matrix populated with the slopes of the 9450 correlations (values between −1 and 1). Next, we used this matrix to perform an unassisted hierarchical clustering analysis (Ward's algorithm) to determine whether similar metabolites clustered together based on their metabolite–reflectance association patterns. Here, we expected that similar secondary metabolites (e.g., sesquiterpenes) would likely affect a species foliar spectral profile in a similar fashion and thus group together.

Results

We found a very strong association ($R = 0.72$, $P < 0.001$) between the *Protium* species' total phenolics content from the Spectranomics dataset and the phenolics fraction from the metabolomics dataset (Fig. 2). This consistency was highly significant despite the difference in light environment (canopy versus understory), life stage (adult tree vs. juvenile sapling), and method to quantify phenolics in the two projects.

We found highly non-random correlation patterns between the spectral data and the foliar content of different secondary metabolites (Fig. 3). To help visualize the results of the analysis, the correlation slopes between each metabolite and all the sampled wavelength band signals are plotted in a heatmap of wavelength versus metabolite (Fig. 3, Appendix S2: Tables S1–S4). Colors on the heat map correspond to the association between the spectroscopic values (at specific wavelength bands) and the concentration of a specific metabolite independent of species identity. The color of the pixel is the strength of the association between wavelength band signal and the foliar metabolite abundance. Red colors represent a positive association (correlation slope close to 1), blue colors show a negative association (correlation slope close to −1), and light colors represent a weak association (correlation slope close to 0). Thus, for each metabolite, wavelength bands with a deeper shade of red indicate that *Protium* species with a higher concentration of a particular metabolite also show higher reflectance signals at this wavelength band than species that do not express this metabolite (or

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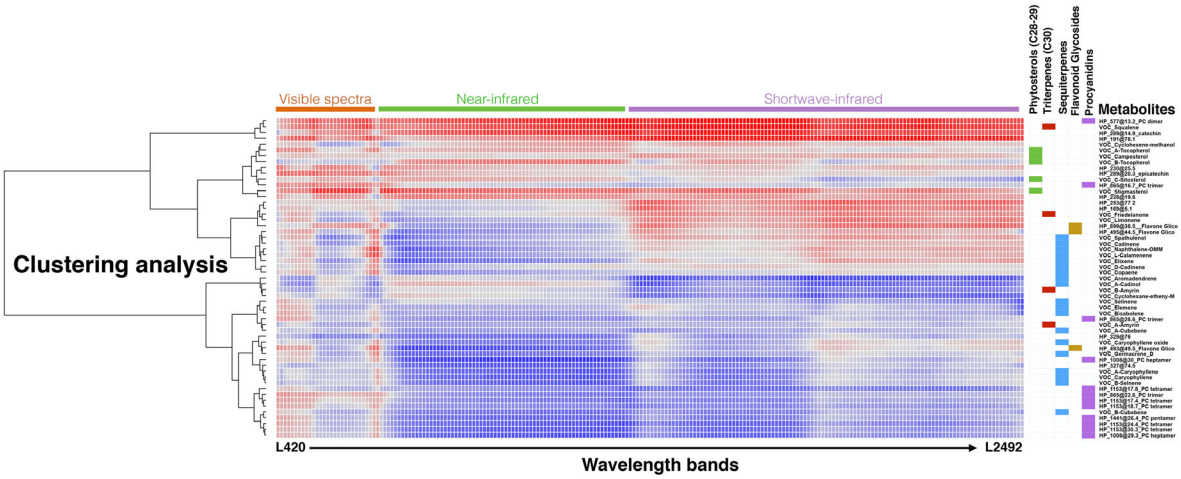

Fig. 3. Heat map displaying the association between spectral bands, grouped by wavelength in the spectral dataset, and specific metabolites from the metabolomics dataset. These correlations were derived from a *Protium* species metabolite-by-spectral band matrix. Red colors represent positive correlations between spectral bands and specific metabolites, and blue colors represent negative correlations. Darker colors indicate stronger correlations and lighter colors weaker correlations (see color scale bar at left). Left: Clustering analysis (Ward's algorithm) highlights four groups of chemicals that exhibit particularly strong signals: the phytosterols (C28-29), the sesquiterpenes, the triterpenes (C30), and the procyanidins.

only express it in very small amounts). Conversely, deeper shades of blue indicate that species with a higher metabolite foliar content will show lower reflectance signals at this wavelength bands than species that have a lower content of this particular metabolite. Finally, light shades suggest that particular wavelength bands are not informative with respect to foliar content of a specific secondary metabolite.

Next, we conducted a clustering analysis across all secondary metabolites to determine whether chemically similar secondary metabolites show similar spectral correlation patterns (Fig. 3). The results of the ordination (clustering analysis) showed a clear clustering pattern for some chemical groups. Although the clustering does not show perfect alignment of groups of chemical classes, each cluster is dominated by one class of secondary metabolites. For example, most of the procyanidin oligomers (condensed tannins) and all phytosterols are clustered in their own group and showed very similar spectral correlation patterns. Moreover, procyanidin oligomers showed a negative correlation with wavelength band values across almost the entire near-infrared and short infrared spectra (Fig. 3, violet boxes). However, some procyanidin compounds in our dataset were placed into four different clusters by Ward's algorithm, mainly due to differences in the visible part of the spectra (Fig. 3, violet boxes). Phytosterols showed a small positive correlation across the near-infrared and short infrared spectrum (Fig. 3). Conversely, although sesquiterpenes did not show a clear clustering pattern, most show a negative association at the near-infrared spectrum, a positive association between the 400 and 500 nm of the visible spectrum, and a negative association between the 510 and 650 nm of the visible spectrum. Interestingly, despite their high abundance in *Protium* tissues, triterpenes did not show any particular correlation pattern with any spectra.

Discussion

The results of our analysis indicate clearly that spectroscopic data contain valuable information about a plant species' secondary chemical composition although not all secondary metabolite groups showed an effect on VSWIR spectral signatures in our focal taxa. Our data also suggest

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that different compounds from the same chemical groups are also likely to affect the foliar VSWIR spectrum in a similar fashion.

We found a highly significant correlation between total phenolics investment from the Spectranomics dataset and the phenolics fraction from the metabolomics dataset. That species-level amounts of phenolics would be so highly correlated between the two datasets is surprising given the different environments these plants inhabit and the expected differences due to additional non-phenolics metabolites expected to be present in the phenolics fraction of the metabolomics dataset. All of the juvenile plants from the metabolomics project were between 1 and 2 m tall, and found in the shaded understory, while all of the leaves sampled for the Spectranomics dataset were sun leaves from adult trees that reach the canopy in the field. These results suggest that phenolics investment within a species is likely to be rather robust to environmental differences in light, nutrient, and water availability and stay reasonably constant from juvenile to adult life stages. This is remarkable given the different challenges that juvenile and adult trees face, ranging from different pathogens and herbivores (Basset 1999), not to mention the large differences in temperature and light availability, among other factors (Boege and Marquis 2005). We are aware of only one study that has compared the secondary metabolites of juvenile and adult tropical trees. Lokvam et al. (2015) studied *Protium subserratum* and found that while concentrations of procyanidins did exhibit variation between juveniles and adults, there were no qualitative differences in chemical composition. Such consistency of species-level foliar chemistry also agrees with recent studies of tropical trees that reported much less intraspecific than interspecific variation in foliar chemistry across heterogeneous environments (Asner et al. 2014*a*, *b*, Sedio et al. 2017).

Although defense chemistry could be expected to vary in response to different environmental conditions and different assemblages of natural enemies in different sites and times, several recent studies show that constitutive defenses such as phenolics and the other secondary metabolites we investigated in this study do not vary substantially in tropical woody plants species even in response to differences in microclimate, season, and across large geographic distances. Endara et al. (2018) reported consistent chemical investment and a readily identifiable chemocode in several *Inga* (Fabaceae) species that have large geographic distributions across five Neotropical sites in five different countries (Panama, Peru, Ecuador, Brazil, and French Guiana). Other studies of *Inga* and *Psychotria* (Rubiaceae) compared metabolomes of tropical woody plants and found little qualitative variation within species with respect to light environment (Sinimbu et al. 2012, Bixenman et al. 2016, Sedio et al. 2017), wet season versus dry season (Sedio et al. 2017), and induction by herbivory (Bixenmann et al. 2016). Although induction of defenses is well known from many annual plants and temperate trees that experience unpredictable outbreaks (Bixenmann et al. 2016), tropical woody plants must deal with a high and fairly predictable amount of attack from a large diversity of different natural enemies across their ranges and often have long-lived evergreen leaves. Perhaps in such circumstances, having a large diversity of defenses is the plant's best strategy against a large number of enemies that consistently attack a plant species (Bixenmann et al. 2016, Salazar et al. 2018).

It is clear that our study could be improved by measuring the VSWIR spectral signature in the same individuals and tissues used for the metabolomics analysis. Nevertheless, datasets that include the VSWIR or the secondary metabolite profiling of a large number of species are not common, especially with multiple individuals for each species; therefore, the combination of these two large datasets represented a great opportunity to explore this approach to link metabolomics and spectranomics.

We also found a significant association between the presence of specific secondary metabolites and the VSWIR spectral signature of *Protium* species, even though the two datasets comprised samples from different individuals and different life stages. This result suggests that molecules that have important functional attributes could be identified by VSWIR spectroscopy, which would save a lot of time and expense as compared to wet laboratory assays (Asner et al. 2014*b*). Chemists have used spectral analysis to identify chemical compounds since the pioneering work of Gustav Kirchhoff and

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Robert Bunsen in the 1860s. Even today, chemists use chromatographic detectors such as the diode array detector (DAD) to determine the chemical spectral properties of specific secondary metabolites. Similarly, our finding that different compounds will have similar spectral properties compared to other compounds in the same chemical groups is a well-known fact among chemists. Nevertheless, although chemists have used these spectral approaches on extracted and purified plant metabolites or in combination with separatory chromatographic approaches (HPLC or TLC), our data suggest that it is possible to detect the presence of at least some secondary metabolites in situ, within the tissues of a plant, and without a preliminary extraction or isolation, despite the presence of dozens of other chemical compounds and proteins.

It is likely that metabolites that are clustered together in Fig. 3 like all of the different kinds of procyanidin oligomers have a relatively similar and direct effect on the spectroscopic characteristics of the leaf, and that this effect is related to the common structure and functional groups present in these compounds. Alternatively, the common association that these compounds have with the leaf's spectroscopic characteristics could also be due to shared metabolic pathways and the chemical precursors involved in these pathways. In contrast to procyanidin oligomers and phytosterols, sesquiterpenes and triterpenes did not show consistent spectroscopic patterns despite the fact that individual compounds from these chemical classes did show distinctive patterns of spectroscopic absorbance or reflectance. This could suggest that the association between the presence of these metabolites and the patterns of the leaf spectroscopic data could be indirect (via a correlation with other leaf traits) or could be due to important differences in molecular structure across sesquiterpenes or triterpenes. For example, some terpenes are cyclic, while others are non-cyclic, among many different kinds of structural variation present in terpene scaffolding. Approaches using liquid chromatography–tandem mass spectrometry (LC-MS/MS) networking (Sedio 2017) could be used to test the hypothesis that correlations between spectra and functional group are influenced by the intrinsic differences in molecular structure within these groups.

Because the leaf reflectance spectrum is an integrated measure of the metabolites in the whole leaf, we do not know the relative contribution of compounds located in various leaf parts (i.e., surface waxes, resin canals, the leaf matrix), and more in-depth studies will be needed to determine the mechanisms underlying these patterns. Furthermore, we believe that a tailor-made study using these techniques on the exact same set of leaves, designed specifically to determine the association between secondary metabolites and the VSWIR spectrum, is needed to best assess how much information about a plant's secondary metabolite composition can be detected within its VSWIR spectral signature. The ideal dataset would be one where intra- and interspecific variation of secondary chemistry could be used to evaluate the relationship between VSWIR and plant metabolite composition by sampling multiple individuals of many species using both VSWIR and the total metabolomics approach on the same tissues from the same individuals. This would allow quantifying the independent effect of each metabolite on VSWIR patterns. These data could also facilitate the efficacy of using VSWIR data with statistical learning classification models (e.g., random forest, stochastic gradient boosting; Hastie et al. 2009) to quantify both plant species metabolite composition and abundance. The analysis shown in Fig. 3 strongly suggests that this approach will be fruitful, and we will be able to identify many molecules of functional significance with high-resolution reflectance spectra.

The spectral data analyzed in this paper were manually collected from each tree and analyzed in a field laboratory. However, the Carnegie Airborne Observatory (Asner et al. 2012), which is the airborne system used to develop and apply the spectranomics approach, collects spectral data from 2000 to 5000 m above ground level that yield results showing signals consistent between field- and laboratory-based approaches (Asner et al. 2015). These spectral data can be combined with other remote-sensing approaches to identify tree species (e.g., Baldeck et al. 2015, Paz-Kagan et al. 2017). Our results here suggest that even within one diverse clade of closely related trees, sufficient variation exists in spectral–chemical signatures to discriminate among different species of *Protium* with remote-sensing technology.

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These results have profound implications for future research. The speed and power of spectranomics approaches means we could amass extremely large datasets of plant defense chemistry for many species. These can be combined with studies documenting the feeding records of insect herbivores or fungal pathogens at broad scales to understand how variation in foliar chemistry is partitioned within and among species across geographic gradients, and how variation in foliar chemistry may be driven by variation in assemblages of natural enemies. Being able to use spectranomics to inform remote sensing may allow us to quantify species' geographic distributions and relative abundance at unprecedented levels of detail, giving us the power to understand the role of foliar chemistry in maintaining tree species diversity and driving commonness and rarity of tropical trees across multiple scales.

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