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Research

Functional and biological diversity of foliar spectra in tree canopies throughout the Andes to Amazon region

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Summary

- Spectral properties of foliage express fundamental chemical interactions of canopies with solar radiation. However, the degree to which leaf spectra track chemical traits across environmental gradients in tropical forests is unknown.
- We analyzed leaf reflectance and transmittance spectra in 2567 tropical canopy trees comprising 1449 species in 17 forests along a 3400-m elevation and soil fertility gradient from the Amazonian lowlands to the Andean treeline. We developed quantitative links between 21 leaf traits and 400–2500-nm spectra, and developed classifications of tree taxa based on spectral traits.
- Our results reveal enormous inter-specific variation in spectral and chemical traits among canopy trees of the western Amazon. Chemical traits mediating primary production were tightly linked to elevational changes in foliar spectral signatures. By contrast, defense compounds and rock-derived nutrients tracked foliar spectral variation with changing soil fertility in the lowlands. Despite the effects of abiotic filtering on mean foliar spectral properties of tree communities, the spectra were dominated by phylogeny within any given community, and spectroscopy accurately classified 85–93% of Amazonian tree species.
- Our findings quantify how tropical tree canopies interact with sunlight, and indicate how to measure the functional and biological diversity of forests with spectroscopy.

Introduction

The spectroscopy of foliage has long been recognized as fundamental to understanding the interaction of plants with solar radiation, as well as for monitoring vegetation with remote sensing (Gates *et al.*, 1965; Goetz *et al.*, 1985). Spectral reflectance and transmittance properties of leaves are determined by plant chemical adaptations to environmental conditions including climate, nutrient availability and biotic interactions. Foliar nitrogen (N), photosynthetic pigments including chlorophylls and carotenoids, structural compounds such as lignin and cellulose, soluble carbon (C), and water largely control foliar spectral properties (Curran, 1989). Additional nutrients such as phosphorus (P), base cations (calcium (Ca), potassium (K), and magnesium (Mg)) and a suite of micronutrients play an indirect role in determining foliar spectra (Ustin *et al.*, 2004). An overarching additional control is leaf mass per unit area (LMA; units of $g\ m^{-2}$), which expresses a trade-off between the energetic cost of leaf construction and the achieved light intercepting area (as reviewed by Poorter *et al.*, 2009). Long-term adaption of plants to particular environmental conditions may also impart phylogenetic patterns in these chemical traits (Kursar *et al.*, 2009), which could translate to phylogenetic patterns in foliar spectral properties. Currently, however, the linkages between environment, phylogeny, and the spectroscopy of plant foliage remain poorly understood for most ecosystems.

Humid tropical forests blanket an enormous range of environmental conditions mediated by geologic substrate, soils, elevation and climate. Recent studies highlight the interplay between these environmental factors and phylogeny in creating chemical diversity among tropical forest canopies (Fyllas *et al.*, 2009). At the growth-form level, canopy trees invest more in chemicals supporting longevity and defense, such as lignin, cellulose and polyphenols, than do canopy lianas (Asner & Martin, 2012). By contrast, lianas invest more than trees in light capture and growth chemicals such as N, P, chlorophyll and carotenoids, but they do so primarily in warmer and sunnier environments. Within a given growth form, soil fertility also imparts major differences in foliar chemical investment. For example, dystrophic soils common to tropical lowlands in Amazonia contain tree species that invest more in longevity and defense chemicals than do other tree species on neighboring high-fertility soils (Fine *et al.*, 2006).

A recent study of thousands of tree species in Peru revealed that 3400 m of elevation gain from the Amazonian lowlands to the Andean treeline imparts a nested pattern in forest canopy chemical traits (Asner *et al.*, 2014). This pattern links variation in soils and elevation to rock-derived foliar nutrients, and foliar nutrients to divergent strategies of C and defense compound

allocation among co-existing species. Despite strong elevation-dependent changes in multiple foliar chemicals, the phylogenetic organization of chemical traits remains very strong in any given forest type along the Andes-to-Amazon elevation gradient. Given the existence of these chemical variations within and across Amazonian communities, we think that a similar pattern may exist in leaf optical traits, if the spectra are quantitatively linked to chemistry. Moreover, if the optical properties of canopy foliage track both environmental and taxonomic variation in forest composition, then the spectra may allow us to quantify functional and biological diversity from leaf-, canopy- and stand-level remote sensing. Currently, however, the degree to which leaf spectral properties track changes in chemical traits and taxonomic composition on tropical elevation and soil gradients is unknown.

We analyzed leaf reflectance and transmittance spectra at 17 forest sites along an Andes-to-Amazon elevation and soil gradient described by Asner *et al.* (2014). We asked: How do leaf spectra change across tree communities at different elevations and on different soils? How does the optical variability within species (intra-specific) compare to the variability between them (inter-specific), at site and regional levels? Do the leaf spectra quantitatively represent the chemical traits in canopy leaves collected along elevation and soil fertility gradients? For chemical traits linked to the spectral properties of Amazonian canopy leaves, how strong a role does phylogeny play in explaining the variation?

Materials and Methods

Field sites

We collected top-of-canopy leaf samples from 2567 individual trees comprising 1449 species (of these species, 557 had two to 13 replicates) in 17 forests arrayed by elevation and soil type in northern, central and southern Peru (Table 1, Supporting Information Table S1). Our collection represents the majority of canopy tree species found throughout the western Amazon (Gentry, 1993). Along the elevation gradient, mean annual precipitation varies from 2448 to 5020 mm yr^{-1}. Mean annual temperature changes from 26.6°C in the warmest lowland Amazonian site to 8.0°C at the Amazonian treeline in the Andes. There is a negative linear relationship between mean annual temperature and elevation (Table 1).

Soils at elevations > 600 m are comprised of relatively high-fertility Inceptisols and Entisols (Table 1). In the lowlands (< 600 m above sea level (asl)), soils vary among three taxonomic orders: Ultisols on low-fertility *terra firme* clay substrates, Inceptisols on inactive high-fertility floodplains of late Holocene age, and Entisols in two regions in northern Peru. The lowland Entisols are white sand substrates associated with very low nutrient availability (Fine *et al.*, 2004). We analyzed the canopy foliage with respect to all sites, as well as when considering only higher fertility substrates. These higher fertility sites have a history of scientific study (Quesada *et al.*, 2009), indicating that they can be treated as nutrient-rich relative to the remaining lower fertility sites. Our delineation of a subset of higher fertility sites is also supported by site-averaged foliar N : P values (Table 1); N : P ratios below 14–16 suggest weak P limitation of primary production (Townsend *et al.*, 2007).

Leaf collections

Our sampling strategy focused on exhaustive surveys of sunlit canopy tree species, both common and rare, over forest community areas of up to 600 ha, directed by historical surveys from

Table 1 Description of 17 sites studied for tree foliar spectroscopy and chemistry in the Andean-Amazon region, sorted by mean elevation above sea level (asl)

Site name	Code	Center latitude	Center longitude	Elevation (m)	MAP (mm)	MAT (°C)	Soil order	Foliar N : P
Allpahuayo 1	ALP1	−3.963324	−73.42315	123	2760	26.3	Ultisol	23.3
Jenaro Herrera 1	JH1	−4.899687	−73.65045	124	2700	26.6	Ultisol	27.9
Jenaro Herrera 2	JH2	−4.902867	−73.63391	124	2700	26.6	Entisol	22.7
Jenaro Herrera 3*	JH3	−4.912412	−73.72774	124	2700	26.6	Inceptisol	12.2
Allpahuayo 2	ALP2	−3.963117	−73.42817	130	2760	26.3	Entisol	21.8
Inkaterra*	INK	−12.53272	−69.04776	180	2600	24.7	Inceptisol	13.6
Tambopata 1	TAM	−12.96661	−69.48691	213	2600	24.0	Ultisol	16.9
Los Amigos 1*	LA1	−12.56920	−70.09325	235	2700	24.0	Inceptisol	13.8
Los Amigos 2	LA2	−12.56019	−70.10145	260	2700	24.0	Ultisol	19.8
Paujil 1	PJ1	−10.32572	−75.26213	420	5020	23.1	Ultisol	24.3
Paujil 2	PJ2	−10.33080	−75.26130	632	5020	23.1	Entisol	30.3
Huampal*	HPL	−10.18659	−75.57680	1040	2380	22.6	Inceptisol	12.9
San Pedro 1*	SP1	−13.05084	−71.53432	1500	4628	18.5	Inceptisol	14.4
San Pedro 2*	SP2	−13.04718	−71.54083	1618	4341	18.5	Inceptisol	14.6
Tres Cruces 1*	TC1	−13.10920	−71.60148	3093	2678	13.0	Inceptisol	11.6
Tres Cruces 2*	TC2	−13.11188	−71.60691	3370	2448	13.0	Inceptisol	8.7
Tres Cruces 3*	TC3	−13.12909	−71.61689	3650	2448	8.0	Inceptisol	10.9

Soil orders follow the US Department of Agriculture (USDA) soil taxonomy system. See Supporting Information Table S2 for taxon identifications for all study sites. An asterisk (*) is placed next to site names considered to be 'higher fertility' in this study, as indicated in the literature and supported by our average foliar nitrogen: phosphorus (N : P) ratios shown. Mean annual temperature (MAT; °C) is negatively related to elevation across the study sites (MAT = 26.1 − 0.004 × elevation; $R = -0.96$; $P < 0.001$; Hijmans *et al.*, 2005). MAP, mean annual precipitation.

similar locations (Gentry, 1988). Only fully sunlit top-of-canopy leaves were included in this study because many foliar spectral and chemical traits are highly sensitive to vertical light gradients within forests (Poorter *et al.*, 1995, 2009; Niinemets & Fleck, 2002). Combining sun and shade leaves confuses spectral and chemical trait comparisons within species, among species, and between communities. Leaf collections were conducted using tree-climbing techniques with strict leaf selection standards. Specimen vouchers were prepared and matched to type specimens kept at the National Agrarian University La Molina Herbarium in Peru, Missouri Botanical Garden and Kew Botanic Gardens. Taxonomy followed the Angiosperm Phylogeny Group 3 (Stevens, 2001–present), which utilizes available genetic information.

The foliar database incorporates 102 families, 393 genera and 1449 species (Table S2). Because of high species turnover between forest communities, the taxonomic partitioning within the sites ranged from six to 53 families, from seven to 142 genera and from eight to 268 species. Analyses of intra-specific variation were performed on a subset of 557 species containing two to 13 individuals. Detailed information for all taxa and sites is provided on the Carnegie Spectranomics website http://spectranomics.ciw.edu.

Leaf spectroscopy

Hemispherical reflectance and transmittance were measured on the adaxial surface of six randomly selected leaves immediately after acquiring each canopy branch in the field. The spectral measurements were taken at or close to the mid-point 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 were averaged; the same was done with each set of six transmittance spectra.

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, Inc., Boulder, CO, USA), an integrating sphere designed for high-resolution spectroscopic measurements, and an illumination collimator (Asner & Martin, 2011). The spectrometer records in 2151 bands spanning the 350–2500-nm wavelength region. 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 Inc., Durham, NH, 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.

Chemical assays

Branches of mature leaves were sealed in polyethylene bags in the field to maintain moisture, stored on ice in coolers, and transported to a local site for processing within 3 h, and usually < 30 min. A subset of leaves was selected from the branches for scanning and weighing. Leaf area was determined on a 600 dots-per-inch flatbed top-illumination optical scanner, using enough leaves to fill 1–2 scan areas each of 21 cm × 25 cm (up to *c.* 75 leaves per sample depending on leaf size). Petioles were removed from each leaf before scanning, and mid-veins were removed when they exceeded 1 mm in diameter. Leaves exceeding the surface area of the scanner were cut into sections until 1–2 full scan areas were completed. The scanned leaves were dried at 70°C for 72 h before dry mass (DM) was measured. LMA was calculated as g DM m^{-2}. From a subset of leaves, leaf discs (at least 30 per leaf) were immediately taken from 12 randomly selected leaves and transferred to −80°C cryogenic shipping containers. The remaining leaves were detached from the branches and subsamples were selected to represent the range of colors and conditions found among all leaves collected. When epiphylls were encountered, they were removed, along with dust and debris, before drying in mobile ovens at 70°C for 72 h followed by vacuum sealing for transport.

Chemical analysis protocols, along with instrument and standards information, are detailed in Asner *et al.* (2014) and in documents available on the Carnegie Spectranomics website (http://spectranomics.ciw.edu), which are summarized here. Dried foliage was ground in a 20-mesh Wiley mill. Concentrations of P, Ca, K, Mg, boron (B), iron (Fe), manganese (Mn), and zinc (Zn) were determined on 0.4 g of dry leaf tissue by inductively coupled plasma spectroscopy (ICP-OES; Therma Jarrel-Ash, Waltham, MA, USA) after microwave digestion (MARSXpress; CEM, Matthews, NC, USA). Carbon fractions including lignin, cellulose, hemi-cellulose and soluble C (composed of amino acids, pectins, simple sugars, starch and waxes) were determined in 0.5 g of dry ground leaf tissue using sequential digestion of increasing acidity (Van Soest, 1994) in a fiber analyzer (Ankom Technology, Macedon, NY, USA). A subset of the ground material was further processed to a fine powder for the determination of total C and N concentrations by combustion-reduction elemental analysis (Costec Analytical Technologies Inc., Valencia, CA, USA).

Photosynthetic pigment concentrations (chlorophyll *a* and *b* and total carotenoids) were quantified using two frozen leaf discs (0.77 cm^2 area each). Following preparation, the absorbance of the supernatant was measured using a UV-VIS spectrometer (Lambda 25; Perkin Elmer, Beaconsfield, UK). Chlorophyll *a* and *b* and carotenoid concentrations were calculated using multi-wavelength analysis (Lichtenthaler & Buschmann, 2001) at 470, 645, 662, and 710 nm. We report total chlorophyll as the sum of chlorophyll *a* and *b*. Additional frozen leaf discs were used for the total phenolic and tannin determinations using the Folin–Ciocalteau method (Ainsworth & Gillespie, 2007).

Analyses

Our analysis follows Fig. 1, showing the spectranomics approach introduced by Asner & Martin (2011). The approach incorporates the full spectral information embedded in each leaf reflectance and/or transmittance measurement into chemical and

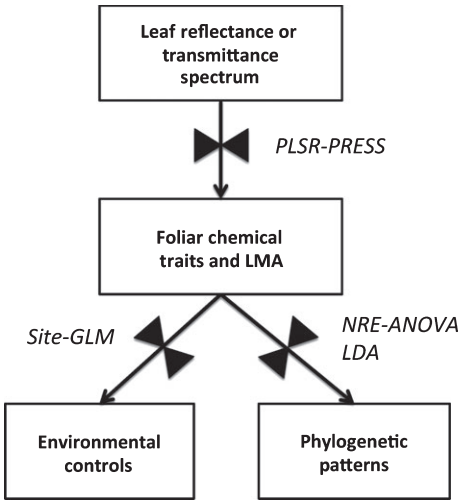

Fig. 1 Schematic of the spectranomics approach. The analysis begins with high-fidelity leaf spectral reflectance and transmittance measurements spanning the 400–2500-nm wavelength range. Partial least squares regression (PLSR) with predicted residual sum of squares (PRESS) analysis is used to convert the leaf spectra from individual trees to multiple foliar chemical traits and leaf mass per unit area (LMA). Spectroscopically derived chemical traits and LMA are compiled by species at the site level (Table 1), and general linear modeling (GLM) is used to assess environmental drivers such as elevation and soil fertility. Nested random effects–analysis of variance (NRE-ANOVA) and linear discriminant analysis (LDA) are used to assess the phylogenetic structure of individual and multiple chemical traits, respectively.

functional trait diversity analysis. An important difference between our approach and others such as band-by-band spectral indices is that it depends on the relative absorption and scattering features expressed across the entire spectrum (Kokaly *et al.*, 2009). When applied using high-fidelity spectrometers and laboratory chemical assays, our approach yields accurate and consistent results within and across vegetation types and ecosystems (Asner *et al.*, 2011).

An important first step in the spectranomics approach involves the conversion of each leaf reflectance and transmittance spectrum to an estimate of multiple chemical concentrations and LMA. This is achieved using partial least squares regression with predicted residual sum of squares (PLSR-PRESS) analysis (Fig. 1). This chemometric method has emerged as a viable approach for chemical interpretation of spectroscopic data (Smith *et al.*, 2002; Townsend *et al.*, 2003; Martin *et al.*, 2008). We used PLSR (Haaland & Thomas, 1988; Feilhauer *et al.*, 2010) to determine which leaf traits are quantitatively determined by spectroscopy. The PRESS step minimizes exposure to statistical over-fitting (Chen *et al.*, 2004), which has been a problem in past studies (Kokaly, 2001). The PRESS statistic was calculated by iteratively generating models while reserving 10% of the samples from the input data set until the root mean square error (RMSE) for the PRESS statistic was minimized. Following conversion of the leaf reflectance and transmittance spectra to chemical traits and LMA, the results were averaged at species, genus, family, and site levels. It is critically important to recognize that we are working with chemical traits and LMA derived from leaf spectra, which we term 'spectranomic traits'.

We assessed spectranomic trait differences among the lowland lower and higher fertility sites (Table S1). The need for this was made apparent in preliminary observations of a potential dichotomy between the lowland spectra based on soil fertility. Next we applied single-variable general linear models (GLMs) to examine the relationship between elevation and spectranomic traits (Fig. 1). We did not assess temperature or precipitation as factors independent of elevation. Temperature is strongly correlated with elevation, as shown earlier, and precipitation, though not correlated with elevation, did not explain variation in canopy chemistry in a previous study (Asner *et al.*, 2014).

With the goal of examining how variance in foliar spectranomic traits can be explained by phylogenetic grouping, we developed nested ANOVA models with random effects using the Residual Maximum Likelihood package in R (lme4; Faraway, 2005; Bates & Maechler, 2009). We included the phylogenetic levels of family (f), genus nested within family (g), and species nested within genus within family (s), as well as an environmental component incorporated as site (T). All effects were treated as random. In each model, y is any spectranomic trait for each canopy sample. This value was modeled as the sum of the mean value for the entire data set μ (or subset, when specified), the nested genetic effects (family i, genus j within family i, and species ijk within genus j), the site effect (T), and the residual error of the measurement e:

$$y = \mu + f_i + g_{ij} + s_{ijk} + T_l + e_{ijkl} \quad \text{Eqn 1}$$

The total variance about the mean for a given trait was therefore quantitatively parsed into the variance explained by families (σ^2_f), genera within families (σ^2_g), species within genera (σ^2_s), site (σ^2_T), and specimens within species (σ^2_e):

$$\sigma^2_{\text{total}} = \sigma^2_f + \sigma^2_g + \sigma^2_s + \sigma^2_T + \sigma^2_e \quad \text{Eqn 2}$$

If in a given model, the residual (σ^2_e) accounted for a high percentage of the total variance, then we concluded that site characteristics and taxonomy did not explain the data well.

One limitation of this analysis is that it describes the overall variation explained by each input variable. Not all taxa have equal variance; some may have tightly clumped chemical signatures whereas others may vary widely. This analysis will not pick up such trends, but instead it quantifies the overall pattern of phylogenetic grouping or lack thereof relative to site and residual effects. Previous work successfully tested this approach for phylogenetic partitioning analyses of chemical traits (Fyllas *et al.*, 2009).

Finally we used linear discriminant analysis (LDA) on the subset of data containing two or more individuals per species to understand how combinations of spectranomic traits relate to taxonomic identity. In LDA, the independent variables are the predictors and the dependent variables are the groups, thereby permitting the use of multiple chemical variables in combination to explain taxonomic groupings. LDA is ideal for this analysis because, in contrast to logistic regression where the classification variable is random and predicted by the continuous variables,

LDA classifications are fixed and the groupings are explained by a set of random variables. LDA was performed using a common covariance matrix and Mahalanobis distance as the distance measure. LDA calculates the distance between each point and the group multivariate mean, and then classifies the point to the closest group. To understand the relative significance of chemical variables in explaining taxonomic grouping, we performed LDA in a stepwise fashion, recording the percentage of the data set correctly classified with the addition of each chemical variable into the model. The ordering of chemical variables into the analysis was determined automatically by comparing and maximizing the classification power of each leaf property in combination with other properties at each step in the analysis. The chemical variable best explaining the grouping was entered first into the model, the second most important was entered second, and so forth, until all variables were entered or the significance level of the model reached 95%.

Results

Variation in leaf spectral properties

Leaf reflectance and transmittance variation was enormous among individual species collected throughout the region (Fig. 2a,b). Variation peaked in the near-infrared (NIR; 720–1400 nm) region, followed by strong variation in the shortwave-infrared (SWIR; 1400–2500 nm), and was lowest in the visible wavelength region (400–700 nm). After the spectra were averaged to the site level, the highest absolute spectral sensitivity to our regional-scale gradient of soils and elevation was observed in the SWIR (Fig. 2a,b). The NIR followed the SWIR in terms of overall sensitivity to the environmental gradient. In comparison, absolute sensitivity was low in the visible region. Variation within sites, shown as the standard deviation of reflectance and transmittance (Fig. 2c,d), was also highest in the NIR and SWIR, and much lower in the visible region. The highest elevation sites (TC1–TC3) harbored trees with the highest NIR and lowest SWIR reflectance, as well as systematically lower NIR and VSWIR transmittance (Fig. 2a,b). However, the variance in the NIR and portions of the SWIR was also higher in these upper elevation forests (Fig. 2c,d).

Inter-specific variation in leaf reflectance was wavelength dependent (Figs 3a, S1 for 400–800 nm), with maximum values of 23% expressed as coefficients of variation (CVs) in the SWIR range among co-existing species occurring in the highest elevation forests (TC1–TC3). On average, inter-specific variation in transmittance was 200–300% greater than that of reflectance (Figs 3b, S1 for 400–800 nm). Again the greatest transmittance variability among species was observed in the highest elevation forests, and in the SWIR region, with additional variation in the visible spectrum. Intra-specific variation in reflectance and transmittance averaged 50% lower than inter-specific variation (Figs 3c,d, S2). While there was no clear pattern in this ratio with elevation, the highest elevation forests tended to harbor tree species with the most distinctive leaf spectral properties, as indicated by relatively low ratios of intra- to inter-specific variation (Fig. S2).

Chemical determinants of leaf spectral properties

Our data revealed high levels of foliar chemical and LMA variation both within and between forested sites throughout the region (Table S3). Leaf traits showing the widest relative distributions among sites included LMA, P, Ca, K, Mg, lignin, phenols, cellulose, soluble C, and all micronutrients (B, Fe, Mn, and Zn). Within-site variation (expressed as standard deviations in Table S3) was also high, yet it represented just 25–50% of the variation found across sites.

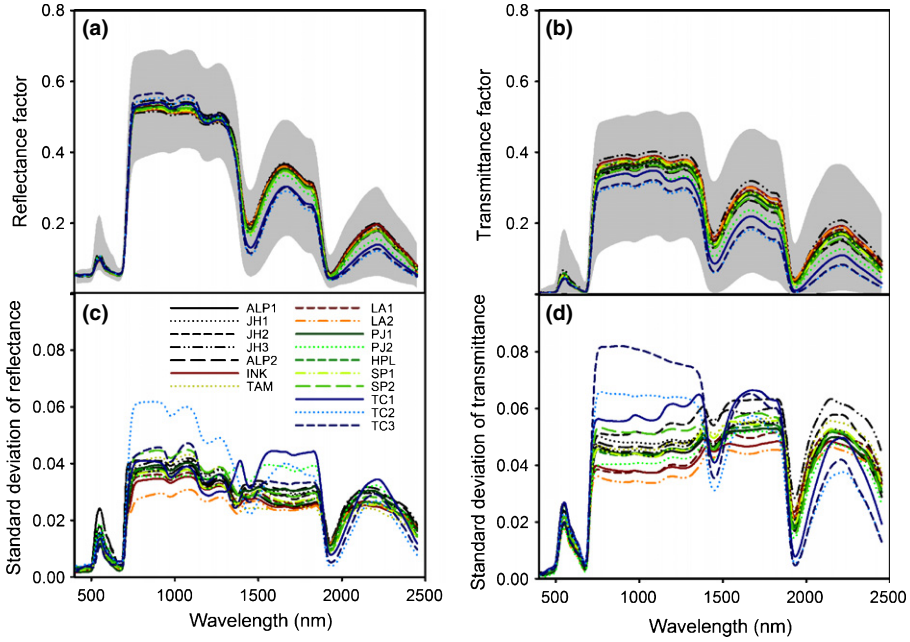

Fig. 2 High-fidelity leaf (a) reflectance and (b) transmittance spectra for trees measured in each site spanning gradients of elevation and soil fertility (Table 1). The colored lines are the mean values for each site. Site codes are listed with full names provided in Table 1. The gray areas indicate the total range among all species at all sites. Lower panels show the standard deviation of leaf (c) reflectance and (d) transmittance.

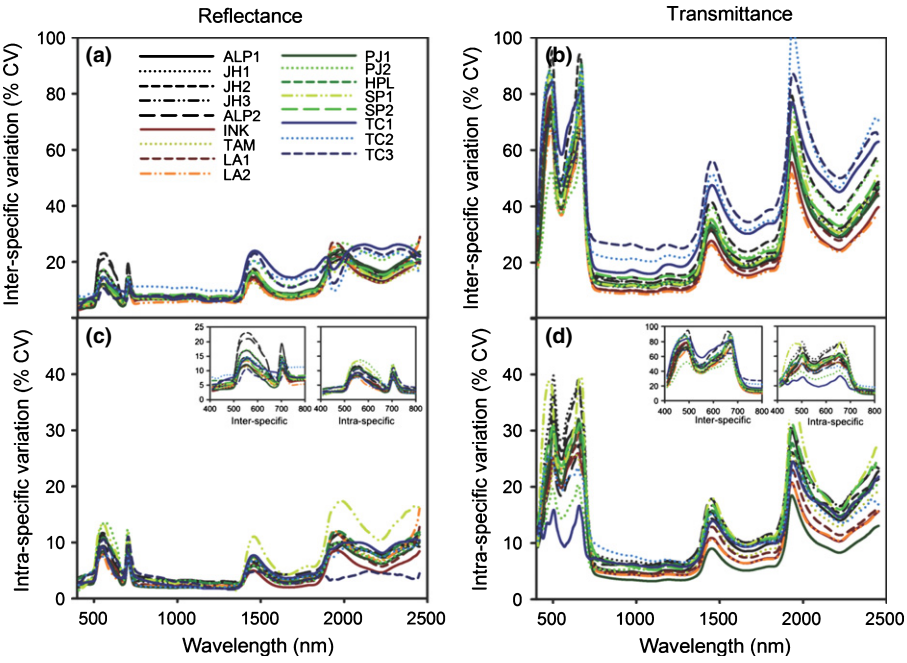

Fig. 3 (a) Intra-specific and (b) inter-specific variation in leaf reflectance spectra at the site level. Site codes are listed with full names provided in Table 1. (c, d) Similar for leaf transmittance spectra. Insets show the inter- and intra-specific variation of reflectance and transmittance from 400 to 800 nm (also included in more detail in Supporting Information Fig. S1).

The highly varying foliar chemical traits and LMA provided input to the PLSR-PRESS models developed for each of the 2567 individual trees surveyed (Table 2). These analyses revealed multiple quantitative links between leaf reflectance and transmittance signatures and foliar traits. Performances of chemical predictions were similar for reflectance- or transmittance-based models, and the PLSR weightings or latent vectors indicated that most of the spectrum was required to predict the chemical traits and LMA (Figs S3, S4). There were approximately three levels of performance in the PLSR-PRESS models (Table 2). Traits retrieved with high precision (high R^2; > 0.65) and high accuracy (low RMSE; 0–20%) included LMA, water, N, P, total chlorophyll, total and soluble C, cellulose, and Mg. Traits estimated with high precision but lower accuracy (RMSE > 20%) were

Table 2 Performance of leaf reflectance and transmittance spectral estimates of chemical traits and leaf mass per unit area (LMA) in trees of the Andes-to-Amazon region using partial least squares regression (PLSR) analysis

	Reflectance				Transmittance			
	R^2	RMSE	%RMSE	No. of PVs	R^2	RMSE	%RMSE	No. of PVs
LMA	0.87	0.11	2.5	75	0.89	0.10	2.3	91
Water	0.88	2.66	4.6	48	0.89	2.50	4.3	70
Nitrogen (N)	0.81	0.14	21.2	93	0.84	0.13	19.5	94
Phosphorus (P)	0.68	0.28	12.3	81	0.69	0.27	11.9	75
Chlorophylls	0.77	0.17	9.0	54	0.78	0.17	8.8	46
Carotenoids	0.72	0.17	44.9	43	0.73	0.17	43.6	39
Phenols	0.72	28.33	26.7	55	0.72	28.18	26.6	58
Tannins	0.59	16.86	35.5	57	0.58	17.19	36.2	38
Total carbon	0.74	1.69	3.4	80	0.77	1.59	3.2	79
Soluble carbon	0.66	6.59	15.1	81	0.68	6.36	14.6	86
Hemi-cellulose	0.63	2.97	25.4	77	0.63	3.00	25.3	69
Cellulose	0.81	2.35	12.4	93	0.85	2.11	11.1	88
Lignin	0.67	5.79	22.7	86	0.68	5.68	22.3	83
Calcium (Ca)	0.78	0.53	101.9	96	0.77	0.53	103.6	98
Potassium (K)	0.61	0.33	73.2	72	0.62	0.32	71.9	87
Magnesium (Mg)	0.70	0.32	20.9	60	0.71	0.32	20.7	74
Boron (B)	0.39	0.48	16.6	49	0.42	0.47	16.2	56
Iron (Fe)	0.58	0.33	8.7	34	0.61	0.31	8.3	78
Manganese (Mn)	0.34	1.09	24.8	47	0.39	1.05	23.9	89
Zinc (Zn)	0.26	0.48	19.5	46	0.30	0.47	18.9	81

The regression coefficient, root mean square error (RMSE), RMSE as a percentage of the leaf trait (%RMSE), and the number of predictor variables (PVs) determined for each trait are given.

carotenoids, phenols, and Ca. Those retrieved with lower yet still reasonable precision and accuracy were tannins, hemi-cellulose, K, B, Fe, Mn and Zn.

Elevation and soil controls on spectranomic traits

Following conversion of each reflectance and transmittance spectrum to chemical values, again referred to here as spectranomic traits, we calculated site-level statistics and plotted them against elevation. Fig. 4 shows the elevation dependence of spectranomic traits based on leaf reflectance; Fig. S5 of the online material provides a similar set based on transmittance. Most traits were highly separable by lower and higher fertility soil classes in the lowlands, shown as open versus closed circles in Fig. 4. Independent of whether the results are based on reflectance or transmittance, LMA was higher and N, P, water and photosynthetic pigments were lower on the lower fertility soils (Table S4). Among structure and defense traits, phenols, tannins, total C, cellulose, hemi-cellulose and lignin were higher in the low-fertility sites, whereas soluble C was lower (Figs 4, S4; Table S3). Lowland low-fertility sites also harbored canopies with consistently lower concentrations of Ca, K, Mg, Fe, B, and Zn.

With increasing elevation among higher fertility forests (closed symbols in Fig. 4), we spectrally measured site-level increases in LMA, water, soluble C, and Mn (Table S5). LMA, soluble C and Mn were the most sensitive to elevation, increasing by 100%, 50%, and 1000%, respectively. Conversely, increasing elevation was highly correlated with decreases in N, Ca, chlorophylls, carotenoids, cellulose, and lignin. Specifically, N and Ca decreased by 50% and 75%, respectively. The variance in foliar N increased noticeably in the highest elevation sites, whereas Ca variation remained low in all forests from lowland to treeline. Spectrally derived foliar structural traits such as cellulose and lignin also declined by 15% to 50%, but notably we found no decline in chemical defense traits such as phenols and tannins. Transmittance-based traits followed similar soil and elevational patterns to those of reflectance-based traits (Fig. S5, Table S6).

Phylogenetic partitioning of spectranomic traits

Beyond the average site-level changes in spectranomic traits measured throughout the region, we also found strong phylogenetic partitioning of the trait variance within forest communities and across the elevation gradient (Figs 5 and S6 for reflectance- and transmittance-based results, respectively). Structure and defense compounds including lignin, cellulose, hemi-cellulose, tannins and phenols, as well as water, N, and total and soluble C, displayed the strongest taxonomic partitioning (> 60%). Among

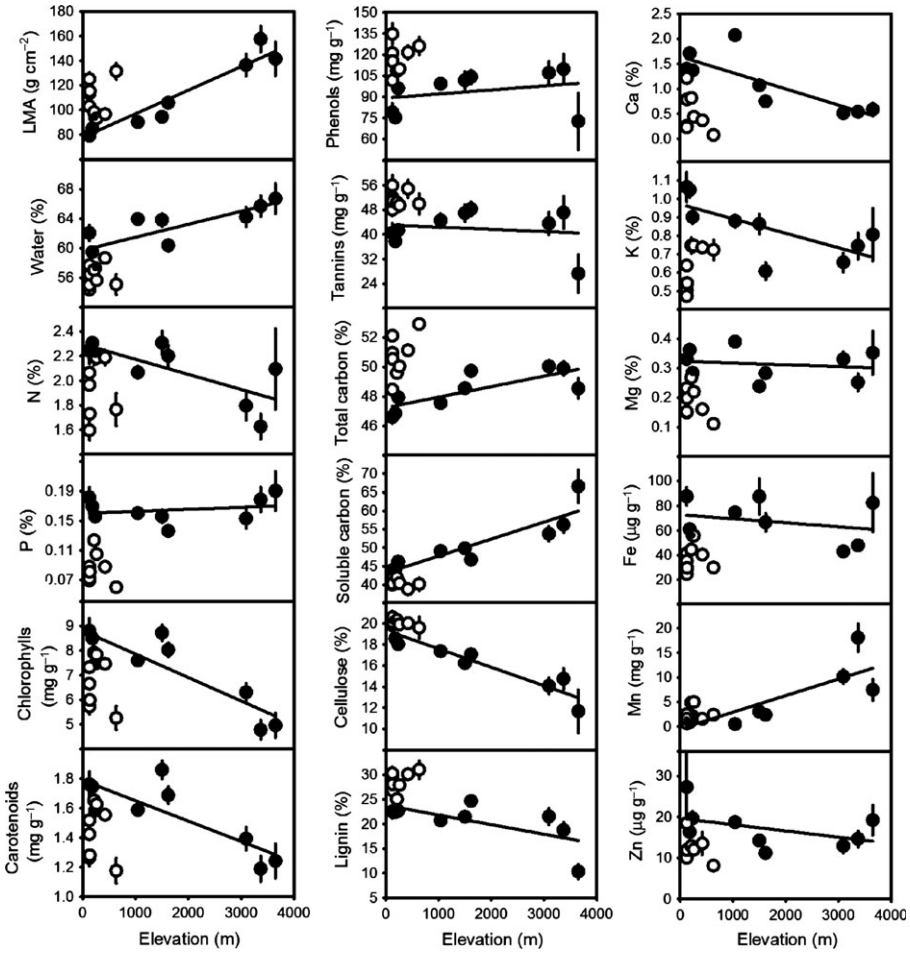

Fig. 4 Effects of elevation and soil fertility on site-averaged spectranomic traits derived from leaf reflectance spectroscopy. See Supporting Information Fig. S5 for the version based on leaf transmittance spectroscopy. Bars indicate ± 1 SE of the mean of spectranomic traits at the site level. Open symbols, lower fertility sites; closed circles, higher fertility sites (Table 1). Regression lines are for the higher fertility sites only. All traits are calculated on a dry weight basis.

these chemicals, the partitioning of variance was driven mostly at family and species levels of organization. The strength of taxonomic partitioning increased further when considering only the higher fertility sites (Fig. 5). For example, phylogenetic partitioning of the spectroscopically derived P variation throughout the region increased from *c.* 32% among all sites to 73% among higher fertility sites alone. In general, rock-derived nutrients were more strongly organized by 'site' than by phylogeny, particularly for P and Ca, which are the two nutrients known to control C uptake in humid tropical forests. We also found strong site-level control of the regional Mn pattern. Despite the effects of 'site' on several rock-derived nutrients, it remained a somewhat small contributor – often < 15% – to the explained variance in most spectranomic traits (Figs 5, S6), indicating that, within any given community along the elevation gradient, phylogeny dominates over local differences in soils, microclimate, and other factors. Here 'site' also incorporates variation among replicates within species, including variability caused by leaf, branch or canopy selection during our field collections.

Using LDA to combine spectranomic traits into multi-trait signatures, we found that up to 85.5% of all species could be accurately classified based on our complete 20-trait spectranomic signature for leaf reflectance (Fig. 6). We achieved higher classification accuracies of 93.1% and 89.8% for species in higher and lower fertility forests, respectively (Table 3). Consistently, the foliar traits most important to determining taxonomic identity included tannins, N, soluble C, total C and water. The least important included Ca and photosynthetic pigments. Genus- and family-level determinations generally tracked those of species, but at lower levels of accuracy. Genera could be predicted from spectranomic signatures with 67.6–79.2% accuracy; families were limited to 47.0–64.2% accuracy. Finally, transmittance-based results generally tracked those of reflectance (Table S6).

Discussion

Our results reveal enormous spectral and chemical variation among canopy trees found throughout the western Amazon. We also discovered quantitative links between spectral and chemical traits, and developed a causal chain leading from foliar spectral diversity to highly accurate classification of tree taxa found throughout the region. In combination, our findings suggest that the spectroscopy of canopy foliage is organized in a nested pattern determined regionally by soil fertility and elevation, and locally to regionally by phylogeny. This finding sheds new light on the degree and causes of variation in how tree canopies interact with sunlight, as well as the potential use of this information for mapping functional and biological diversity in humid tropical forests with spectroscopy.

Spectral variability among Amazonian trees

Among the 1449 canopy tree species we assessed, variation in the leaf reflectance and transmittance properties of mature, green canopy foliage was extremely high compared with other biomes (Williams, 1991; Peñuelas *et al.*, 1997; Roberts *et al.*, 1998; Asner *et al.*, 2000; Castro-Esau *et al.*, 2004). Critically, the majority of the measured spectral diversity was driven by inter-specific rather than intra-specific variability. While some studies have focused on how intra-specific variation in leaf optical properties can dominate the spectral dynamics of temperate ecosystems due to phenology (Demarez *et al.*, 1999; Kodani *et al.*, 2002), along vertical canopy light gradients, and with leaf age (Poorter *et al.*, 1995; Roberts *et al.*, 1998), our results suggest that canopy foliage in western Amazonian forests follows a somewhat different set of rules. The upper canopy is much drier and less susceptible to epiphylls, herbivory, and other factors that often enhance intra-specific variation in leaf spectral signatures (Vourlitis *et al.*, 2008). During foliar collections, we documented the condition of leaves on branches as they were harvested from the treetops, finding an average of 9% with damage or epiphyll growth substantial enough to affect reflectance or transmittance (G. P. Asner, unpub. data). Such effects were more common in the humid submontane sites between 1000 and 2000 m elevation (up to 23%), but not systematically so, and they occurred in discrete landscape patches comprised of

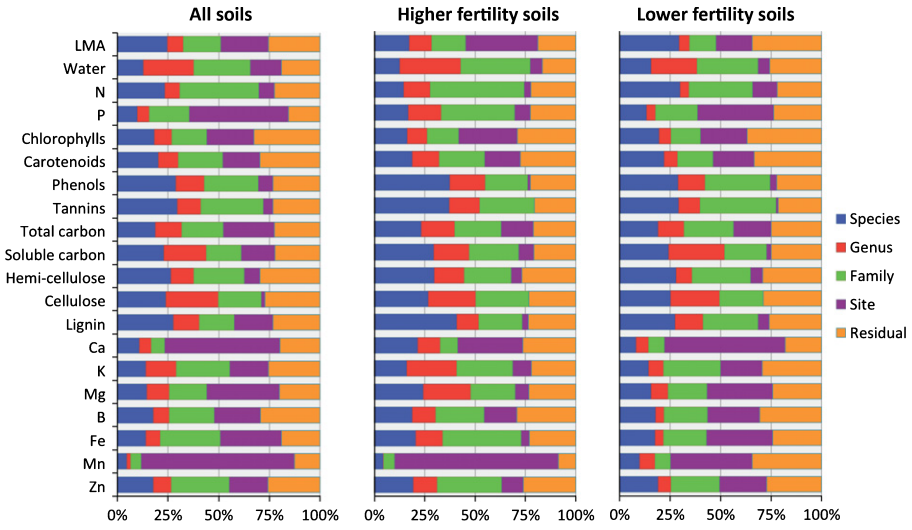

Fig. 5 Partitioning of the variance for spectranomic chemical traits derived from leaf reflectance spectroscopy into phylogenetic (family/genus/species), site, and unexplained residual components for: (left) all sites on all soil types; (center) sites on higher fertility soils; (right) sites on lower fertility soils. The 'site' component incorporates variation in soils, geology, and topography, as well as tree and foliage selection in the field, among other factors. Unexplained residuals are comprised of measurement error and other non-site-related sources of uncertainty. A similar result for chemicals and leaf mass per unit area (LMA) derived from leaf transmittance spectroscopy is shown in Supporting Information Fig. S6.

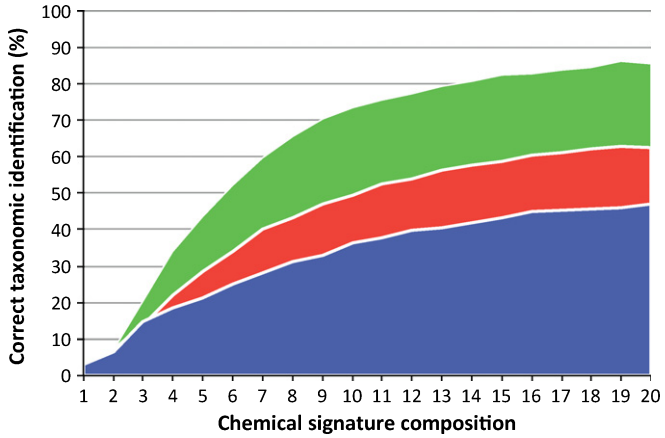

Fig. 6 Increase in the accuracy of spectranomic classification of tree families (blue), genera (red) and species (green) using stepwise linear discriminant analysis (LDA) based on foliar reflectance of species containing two or more individuals. Spectranomic composition steps (1–20) map to specific chemical elements, molecular compounds and leaf mass per unit area (LMA) listed in Table 3. A comparable set based on foliar transmittance is provided in Supporting Information Table S5.

a few crowns at the time. These patches did not display a relationship with site fertility. Our sampling focused on the outer 'skin' of the canopy, a strategy that surely accentuated spectral differences between, rather than within, species and foliated branches. Yet these are the portions of the canopy that harvest the vast majority of the energy input to the ecosystem (Saldarriaga & Luxmoore, 1991; Doughty & Goulden, 2008), and which also dominate remotely sensed optical measurements of highly foliated canopies from aircraft or satellites (Asner, 2008). As such, our results of 2–3 times greater between-species than within-species spectral variation indicate that phylogeny is a key determinant of spatial variation in the reflectance properties and solar radiation use of Amazonian forests.

Chemical determinants of leaf spectra

The measured spectral variation was linked to multiple chemical traits and LMA (Table 2). The traits that were best retrieved from the spectra ranged from those that mediate light capture and CO_2 uptake, to longevity and defense, as well as a plethora of foliar metabolic processes. The best spectroscopic determinations were LMA, water, N, chlorophylls, carotenoids, total C, cellulose and several base cations and micronutrients. Defense compounds including lignin, phenols and tannins were also well estimated from spectroscopy, albeit with increased noise that parallels the uncertainty inherent in laboratory assays of these chemicals (Ainsworth & Gillespie, 2007). We note that many of these foliar traits have been estimated from spectroscopy in the past (see recent reviews by Jacquemoud *et al.*, 2009; Kokaly *et al.*, 2009; Ustin *et al.*, 2009). Others, such as the micronutrients B, Fe, Mn, and Zn, although less precisely quantified by spectroscopy, were also retrieved with surprising accuracy. This finding supports the hypothesis that leaf reflectance and transmittance signatures are an expression of both the direct elemental and molecular composition of specific chemicals such as water, pigments, N, and C

Table 3 The cumulative effect of combining leaf spectranomic traits based on reflectance in the prediction of tree canopy species using linear discriminant analysis (LDA) (Fig. 6)

	Species			Genera			Families		
LDA step	All sites	High-fertility	Low-fertility	All sites	High-fertility	Low-fertility	All sites	High-fertility	Low-fertility
1	C	Tannins	Tannins	Water	Water	Water	C	C	Water
2	Water	Sol-C	Lignin	C	N	C	Water	Sol-C	C
3	Tannins	Water	N	Sol-C	C	Tannins	Sol-C	N	Tannins
4	N	LMA	Water	Tannins	Sol-C	Sol-C	N	Tannins	Sol-C
5	Sol-C	C	C	N	Tannins	N	Tannins	Mn	P
6	P	Mn	Cellulose	Mg	Ca	Mg	Mn	Water	Mg
7	Cellulose	N	LMA	Hemi	Hemi	Phenols	Mg	B	Hemi
8	LMA	Cellulose	P	Mn	Fe	Hemi	B	Mg	B
9	Mn	K	Phenols	Fe	K	LMA	Hemi	Hemi	Lignin
10	Hemi	Hemi	Zn	B	B	Lignin	Zn	Fe	LMA
11	K	Mg	Mg	Zn	Mn	Zn	P	Zn	Zn
12	Mg	Fe	Hemi	LMA	Mg	B	LMA	K	N
13	Zn	Zn	K	Cellulose	Zn	Fe	Cellulose	Ca	Phenols
14	B	B	B	Phenols	Cellulose	K	Phenols	Car	Ca
15	Phenols	P	Car	P	Car	P	Ca	Chl	Chl
16	Fe	Phenols	Chl	K	Chl	Car	K	Cellulose	Car
17	Car	Ca	Mn	Car	P	Chl	B	P	K
18	Chl	Car	Ca	Chl	Phenols	Mn	Chl	Phenols	Mn
19	Ca	Chl	Fe	Ca	LMA	Ca	Car	LMA	Fe
20	Lignin	Lignin	Sol-C	Lignin	Lignin	Cellulose	Lignin	Lignin	Cellulose
%ID	85.5	93.1	89.8	62.6	79.2	68.3	47.0	64.2	55.1

Results are shown for species, genus and family levels, and by all sites, higher fertility sites, and lower fertility sites. A comparable set of results based on foliar transmittance is provided in Supporting Information Table S6. Car, carotenoids; Chl, chlorophylls; Hemi, hemi-cellulose; LMA, leaf mass per unit area; Sol-C, soluble carbon.

(Curran, 1989), and the indirect expression of the constellation of other chemicals that support whole-leaf stoichiometry and functional processes (Porder *et al.*, 2005; Martin *et al.*, 2007). Critically, all 19 chemical traits and LMA were predicted from spectroscopy at the $P < 0.05$ significance level (Table 2), allowing us to test combinations of spectranomic traits for use in taxonomic classification.

Environmental controls on spectranomic traits

The leaf spectranomic traits, derived by converting spectra to chemicals, were well organized by soil fertility in the Amazonian lowlands and by elevation to the Andean treeline. Here we emphasize that all findings are based on chemical and LMA estimates derived from spectroscopy; that is, we calibrated the spectra to laboratory-assayed chemicals and LMA, and then predicted the chemical portfolios of all 2567 individual trees from the spectroscopy, followed by statistical analyses of species, genera, and families at site and regional levels. We found that lower fertility forests contain tree canopies with elevated concentrations of defense compounds including phenols, tannins, and lignin, as well as higher LMA (Fig. 4). These trees have lower concentrations of every macro- and micro-nutrient known to underpin growth and multiple metabolic processes. Additional review of the lower fertility sites in the lowlands indicates that trees found on the dystrophic white sand soils in northern Peru maintain the absolute highest levels of defense and lowest nutrient investment of all forests in the study, including the highest altitude sites (Tables 1, S3). Such uniquely evolved chemical, and thus spectral, traits may help explain the functional divergence of tree canopies on these unique white sand ecosystems.

Elevation was another strong driver of change in community-averaged spectranomic traits (Fig. 4). Recent elevation studies in the western Amazon suggest that increasing altitude is associated with decreasing gross and net primary production (Girardin *et al.*, 2010; Moser *et al.*, 2011; Huasco *et al.*, 2013). Our results support this finding by revealing declines in foliar N and photosynthetic pigment concentrations, and increasing LMA, with elevation among the higher fertility sites, which in combination suggest declines in photosynthesis in higher altitude forests in the Andes-to-Amazon region (see Wright *et al.*, 2004). The elevational decline in Ca concentrations revealed in our data may also be linked to suppressed growth.

In contrast to growth-related traits, defense compounds such as phenols and tannins showed no trend with elevation. However, we did observe a strong shift in C allocation, with soluble C increasing substantially at higher altitudes while cellulose concentrations declined. Lignin also showed a modest decline at higher altitudes. Increasing soluble C with elevation gain is probably related to increased allocation to waxes that protect high-elevation foliage (Asner *et al.*, 2014). However, decreasing cellulose relative to soluble C also suggests a possible bottleneck in the processing of sugars and starches to cell walls. Such a bottleneck might be caused by Ca deficiency at higher elevation (Grubb *et al.*, 1963; Demarty *et al.*, 1984).

Phylogenic pattern in spectranomic traits

Despite the clear regional effects of soils and elevation on average spectranomic trait values, we also found strong evidence for phylogenetic organization of many of the traits derived from spectroscopy (Fig. 5). This applied primarily to leaf structure and defense compounds such as lignin, cellulose, phenols and tannins; the phylogenetic partitioning of variance among these chemicals was strongly determined at family and species levels. These findings may reflect selective pressure among co-existing tree species to diverge in C and defense allocation, thereby maintaining contrasting strategies in the presence of host-specific herbivores (Marquis, 1984; Fine *et al.*, 2006).

Foliar N and water displayed surprisingly strong phylogenetic organization, whereas rock-derived nutrients including P, base cations and micronutrients all showed greater sensitivity to site and unknown residuals than to phylogeny (Fig. 5). The pattern for spectroscopically derived N may be driven, in part, by the hyper-abundance of taxa in the N-fixing family Fabaceae (Table S2), with its highly variable N concentrations and low intra-specific variation among genera and species (Bustamante *et al.*, 2006; Nardoto *et al.*, 2008; Asner *et al.*, 2014). By contrast, high phenotypic plasticity in P and other rock-derived nutrients probably reflects a need to negotiate resource scarcity and patchiness in highly weathered soils (Correa & Reichardt, 1989; Quesada *et al.*, 2009). This hypothesis is strongly supported by an observed doubling of the phylogenetic attribution of variance in foliar P and Ca, and to a lesser extent Mg and Fe, when we constrained the analysis to high-fertility sites alone (Fig. 5). However, a lack of response in phylogenetic organization of K, B, and Zn on higher versus lower fertility sites suggests that these micronutrients do not limit productivity or some other metabolic function to the degree in which it would be expressed as an evolved spectranomic trait.

The possibility of spectranomic signatures – combinations of chemical traits – creating linkages between phylogeny and spectroscopy remains a new scientific area of inquiry. In high-diversity, humid tropical forests, an evolutionary history of intense biotic interactions (e.g. competition, mutualism, and pest defense) and generally good growth conditions (e.g. high net primary production) should theoretically create highly organized canopy spectranomic signatures. Our LDA modeling results strongly support this hypothesis: multi-chemical signatures derived from foliar spectroscopy are highly unique among co-existing tree species found on each soil type and throughout the region as a whole (Fig. 6, Tables 3, S6). In turn, this suggests the existence of a wide range of strategies played out on the forest landscape to negotiate varying niche conditions and biotic interactions over time.

Spectral assembly of the Amazonian canopy

The functional and biological diversity of the Amazonian forest canopy is sometimes portrayed as relatively homogeneous, or somewhat smoothly varying, throughout the lowlands (Condit *et al.*, 2002; ter Steege *et al.*, 2006, 2013). However, our results

indicate that tree canopy spectral and chemical composition varies strongly within and across communities in the western Amazonian lowlands. In fact, spectrally derived chemical variation across lowland communities meets or exceeds that which is achieved across > 3000 m of elevation change (e.g. Fig. 4). With foliar spectroscopy closely tracking multiple chemicals throughout these forests, we contend that the fundamental interaction of the canopy with incoming light also changes by soil type in the lowlands. Although traditional optical metrics such as total absorbed photosynthetically active radiation (APAR) are saturated and fail to produce a pattern across our lowland sites (data not shown; derived from Fig. 2, 400–700 nm range), other parts of the optical spectrum, particularly the NIR and SWIR (700–2500 nm), are highly sensitive to changing lowland sites (Fig. 2). This finding suggests not only multiple chemical responses to lowland soil fertility but also that lowland forest albedo, which is largely determined by NIR and SWIR spectroscopy (Ollinger, 2011), is linked to soil fertility. This finding also provides a mechanistic link between canopy brightness patterns observed in broadband NIR and SWIR reflectance of Landsat imagery, and changes in soil fertility and floristic composition reported for northwestern Amazonian forests (Tuomisto *et al.*, 1995; Higgins *et al.*, 2011).

Transitioning from the lowlands to the Andean treeline, field plot data suggest a pattern of decreasing productivity and soil N availability with increasing elevation (Fisher *et al.*, 2013; Girardin *et al.*, 2013; Huasco *et al.*, 2013). A recent study also showed that foliar N : P decreases with elevation (Metcalfe *et al.*, 2013), yet this pattern is dominated by a few white sand and highly weathered clay sites in the lowlands that locally suppress foliar P concentrations (Asner *et al.*, 2014). Removing these dystrophic sites from the analysis reveals that P does not change with elevation alone (Fig. 4), and thus foliar spectral properties are not changing in response to changing P with elevation. Instead, our results indicate that average foliar spectral properties along the elevation gradient are driven strongly by changes in LMA, N, photosynthetic pigments, soluble C, and cellulose, among others (Table S3). In other words, light capture and growth traits are clearly responsible for changes in foliar spectra with elevation. These findings agree with and explain recent results from a top-down analysis of 25-ha forest landscapes along an Andes-to-Amazon elevation gradient as sensed in airborne whole-canopy reflectance measurements (Asner *et al.*, 2013).

Despite the differential effects of soil fertility in the lowlands and elevation to the Andean highlands, our results reveal the existence of a kaleidoscope of spectral variation within each forest community. From the lowlands into the montane region, high spectral diversity is predominantly driven by between-species rather than by within-species variation in leaf reflectance and transmittance in the upper canopy. Although we do not yet know the relative importance of drivers of the chemical diversification underpinning this spectral diversity among co-existing trees, they are likely to be the same drivers often invoked to explain the existence of high biological diversity, such as phylogenetic niche conservatism (Wright, 2002; Brown, 2014). Our results strongly suggest that high spectral diversity is both part and parcel of high functional and biological diversity, perhaps by way of chemical defense trait evolution in response to host-specific pest and pathogen pressure (Janzen, 1970; Coley & Barone, 1996; Fine *et al.*, 2006; Coley & Kursar, 2014). In turn, local-scale phylogenetic control of leaf spectroscopy probably mediates each taxon's contribution to ecosystem processes ranging from C cycling to hydrologic functioning and nutrient dynamics. The spectroscopy of canopy foliage thus expresses spatial variation in the biogeochemical processes that underlie the Amazonian forest function.

Finally, our results demonstrate that high-fidelity optical spectroscopy might serve as a quantitative surrogate for laborious wet chemical assays, thereby saving much time and expense. Measuring full-range (400–2500 nm) spectral reflectance and transmittance with very low noise and high signal performance means that we can derive chemical estimates with laboratory precision and accuracy in the field. In turn, this could advance our ability to modify field data collections 'on the fly', thereby improving efficiencies that may support more rapid discoveries. Our findings also strongly support the need for a spaceborne high-fidelity imaging spectroscopy mission to probe the spectral, chemical and biological composition of our biosphere. Without a high-fidelity imaging spectrometer in Earth orbit, opportunities to understand functional and biological diversity change are currently unrealized, ironically in an era of the most rapid changes thought to be underway since the last ice age (Schimel *et al.*, 2013).

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Intra-specific and inter-specific variation in leaf reflectance and transmittance spectra in the 400–800-nm wavelength range.

Fig. S2 Ratio of intra-specific to inter-specific variation in leaf reflectance and transmittance spectra of forest sites.

Fig. S3 Standardized weighting coefficients for partial least squares regression (PLSR) for leaf chemical traits and LMA using foliar reflectance spectra.

Fig. S4 Standardized weighting coefficients for partial least squares regression (PLSR) for leaf chemical traits and LMA using foliar transmittance spectra.

Fig. S5 Effects of elevation and soil fertility on site-averaged chemical traits and LMA derived from leaf transmittance spectroscopy.

Fig. S6 Partitioning of the variance for spectranomic traits derived from leaf transmittance spectroscopy.

Table S1 Taxonomic arrangement of samples collected throughout the Andes-to-Amazon region

Table S2 List of canopy tree taxa measured in the study

Table S3 Mean and standard deviation of tree foliar chemical traits at each study site

Table S4 Analysis of variance tests comparing higher and lower fertility soils

Table S5 Relationships between modeled chemical traits and LMA ('spectranomic traits') and elevation for higher fertility sites

Table S6 The cumulative effect of combining leaf spectranomic traits based on transmittance in the prediction of tree canopy species using linear discriminant analysis (LDA)

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